Immunogenicity of clinically relevant SARS-CoV-2 vaccines in non-human primates and humans

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Abstract

Multiple preventive vaccines are being developed to counter the COVID-19 pandemic. The leading candidates have now been evaluated in non-human primates (NHPs) and human Phase 1 and/or Phase 2 clinical trials. Several vaccines have already advanced into Phase 3 efficacy trials, while others will do so before the end of 2020. Here, we summarize what is known of the antibody and T-cell immunogenicity of these vaccines in NHPs and humans. To the extent possible, we compare how the vaccines have performed, taking into account the use of different assays to assess immunogenicity and inconsistencies in how the resulting data are presented. We also summarize the outcome of SARS-CoV-2 challenge experiments in immunized macaques, while noting variations in the protocols used, including but not limited to the virus challenge doses.

Introduction

The COVID-19 pandemic rages unabated and may continue to do so until there is a safe, effective and widely used protective vaccine. Multiple vaccines to prevent SARS-CoV-2 infection and/or COVID-19 disease are now progressing through pre-clinical testing and Phase 1/2 human trials, while some are already in Phase 2/3 efficacy trials in and outside the USA (https://www.who.int/who-documents-detail/draft-landscape-of-covid-19-candidate-vaccines) (1-17). Several of these mid- to late-stage vaccines are part of the US Government's Operation Warp Speed (OWS), while candidates produced in China are also well advanced in the evaluation and approval process (Table 1). The Russian government has approved an adenovirus-vector based vaccine of uncertain composition after minimal safety testing and with no evidence of protective efficacy. We have ignored this vaccine as there is no published scientific information to review. All the vaccines are based predominantly or exclusively on the viral Spike (S)-protein, which is administered by various methods including expression from non-replicating adenoviruses and nucleic acid vectors, as recombinant proteins and as a component of inactivated viruses (Table 1).

It is of considerable scientific and public interest to know the immunogenicity of the leading vaccines in absolute and, to the extent possible, comparative terms. Here, we have reviewed antibody and T-cell immune response data derived from published studies of vaccines that were tested in non-human primates (NHPs) and then progressed into human Phase 1/2 trials, or that are in human trials without a prior NHP experiment (Tables 2 and 3). We have also evaluated macaque vaccine-challenge experiments, including how they were performed, as the outcomes are relevant to understanding the protective potential of SARS-CoV-2 vaccines (Table 3). The NHP experiments are described in references (1-9), the human trials in references (10-18). Some of the vaccines tested in NHPs were advanced into human trials for which no data have been

released. For example, the Sinovac and both Sinopharm inactivated virus vaccines are now in Phase 3 trials in South America, but Phase 1/2 trial data have been reported only for the Sinopharm/WIBP vaccine (10). The Chinese government approved the CanSino Ad5-nCoV vaccine for military use in June 2020, presumably on the basis of the Phase 1/2 trial data (11, 12).

The immunogenicity of some of the >150 vaccine candidates now in pre-clinical development worldwide has been tested in small animals and, in some cases, NHPs. These reports are beyond the scope of this review, although we summarized several previously (19). Small-animal immunogenicity studies that directly relate to the vaccine candidates we review here are described in several of the papers on NHP experiments and human trials, and also in references (20-22).

Assessing antibody responses to vaccine candidates

Antibodies induced by the S-protein based immunogens are generally measured in two ways. Immunoassays, usually but not always ELISAs, quantify antibody binding to the S-protein or fragments thereof, such as the receptor-binding domain (RBD). Neutralization assays assess the abilities of neutralizing antibodies (NAbs) to inhibit SARS-CoV-2 infection of target cells. The binding and neutralizing antibody assays both have value, and titers derived from them generally correlate reasonably well. However, neutralization assays quantify antibodies that block infection while ELISAs and other binding antibody assays also detect antibodies that lack such properties (non-neutralizing antibodies, or non-NAbs). Other assays are sometimes used, for example to detect antibodies that inhibit the binding of the S-protein or its RBD to a soluble form of the angiotensin-converting enzyme 2 (ACE2), which is the entry receptor for SARS-CoV-2. We restrict our discussion to binding antibodies and NAbs, with some exceptions. A general theme in the papers we summarize is the use of COVID-19 convalescent plasma as comparators for vaccine-

induced antibody responses. We have ignored all of these data sets. The serum panels differ among the various studies, and the range of antibody titers seen in COVID-19 patients can span a 5-log range and vary considerably also during convalescence (19). Accordingly, we have not found the convalescent serum panels helpful when gauging the relative immunogenicity of the various vaccine candidates.

As we have noted previously, different research groups use different assays and measure antibody binding and virus neutralization differently, which greatly complicates comparisons of datasets (19). While binding antibodies are most often measured using ELISAs, which tend to be fairly similar wherever they are performed, different measurements are recorded. Thus, endpoint titers are presented in some reports but 50%-binding titers (ED_{50}, effective serum dilution factor giving 50% of maximum binding) in others. Indeed, in some high-profile descriptions of Phase 1 human trials ELISA data are presented in idiosyncratic formats that compromise attempts to cross-compare vaccine immunogenicity. NAbs are measured using either replicating viruses (RV assays) or S-protein pseudotyped viruses that do not complete an infection cycle (PV assays). Usually, but not always, PV assays are a few-fold more sensitive than RV assays and therefore generate higher titer values. The different conditions used to perform RV and PV assays can, however, also affect their sensitivity. The most common practice is to report neutralization data as 50%-neutralizing titers (ID_{50}, inhibitory serum dilution factor giving 50% infectivity), but this is not always done. Other measurements include the more stringent neutralization ID_{80}, $ID_{>99}$ values, or endpoint binding titers, as well as areas under the curve (AUC). An additional point is that, in some papers, titer values are not listed in Tables, Figures or the text but can only be estimated from diagrams. When we have had to make such estimates, the values we list in the text and Tables are preceded

by an ~ symbol, which we also use when approximating for succinctness. When multi-digit NAb and binding titer values are reported in the original papers, we repeat those numbers in this review but note that it is rarely justified to use more than two significant figures in such circumstances. Finally, we report median and geometric mean values for the study groups as was done in the original papers. The range of SARS-CoV-2 vaccine-induced antibody titers seen in groups of NHPs and humans generally exceeds 100-fold and can be as high as 1000-fold. As noted previously, the existence of such a wide range of responses has implications for the proportion of a population that a vaccine can protect (19). The titer spreads reported in the various primary papers are worth comparing from this perspective, which we do where indicated.

Antibody responses in the various studies are measured for only a short period after the final immunization or, in the case of some macaque studies, after the virus challenge. The same constraint applies also to the T-cell data. Therefore the lack of knowledge of the longevity of vaccine-induced immune responses is a substantial gap that will need filling.

In almost all of the papers we review, antibody responses are measured only in serum. There has been very little attention paid, to date, to mucosal immune responses, which seems unfortunate given how SARS-CoV-2 is transmitted and where it predominantly replicates. Accordingly, we cannot address mucosal immunity in this review, other than by noting that one recent preclinical study of a chimp adenovirus vaccine (different from the AstraZeneca clinical candidate) in mice highlights how important inducing and characterizing mucosal immune responses might turn out to be (23).

In short, it is often difficult to inspect two different papers on vaccines A and B and conclude with certainty which one induces the superior immune response. Knowledge of how

vaccines of different designs generally perform can help form judgements, but there must always be caveats.

Assessing T-cell responses to vaccine candidates

T cell responses to vaccine immunogens are generally measured by quantifying the amount of cytokine expressed by a T cell after specific antigenic stimulation from a peptide, protein or vector delivered antigen. The ELISPOT assay is most often used, or variants thereof, with peripheral blood mononuclear cells (PBMC) being the commonest source for T cells. Interferon gamma secretion is the most commonly chosen cytokine output but other cytokines are sometimes also measured, as is the production of granzyme B. Cytokine flow cytometry (CFC) is often used as a read out, and there is generally a good correlation between ELISPOT and CFC assay results. An advantage of the CFC assay is that it can directly identify the phenotype of responding T cells, which requires depletion procedures when ELISPOT is used. Assays for antigen-specific CD4+ T cells sometimes measure the upregulation of surface activation induced markers (AIM). However, these methods do not usually measure T cell avidity or test the potency of cells in viral inhibition assays (24). Here, we confine our discussion to ELISPOT assays, with some exceptions.

Depending on the vaccine candidate antigen, a T cell assay can use individual peptides, pooled or matrix-pooled peptides, protein or vector-expressed antigen as a source of peptides to bind to the MHC molecules that are expressed on the cell surface and recognized by a specific T cell receptor. As cross-reactive T cells are known to occur, most assays will not specifically identify a response that was elicited by prior exposure to a cross-reactive pathogen (or a different vaccine). For example, an earlier infection with one of the common cold coronaviruses might lead to a secondary memory response that could skew the outcome of the SARS-CoV-2 vaccine trial

analysis, unless prior infection by those other coronaviruses is an exclusion criterion (which is rarely if ever the case).

ELISPOT results are usually expressed as spot forming cells (SFC) per 10^6 input PBMC, but this is not a uniform practice. For example, some investigators use SFC/10^5 cells as their read out; we multiply their values by 10 and report them as SFC/10^6 cells. We also use the abbreviation SFC rather than SFU when the latter is used in the original paper. There are also variations in methodologies, including the length of time between blood draw and cryopreservation, the method used for thawing, the peptide concentration used, the duration of peptide incubation with the cells, the time taken to complete the assay, and whether responding T cells are separated. All of these factors can affect an ELISPOT result, and need to be considered when comparing different studies. A general feature of the papers we have summarized is a lack of detail on how the assays were performed. It would also be useful if images of key ELISPOT plates were provided as raw data, to allow the spots to be re-counted. The timing of when cell samples are collected after a vaccine prime or boost is also relevant. Thus, the time-dependent decay of circulating T-cells affects the magnitude of the responses measured *in vitro*, to a greater extent than applies to the more stable antibody responses.

Concerns have been expressed that SARS-CoV-2 vaccines may cause enhanced disease in infected animals, based on data arising in earlier animal model experiments with vaccines against other coronaviruses (19, 25-28). One particular potential problem is referred to as Vaccine-Associated Enhanced Respiratory Disease (VAERD; 28). While it is not possible to determine whether VAERD will be a problem with SARS-CoV-2 vaccines prior to the outcome of efficacy trials and post-licensure safety assessments, the pulmonary dysfunctions are associated with

increased production of IL-4, -5 and -13, eosinophil recruitment and impeded CD8+ T-cell responses (27-29). This pattern of immune responses is indicative of Th2-polarization. Accordingly, some of the NHP and human experiments include analyses of *in vitro* cytokine release profiles, to seek signs of unwanted, Th2-biased responses. To date, Th2 responses have rarely been seen. We briefly note the outcomes of such analyses when they were performed.

Immunogenicity of vaccine candidates in NHPs

Immunogenicity studies have been performed in rhesus or cynomolgus macaques or, in one case, baboons (1-8). The immunogens were generally tested beforehand in small animals, often but not always mice, to provide initial assessments of their performance and to provide some indication of the dose or dose range to then evaluate in NHPs. Here, we focus only on the NHP studies themselves; the primary papers should be consulted for the small animal data. In general, the NHP experiments also involved safety assessments. The outcomes were unexceptional in that no significant problems were reported in the primary papers, which should, again, be consulted for details. Key serum antibody titer values recorded in this section are summarized in Table 2 and, for data obtained at the time of closest to challenge, also in Table 3. T-cell response data are similarly summarized and tabulated, although these assays were not performed in several of the studies. In all cases, the vaccines were administered intramuscularly (i.m.), which also applies to the human clinical studies (see below). However, recent small-animal studies of a chimp adenovirus vaccine and an Ad5 vaccine (not the AstraZeneca/Oxford and CanSino clinical candidates, respectively) suggests that viral vectors might be very fruitfully delivered by the intranasal route instead (23, 30).

Usually, one or more sub-groups of macaques were rolled over into a SARS-CoV-2 challenge study, or the optimal regimen was tested in a *de novo* experiment. Some details and the outcomes of the virus challenges are summarized separately below and in Table 3.

The first macaque immunogenicity paper to appear described PiCoVacc, the Sinovac ß-propiolactone-inactivated, Vero cell-produced virus vaccine (1). Two vaccine doses (3 μg and 6 μg of viral protein) with an Alum adjuvant were tested on groups of 4 rhesus macaques via three immunizations on days 0, 7 and 14. The 6 μg dose elicited slightly the stronger antibody responses on day 21, when the anti-S protein geometric-mean (GM) ELISA endpoint titers were ~12,800 and NAb GM ID_{50} values were ~50 in an RV assay (Table 2). Antibodies specific for the RBD dominated the antibody response to the inactivated virus vaccine, which is relevant to understanding the outcome of the challenge experiment (see below).

The Sinopharm/BIBP inactivated virus vaccine, BBIBP-CorV, was also produced in Vero cells and inactivated with beta-propionolactone. Mixed with Alum adjuvant, three different doses (2 μg, 4 μg and 8 μg of viral protein) were administered to groups of 10 cynomolgus macaques on days 0, 7 and 14 (2). The resulting NAb titers, measured in an RV assay, were dose-dependent, with a GM ID_{50} value of ~210 reported for the highest dose group on day 21 (Table 2).

The ChAdOx1nCoV-19 recombinant virus vector expresses the SARS-CoV-2 S-protein (3). Groups of 6 rhesus macaques received this vaccine (2.5 x 10^{10} particles) either once (day 0) or twice (days 0 and 28) in a prime-boost protocol. In the single-dose group, the anti-S protein median endpoint titer on day 14 was ~600 and the median NAb ID_{50} value was ~20 in an RV assay. The second dose boosted these responses to ~28,000 and ~280, respectively, on day 42

(Table 2). The animals were challenged with SARS-CoV-2 on day 28 (one-dose group) and day 56 (prime-boost group), as summarized below.

Rhesus macaques were used to identify and evaluate the optimal design of the Janssen Ad26.COV2.S vaccine candidate (4). First, antibody responses to seven different S-protein variants were compared using a range of assays, leading to the selection of the optimal Ad26 S.PP design. After a single dose of this immunogen, median RBD-ELISA endpoint titers at week 4 were ~4000, while the PV and RV NAb median ID_{50} values were 408 and 113, respectively (Table 2). In an IFN-gamma ELISPOT, at week-4, the median response elicited by the S.PP vaccine was only ~80 SFC/10^6 cells. The data were insufficient to confidently assess the Th1 *vs*. Th2 bias, as only IFN-gamma and IL-4 responses were measured. T-cell response data were presumably not factored into the decision to choose the S.PP construct as the clinical candidate, as this virus was the least immunogenic of the seven variants from the perspective of inducing CD4+ and CD8+ T cell immunity. The antibody responses were clearly prioritized.

DNA vaccines expressing 6 different SARS-CoV-2 S-protein variants, including the full-length S-protein and the RBD, were tested, without adjuvant, in rhesus macaques (5). Median endpoint anti-S protein titers at week 5 varied moderately with the immunogen but were 140-180 for the full-length S-protein and RBD immunogen groups. Midpoint NAb titers at week-5 also varied by immunogen, with median ID_{50} values of ~50-200 and ~30-40 in PV and RV assays, respectively (Table 2). The full-length S-protein construct elicited somewhat stronger NAb responses than its RBD counterpart. At week 5, T-cell responses were detectable in ELISPOT assays with pooled S peptides (~80 SFC/10^6 cells in the S Group, Table 2). Intracellular staining showed IFN-gamma responses both in CD4+ and CD8+ subpopulations; the responses to full-

length S were stronger than to S1 and RBD. Finally, IL-4 responses were barely detectable, which is compatible with a Th1 bias of the cellular immune responses.

Inovio's INO-4800 S-protein based DNA vaccine was given to 5 rhesus macaques in 1 mg doses at weeks 0 and 4 by an i.m. electroporation device that provides a mild electric shock to open membrane channels in muscle cells (6). The peak anti-S protein GM endpoint titer (week 6) was ~130,000, but dropped ~40-fold by the time of challenge at week-17. The binding antibody endpoint titers against the RBD were ~5-fold lower than against the S-protein at week 6. In the PV NAb assay, the peak geometric-mean ID_{50} titers were ~1000 but had declined to ~250 by week-12 (i.e., a 4- or 5-fold titer decrease over 6 weeks against the two PVs tested). Low titers of anti S-protein IgG (~10) were also detected in bronchoalveaolar lavage (BAL) samples from vaccinated animals. An IFN-gamma ELISPOT was used to measure T-cell responses triggered by 5 peptide pools at week 6. Signals were seen with PBMC from 4 of the 5 animals, with a range of 0-518 SFC/10^6 cells and an arithmetic mean of ~140. By week-12, the mean value had declined to only ~30.

The Moderna S-protein based vaccine candidate, designated mRNA-1273, was also tested in rhesus macaques (7) The lipid-encapsulated mRNA formulation was given i.m. at doses of 10 μg or 100 μg to each of two groups of 8 animals, at weeks 0 and 4 Anti-S protein ELISA data were presented only in the form of AUC values, precluding direct comparison with other studies. For the high-dose group at 4 weeks after the second dose, the NAb ID_{50} GM values in PV and RV assays were 1862 and 3481, respectively. In a CFC assay, Th1 responses were dose-dependent, while Th2 and CD8+ T-cell responses were at most minimal. Although all animals responded according to pre-specified criteria, the T-cell assay signals were generally weak, even at the highest

vaccine dose. There were no differences in Th1 or Th2-associated cytokines or chemokines in BAL samples from the vaccine and control animals.

The Novavax NVX-CoV3273 vaccine is an insect cell-derived S-protein that is mixed with detergent to form what are described as nanoparticles (8, 31). That formulation is combined with the Matrix M adjuvant. The immunogenicity study in baboons compared 1, 5 and 25 μg doses of the S-protein in a two-dose (days 0, 21) regimen, while a fourth group received 25 μg with no adjuvant. Antibody assays on days 21, 28 and 35 showed that the optimal dose was 5 μg with adjuvant, with the peak response reached by day 28. The highest GM anti-S-protein GM EC_{50} and NAb $ID_{>99}$ values were 174,000 and 17,000, respectively (8). The same protein/adjuvant combination was then tested in cynomolgus macaques (9). The animals were immunized on days 0 and 21 at different doses, with both the protein and adjuvant amount varying (protein at 2.5, 5 and 25 μg doses). At the highest dose (25 μg S-protein, 50 μg adjuvant, mirroring one of the human study groups, see below), the anti-S protein GM EC_{50} value on day 35 was 469,739. Note that binding antibody data were presented as EC_{50} values, not the more usual endpoint titers, which would be substantially higher (perhaps 10-100 fold). In the RV NAb assay, the CPE_{100} (inhibition of ~100% of the cytopathic effect, approximately equivalent to $ID_{>99}$) was measured, with GM values ranging from 17,920-23,040 in the different dosing groups. It should be noted that the neutralization titers for near complete efficacy of neutralization measured in these studies (CPE_{100} or $ID_{>99}$) will be substantially lower than the more conventional IC_{50} values, although we cannot estimate by how much. In summary, the NAb titers in these papers are based on highly stringent

assessments of virus neutralization, which should be borne in mind to avoid underestimating the clearly strong antibody immunogenicity of NVX-CoV3273.

Outcome and interpretation of macaque challenge experiments

When interpreting the outcome of macaque challenge experiments, it should be borne in mind that SARS-CoV-2 does not cause a lethal COVID-19 like disease in these animals. The macaques do become sick, rhesus perhaps more so than cynomolgus, but the disease course is generally mild, self-limiting and overcome within ~2 weeks (32-34). In general, the various vaccines reduce the severity of this mild disease, including by reducing or even preventing the transient lung damage that can be seen in post-mortem samples taken from control animals. No signs of vaccine-mediated enhancement of infection, including VAERD, were reported. The most common way in which vaccine efficacy is assessed is by determining the viral load (VL) in samples from various locales and tissues at short intervals during the week after challenge. In some experiments, both viral RNA copies per ml and subgenomic RNA copies per ml are determined, the latter avoiding problems associated with the presence of residual challenge virus and more unambiguously demonstrating *de novo* replication in the infected animal (5, 35) (Table 3). Lung pathology was also generally assessed, although the criteria chosen tends to vary among the different experiments.

Antibody titers in the animals on or very close to the day of challenge were reported in some of the papers and are summarized below and also in Table 3. In the other papers, the antibody data were derived at an earlier time point (Table 3). The inconsistencies in how the different studies were conducted and/or reported is another factor that blurs attempts to compare and interpret the performances of the different vaccines. Only three of the reports include data on T-cell responses at any time point prior to challenge, which limits understanding of any role they may play (Table

2) (4-6). In a separate section, we discuss what, if any, correlates of protection (CoP) can be inferred from some of the challenge experiments.

Most of the experiments involved SARS-CoV-2 challenges within a few weeks of the final (or only) vaccine dose, i.e., at a time when the immune response is likely to be close to its peak. The exception is the Inovio DNA vaccine study in which the challenge was delayed by 13 weeks (6). The next longest delay is the 6 week period after the delivery of a single dose of the Ad26.COV2.S vaccine (4). Thus, it is not yet known whether these various vaccines would be as effective against challenges conducted many months after the immunization protocol was completed. Extrapolating to what might happen when vaccinated humans become exposed to SARS-CoV-2 over the subsequent months or years is not possible.

Another issue when considering these macaque experiments is the SARS-CoV-2 challenge itself. There is no generally accepted standard, and various different challenge virus stocks (in several cases, of unspecified origin) were used. The challenge dose also varies by 100-fold and the route of challenge is another variable (Table 3). In one experiment, the virus was even administered by four different routes (3). All of these protocol variations constitute yet another factor hindering cross-study comparisons. As a general principle, it will be easier to protect against a low dose of a challenge virus than a higher one, all other things being equal. Thus, would a vaccine that protected against a relatively low challenge dose be as protective against the 100-fold higher dose used in other experiments? Or would its protection break down under those conditions? We return to this point at the end of this section. Challenge doses for vaccine experiments are traditionally pre-determined in naïve animals, to identify an inoculum size that is neither too low to be consistently infectious nor too high to protect against. It is rarely clear from the papers whether such titrations were performed. In one report, nasal swab VLs taken from SARS-CoV-2-infected humans and

from the virus-challenged macaques soon after infection were said to be comparable (~10^6 RNA copies/ml) (7). However, the initial infection and subsequent replication efficiencies are likely to differ substantially between the two species so it is not clear that this comparison is useful.

In the Sinovac PiCoVacc study, groups of 4 rhesus macaques were immunized with either 3 μg or 5 μg of the inactivated virus vaccine on days 0, 7 and 14 and challenged intratracheally with the CN1 strain of SARS-CoV-2 on day 22 (1). At this time, the anti-S protein GM endpoint titer was ~12,800 while the NAb GM ID_{50} titer was ~50 when measured 7 days earlier. All the vaccinated animals became infected after challenge, but disease severity was reduced compared to the control group (adjuvant-only) as judged by lung pathology assessments. VLs (i.e., viral RNA) was frequently detected at high levels in lung samples from control animals but in none of the high-dose vaccine recipients and only sporadically at significantly lower levels in the low-dose group. Viral RNA) levels in throat swabs were also lower and declined more rapidly, particularly in the higher vaccine-dose group (Table 3). The observed increases in NAb titers after day 7 post-infection may be consistent with an anamnestic antibody response.

The Sinopharm/BIBP inactivated virus vaccine experiment involved two groups of 4 cynomolgus macaques that were immunized with different doses (2 μg or 8 μg of viral protein) on days 0 and 14 (2). Binding antibodies were not measured. The NAb GM ID_{50} values in an RV assay were ~200 and 230 in the low and high dose groups when the animals were challenged on day 24 with a SARS-CoV-2 isolate from the Chinese Center for Disease Control and Prevention, by the tracheal route (Table 3). There were no changes in body temperature in either the vaccine or placebo groups over the next 7 days, which is indicative of a mild disease course. Viral RNA in all lung lobes was analyzed post mortem, but none was detected in any lobe taken from vaccine

recipients (in either dosing group). In contrast, the RNA copy number per ml ranged from ~30,000 to 3,000,000 in the lower lobes of the control animals. Lung pathology was also prevented or reduced in the vaccine groups. Although viral RNA in throat swabs became undetectable 7 days after challenge in the high-dose group, other evidence suggests these animals did become infected, albeit to a much lesser extent than the control and low-dose vaccine groups. Thus, gastrointestinal virus (detected in anal swabs) remained stable in the high-dose at ~ 100 RNA copies per ml from day 3-7, whereas the corresponding values in the two other groups fluctuated in a range around 100,000. While this study only analyzed viral, not subgenomic, RNA, it seems highly unlikely that gastrointestinal viral RNA could simply represent residual challenge virus. Thus, the higher-dose animals were at least strongly, but apparently not completely, protected from infection, and in both dosing group the vaccine reduced the extent of virus replication post-infection (2).

All 12 of the ChAdOx1-vaccinated macaques became infected when they were challenged 28 days after their final immunization (they received either 1 or 2 vaccine doses, see above). The SARS-CoV-2 challenge strain was WA1-2020 (MN985325.1). At the time of challenge, the median binding antibody endpoint titers were ~6,300 with median NAb ID_{50} values of ~60 in a RV assay (Table 3). The vaccinated animals had fewer symptoms than the control group, less lung damage and lower VLs (measurements included subgenomic RNA) in BAL and lung samples. No virus was detected in BAL samples from the vaccinated animals on day 5, but subgenomic RNA could be detected in lung samples from some animals in both groups. No antibody or T-cell data post-challenge were reported, so it is unknown whether there were anamnestic responses to the infecting virus (3).

Seven different Ad26-based vectors were given once to groups of 4-6 rhesus macaques before challenge 6 weeks later with an unspecified isolate of SARS-CoV-2 (4). Compared to a

control group of 20 animals, VLs in BAL and nasal swabs were significantly reduced in each of the 7 Ad26 vector groups, by >5-log in the case of BAL samples. The best performing vector, from this perspective, was the one designated S.PP; it was chosen to become the Ad26.COV2.S clinical vaccine candidate. Overall, the authors assessed that 17 of the 32 vaccinated macaques were protected from infection, judged by the VL data. There was no evidence for anamnestic B- and T-cell responses in the protected Ad26.COV2.S vaccinated animals, although NAb titer increases were seen in other vaccine groups. The strongest, and perhaps complete, protection was seen in the S.PP group. Thus, virus (subgenomic RNA) could not be detected in BAL from 6/6 and in intranasal swabs from 5/6 animals (4).

In another study, rhesus macaques were immunized i.m. with S-protein-expressing DNA plasmids at weeks 0 and 3, and challenged at week 6 with an unspecified SARS-CoV-2 isolate (5). All of the 10 control animals became infected, with BAL and nasal swab peak subgenomic RNA levels copies in the range 10^4–10^7 per ml. However, 8 of the 25 vaccine recipients were RNA-negative in BAL and nasal swab samples while median subgenomic RNA levels in the other 17 macaques were 3-4 logs lower than the median values from the 10 control animals. Even when subgenomic RNA was undetectable in vaccinated animals, the observation of anamnestic antibody and T-cell responses does imply that the animals were not completely protected from infection. Instead, initially replicating virus may have been suppressed by vaccine-mediated immunity (5).

The Inovio INO-4800 DNA vaccine was given to 5 rhesus macaques at weeks 0 and 4 (6). The SARS-CoV-2 challenge (USA-WA1/2020 strain) was then delayed until week-17 (i.e., 13 weeks after the second immunization), a substantially longer period than applies in the other studies summarized here. Upon challenge, all the macaques became infected, judged by VLs in various samples. However, VLs in the vaccinated group were lower and declined more quickly

than in 5 control animals, the reduction in medians being in the <10-300-fold range depending on the sample site and the time point assayed; the difference was significant for BAL but not intranasal swab (INS) samples. Antibody and T-cell recall responses were quantified in the animals after virus challenge. Thus, by 14 days post-challenge anti-S protein antibody and NAb measurements were higher (~10-30 fold) in the vaccinated than control animals, while there was an ~2-fold increase in INF-gamma signals. Overall, the vaccine-mediated reduction in viremia post-challenge was attributed to recall responses (i.e., T- and B-cell memory).

Two dosing groups of 8 rhesus macaques were immunized with the Moderna mRNA-1273 vaccine at weeks 0 and 4 and then challenged with the USA-WA1/2020 strain of SARS-CoV-2 at week 8 (7). Judged by VLs, most (~7 of 8) of the animals in the higher dose group were protected, but most (~5 of 8) of the lower dose group became infected (the exact numbers vary per time point, and depend on the VL sample site). There were indications of anamnestic responses in some animals, including in BAL fluids. Post-mortem analyses of the lungs found little or no signs of inflammation in the higher-dose group, but some indications of pathology in the lower dose animals that became viremic. Neutralization titers in both PV and RV assays correlated inversely with INS viral loads; virus-specific IgG and IgA levels in BAL were elevated in the high dose group. In the high-dose, high-protection group, the GM ID_{50} values from PV or RV NAb assays were >900 in 7/8 animals, whereas the corresponding values were <900 for 7/8 animals in the low-dose, low-protection group. The data pattern implied that NAbs were protective in the high-dose group.

The adjuvanted NVX-CoV2373 recombinant protein vaccine was given to cynomologus macaques on days 0 and 21 before the animals were challenged with the WA1 strain of SARS-CoV-2 via the nasal and tracheal routes on day 35 (9). Judged by VLs (subgenomic RNA) in BAL

and nasal swabs, performed 2 and 4 days later, every animal was virus-negative except for one in the lowest dose group that had a weakly positive BAL sample. Post-mortem lung samples in the vaccine groups showed no sign of the pathologies that were visible in the control animals. To the extent that can be judged, the vaccinated animals may have been completely protected from infection. This outcome may reflect the very high GM antibody titers on the day of challenge (anti-S protein EC_{50} 469,739, NAb CPE_{100}, 23,040) (9).

In summary, all of the vaccines tested to date have conferred a substantial degree of protection to the immunized macaques. In some cases, there is reasonable evidence for complete protection (i.e., 'sterilizing immunity'), but the more common outcome is a reduction in the severity of the already mild disease course seen in control animals. We discuss in the next section what immune factors and other variables may have influenced the outcomes of the different experiments. In respect of what the outcomes may mean for vaccine efficacy in humans, we note that it is generally easier to protect animals against mild infections than severe ones. Hence, it is hard to assess whether and how any of the present findings in macaques might translate to the subset of humans who need protection from severe and lethal COVID-19. Moreover, as noted above, it is not known whether the various vaccines would still protect macaques, and by extrapolation humans, after a substantial period (multiple months) has elapsed.

Towards correlates and mechanisms of protection

It is striking that in the various macaque immunization studies, similar outcomes were associated with substantial (~2000-fold) differences in serum antibody titers to the S-protein, the NVX-CoV2373 recombinant protein vaccine being the strongest immunogen (Table 2) (8, 9). It can also reasonably be concluded the ChAdOx1 vaccine is not a strong inducer of antibody responses to the S-protein, particularly when given only once (Table 2) (3). The same inference

can be made about the DNA plasmid vaccines (Table 2) (5, 6). Are the serum antibody responses induced by the weaker vaccines solely responsible for any protection that was conferred? Perhaps cellular immune responses or some other unmeasured factor, such as mucosal IgA, were contributory? The potential protective role of mucosal immunity is highlighted by the outcomes of experiments involving a ChAd virus vector in mice (23). These inferences are similar to what has been seen in studies of other vaccines, such as HIV-1 Env, where only protein-based immunogens induce very strong antibody titers (36).

What protected the vaccinated animals from SARS-CoV-2 infection and/or disease? CoPs are important in vaccine development, because they can serve as robust predictors of future vaccine efficacy whether they are derived from animal experiments or clinical trials and whether the endpoints involve protection from infection or a reduction in disease severity. There are nuances to the identification of CoPs in population-wide studies that we cannot address here (37, 38). The present macaque-challenge studies are not sufficiently powered and are not wide-ranging enough in scope to allow the identification of CoPs with high confidence. The few attempts to identify CoPs have pointed towards a predominant role for NAbs, which is not unexpected, but it is premature to conclude that NAb titers at the time of challenge (i.e., in humans, virus exposure) tell the entire story. The possible role of recall responses (i.e., T- and B-cell memory) in clearing a transient infection has only been addressed in the Inovio DNA vaccine study, which uniquely involved a markedly delayed challenge. Even in that experiment, the amount of information available is quite limited (6).

Despite the limitations of the available data, we sought hints of CoPs. Thus, we analyzed the relationships between, on the one hand, binding antibody and NAb titers at the pre-challenge peak or within two weeks before challenge and, on the other hand, VL reductions in vaccinated

animals compared with controls (summed for two locales of sampling and based only on subgenomic RNA; Tables 2 and 3). We found no tangible non-parametric (Spearman) positive correlations of any significance between VL reduction and any antibody parameters, which is not surprising given the number of variables between the different experiments. We were also unable to identify any consistent relationship between the challenge virus dose or delivery route and the degree of protection. The challenge dose was not a consistent predictor of the magnitude or duration of high viral loads in the control animals, but we should bear in mind that the challenge virus stocks represent another variable, as does the sub-species (and sources) of the animals involved. Nonetheless, the wide variation in challenge dose between experiments should not be ignored. Would a vaccine that protected against a relatively low challenge dose be as protective against the 100-fold higher dose used in other experiments? Or would its protective capabilities degrade under those conditions? Experimental conditions yielding high VLs in control animals may impede complete protection while giving the potential for greater VL reductions. The smallest VL decrease in the vaccine group compared to control was seen in the Inovio DNA vaccine experiment which involved one of the lowest challenge doses (Table 3) (6). In the report on the Ad26 virus vaccine, where there were indications of sterilizing immunity, an intermediate challenge dose was used (4).

In three studies, some groups of vaccine recipients seem to be completely protected, or nearly so (4, 5, 9). In one experiment, no anamnestic antibody or cellular immune responses were detected in the protected animals, which suggested that immunity was sterilizing (4). However, in another study there were anamnestic immune responses in animals with undetectable VLs, which is more indicative of incomplete but aborted infection (5). Anamnestic responses were not analyzed in the third report (9). In two other cases, lung lobes in the vaccine groups where

protection was strongest were free of viral RNA 7 days after infection, which contrasted to the high levels found in the lower lobes of control animals (1, 2). The criteria for sterilizing immunity, or at least complete protection against persistent infection, are neither defined nor standardized in the SARS-CoV-2 animal model field, which also extends into other protection-challenge systems involving small animals and both vaccines and antiviral antibodies (19, 32-34).

Within individual studies there are fewer confounding factors than in a cross-study meta-analysis. Some evidence was presented that antibodies were the CoP in the Moderna mRNA vaccine study (7). In the report on the Janssen Ad26 vector vaccine, comparing various antibody and T-cell responses with infection outcomes (as judged by VLs) identified NAbs as the strongest CoP, with some possible contribution from Ab-effector functions such as antibody-dependent cellular phagocytosis and antibody-dependent activation of natural killer cells. In contrast, T-cell responses (measured by ELISPOT or ICS) did not correlate with protection (4). Similar inferences about an antibody but not a T-cell CoP emerged from the experiments involving DNA plasmid immunizations (5). But even when significant differences were identified, the ranges of the various measurements were generally overlapping between completely and partially protected animals (4, 5). This degree of variation compromises attempts to identify the threshold response required for protection, particularly in the study of the Ad26 vector vaccine (4). Overall, the available evidence from macacaque-challenge experiments does point towards a protective role for antibody-based immunity (probably NAbs), but not to the extent that a protective titer can be inferred and then extrapolated to the outcome of human efficacy trials.

Immunogenicity of vaccine candidates in humans

As with the NHP studies, the primary papers and reviews should be consulted for additional details of the human trials, most of which are formally classified as Phase 1 studies although at

least one data set derives from a Phase 2 trial (10). Vaccine safety assessments were a key component of these trials; in all cases, reported side effects and adverse events were considered to be minor or moderate; the primary papers contain the details, which we have not attempted to summarize. The antibody titer values reported summarized here are also presented in Table 4.

The human trials have predominantly involved young/middle-aged, healthy adults (see primary papers for details). Only limited information is yet available on age-dependent decreases in immunogenicity. In the CanSino Ad5-nCoV vaccine, participants aged older than 55 responded with weaker antibody responses than their younger counterparts. However, that outcome could reflect either the aging process or time-dependent increases in exposure to other Ad5 viruses that compromise expression of the immunogen from by the vector (12). In a Pfizer/bioNTech mRNA vaccine trial, an ~2-3-fold reduction in antibody responses was seen in older adults (aged 65-85) compared to younger ones (aged 18-55) (18). Moderna reported similar findings for their mRNA vaccine in a press release but has not yet released the data in preprint form. There has also been an under-representation of minority groups in the American and European trials, so again information on how immunogenicity might vary in different populations is lacking. These various lacunae will need to be filled in Phase 3 trials, given that COVID-19 is more severe in older people and in African-American and Latinx populations.

The Sinopharm/WIBP inactivated virus vaccine was delivered in Alum adjuvant. It was first tested in 96 volunteers in a Phase 1 trial and then in 224 more people in a Phase 2 study (10). The study cohorts were based on healthy individuals aged from 18-59. The Phase 1 trial was dose-ranging (2.5, 5, 10 μg of viral protein) and involved i.m. injections on days 0, 28 and 56, while in Phase 2 only the 5 μg dose was tested in two sub-studies that involved immunizations on days 0 and 14 or on days 0 and 21. Immune responses were measured by ELISA using inactivated virus

as the detecting antigen, which does not allow a comparison with other vaccines, and by a RV neutralization assay. For sera collected 14 days after the final dose, the NAb titers (GM ID_{50} values) in the Phase 1 trial were 316, 206 and 297 in the low, medium and high dose groups respectively. Allowing for the titer ranges among participants, the three doses induced similar antibody responses. In the Phase 2 trial, the corresponding NAb titer values were 121 and 247 for the 0, 14 day and 0, 21 day groups, respectively. Anti-virus ELISA endpoint geometric mean titers were also similar among the different test groups in the two trials, and were ~200-300 in Phase 1 and ~90-200 in Phase 2. There were no T-cell data in the paper. Phase 3 trials are now in progress in South America, although the vaccine dose and delivery regimen (i.e., the number and spacing of doses) was not specified in the report on the Phase 1 and 2 trials (10).

In a Phase 1 trial, the immunogenicity of the CanSino Ad5-nCoV vaccine candidate was found to be dose-dependent (11). Doses of 5×10^{10}, 1×10^{11} or 1.5×10^{11} virus particles were given once to three different sub-groups. In the highest dose group, the anti-S protein and anti-RBD GM titers on day-28 were 596.4 and 1445.8, respectively (in references (11) and (12) the cut-offs for titer determinations are not specified; we refer to them as "titers"). The GM NAb titers were 34.0 and 45.6 in RV and PV assays, respectively, and were strongly correlated with anti-S and -RBD titers. A Phase 2 trial was then conducted on 508 participants, of which 126 received a placebo (12). The protocol again involved a single administration of the Ad5 virus, which was tested at doses of 1×10^{10} or 1.5×10^{10} in sub-groups. The anti-RBD GM titers on day-28 was 656.5 range, which is a ~2-fold lower than in the Phase 1 trial. NAb titers in the RV and PV assays were 19.5 and 61.4, respectively, and hence similar to the Phase 1 trial data. T cell responses were measured by ELISPOT on samples taken before vaccination and then on days 14 and 28. Freshly

drawn PBMC were incubated with S-protein peptide pools for >12 h, with the data expressed as SFC/10^5 cells after subtraction of background values derived from unstimulated control cells. (Note that the data in Table 4 have been adjusted to SFC/10^6 cells to facilitate comparison to other datasets). There was no mention of a positive control method, nor of the number of replicates. An ELISPOT result was stated to be positive if the number of IFN-gamma secreting T cells responding to the S-protein peptides was increased 2 times above baseline post vaccination. TNF-alpha, IL-2 and IFN-gamma responses to the vaccine were also assessed by CFC. T cell responses peaked at day 14 post vaccine, and ranged from 200 SFC/10^6 cells in the low dose group to 580 SFC/10^6 cells in the high dose group. In CFC assays, both CD4+ and CD8+ T cells were found to be responsive.

AstraZeneca's ChAdOx1 nCoV-19 recombinant virus vaccine (also known as AZD1222) was tested in a randomized Phase 1/2 trial involving 543 people; another 544 participants were given a meningococcal control vaccine (13). The original protocol involved a single dose of 5 x 10^{10} virus particles, which is twice the amount given to macaques (Tables 2 and 4). However, a decision was taken during the trial to give ten participants a second dose of ChAdOx1 on day 28 in a non-randomized boosting protocol. It is assumed that the decision was taken because of the limited immunogenicity of the single-dose regimen (a modest boosting effect of a second dose was seen in the NHP study, see above; 3). Anti-S protein binding was measured at single dilutions and converted to "ELISA Units", an approach that complicates comparisons with anti-S protein responses to other vaccine candidates in humans and that represents an unexplained change from how the macaque sera were analyzed by titration in ELISA (3). By day 14 and 28, the responses in most of the participants were in the 100-1000 Units range (medians 102.7 and 157.1,

respectively), with little change by day 56 in the sub-group that was assayed at that time point (median 119). After the second vaccine dose in the prime-boost protocol, a ~5-fold increase in median anti-S protein ELISA Units was measured 14 days later (median 997.5), and the levels were largely maintained by day 56 (median 639.2). NAbs were measured 14 days after the booster immunization using one PV and three different RV assays. NAb data from the PV assay and from the only RV assay that reported ID_{50} values are given in Table 4. The primary papers should be consulted for other aspects of the neutralization data from the various assays (13). Overall, the seemingly modest NAb responses to the single-dose vaccine were increased a few-fold by the day 28 boosting immunization, at least in the short term (until day 42). The median titers for the prime-boost group on day 42 were 372-450.9 (Table 4). ELISPOT assays were performed on freshly isolated PBMC at days 0, 7, 14, 28 and 56, and at day 35 for the participants who received 2 doses. Pooled peptides were used as antigens, and data were excluded if the assay background response rate was deemed to be too high. The measured responses peaked at day 14 at a value of 856 SFC/10^6 PBMC in the prime group and 1642.3 SFC/10^6 PBMC in the prime-boost group (i.e., after one dose in either group). The results for other time points are given in Table 4. Of note is that ~10% of recipients of this vaccine appear to generate no measurable T cell response after the first dose. Furthermore, the booster dose given to ten trial participants did not further increase their T-cell responses. This vaccine has now advanced into Phase 3 trials in several international locations, including Brazil and South Africa. It is thought that these trials were initiated as a single-dose regimen but were later changed to incorporate the second, boosting dose. A two dose Phase 3 trial started in the USA at the beginning of September.

The Phase 1 trial of the Moderna mRNA-1273 vaccine involved 45 volunteers in three dosing groups who were given 25, 100 or 250 μg of the immunogen by the i.m. route on days 1

and 29 (14). Antibody immunogenicity was dose-dependent and much stronger after the second dose than the first. The 100 μg, two dose regimen was chosen for the Phase 2 and Phase 3 studies that are now in progress. Anti-S protein GM endpoint titers in the 100 μg group on day 57 (28 days after the second dose) were 782,000, while the corresponding anti-RBD endpoint titers were ~30,000. Most of the NAb data were derived from a PV assay; on day 43, the GM ID_{50} titer for the 100-μg group was 344. An RV assay was also used on a subset of day 43 samples. The resulting ID_{80} titers were 654 for the 100 μg group. Note that these are not ID_{50} values, which would be higher numbers. No detailed data on the longevity of the antibody responses were reported, but inspection of the graphs suggest that the antibody titers on a downward trend at the day 57 time point compared to days 36 and 43. T cell responses were measured only by CFC, and no data on their magnitude was reported. For both vaccine dose groups, the peptide pools activated specific Th1 responses from <0.3% of the CD4+ T cells, and no Th2 responses were detectable. CD8+ T cell activity was, at most, minimal. The Moderna vaccine entered Phase 3 trials in the USA during August 2020, using a two dose regimen.

The Pfizer/BioNtech consortium has conducted three Phase 1 trials of lipid nanoparticle-encapsulated mRNAs that eventually led to the selection of the clinical candidate for now ongoing Phase 2/3 studies (15, 16, 18). In the first trial, the BNT162b1 mRNA expressing a soluble, trimerized version of the RBD was given at two doses (10 μg and 30 μg) on days 1 and 21 to groups of 12 participants, and once at 100 μg on day 1 to a third group of 12. There were also 9 placebo recipients (15). Immunogenicity was assessed by anti-RBD (15,16) or anti-S1 (18) binding Abs on days 7, 21, 28 and 35, although the data were reported in a non-traditional format that does not allow for cross-study comparison (15, 16, 18). All recipients in the two lower dose groups developed anti-RBD antibodies by day 21 that were boosted ~10-20-fold by the second

immunization when measured on day 28 and unchanged by the end of the study on day 35. The 30 μg group was more immunogenic than 10 μg by ~3-fold. The pattern of the NAb data was similar, although fewer time points were studied. In all three groups, the NAb responses to the initial immunization were low, but were boosted by the second dose. On day 28, the GM ID_{50} values in an RV assay for the 10 and 30 μg groups were 168 and 267, respectively (15).

A second Phase 1 trial, conducted in Germany, also explored dosing regimens (16). Multiple doses of BNT162b1 mRNA, in the range 1-50 μg, were tested, as were single doses and a prime-boost protocol involving two doses on days 0 and 21. Overall, and as expected, the immunogenicity data were comparable to what was seen in the first trial. Higher immunogen doses and the prime-boost format were associated with stronger responses, as expected. The anti-RBD ELISA data were again presented in a non-traditional format. After the second dose, NAb ID_{50} GM titers in the higher dose groups were 578 in a RV assay and ~3100 in a PV assay. In an additional analysis, selected sera were tested in the PV-NAb assay against RBD and S-protein sequence variants (including the D614G change); no significant sensitivity differences were observed. T-cell responses were measured by a modified ELISPOT in which either CD4+ or CD8+ T cells were depleted from the effector population, or by CFC. An unpublished 'normalization' method was applied to enable direct comparison of spot counts/strength of response to anti-CD3 stimulation between individuals. Because PBMC were separated into either CD4+ or CD8+ subpopulations in the ELISPOT assay, no direct comparison can be made with ELISPOT data on the other vaccines reviewed here due to differences in methodology. CD4+ and CD8+ T-cell responses were analyzed immediately before vaccination and then on day 29, i.e., 7 days after the booster immunization. The magnitudes of both T-cell responses were dose-dependent. At the highest dose, the majority of participants had T-cell responses >1500 SFC/10^6 cells. The

magnitudes of the CD4+ and CD8+ responses were comparable. Approximately equal proportions of the CD4+ responders fell into groups with <500, 501-1500 and >1500 SFC/10^6 cells. Cytokine secretion profiles showed that CD4+ T cells producing only IL-2 were the most abundant subset, while IL-4 release was minimal. This pattern is of potential concern, as CD4+ cells secreting IL-2 can polarize CD4+ T cells towards the Th2 phenotype that may be associated with VAERD (28-30). The responding CD8+ T cells mostly produced IFN-gamma (16).

The third Pfizer/BioNTech phase 1 trial compared the BNT162b1 RBD-based construct with BNT162b2, an mRNA expressing a full-length, membrane-anchored S-protein (18). The two constructs were comparably immunogenic but BNT162b2 was associated with lower reactogenicity levels. Accordingly, BNT162b2 at a 30 μg dose was selected to progress into Phase 2/3 trials. The Phase 1 trial had two principal sub-components, involving adults aged 18-55 and ones aged 65-85. For each of the two mRNAs, 3 or 4 different doses (10, 20, 30 μg and in one case 100 μg of mRNA, plus placebo) were tested in a two-immunization protocol (days 0, 21), so the 195 participants were split among 13 different groups in all. Here, we list the immunogenicity data only for the clinical candidate (BNT162b2, 30 μg), on day 28. NAb ID_{50} GM titers in a RV assay were 361 and 149 for the 18-55 and 65-85 age groups respectively, while the corresponding geometric mean antibody endpoints to the S1 protein in a Luminex assay were 9136 and 7985 (see also Table 4). Thus, for the clinical candidate, the NAb titers for the older group were 41% of those in their younger counterparts. Visual inspection of other antibody data sets suggests that the age-related reduction is generally ~2-3 fold, a decline that is perhaps meaningful but not catastrophic. No longer term antibody data and no information on T-cell responses were presented (18). The BNT162b2 vaccine candidate is now in Phase 3 trials in the USA and Europe, which involve a 2 dose regimen.

The first report on how a recombinant S-protein performs in humans described the Novavax NVX-CoV2373 vaccine candidate (17). The immunogen is an insect cell-derived soluble S-protein. When mixed with detergent, 5 or 6 S-proteins become attached non-covalently via their bases to the resulting micelles (31). This component of the immunogen was co-administered with Matrix-M adjuvant. Two formulations (5 μg and 25 μg of S-protein) were tested in 106 people with or without adjuvant, in a two-dose regimen on days 0 and 21. In the absence of adjuvant, antibody responses were, as expected, very weak, while the 5 and 25 μg doses performed comparably when the adjuvant was present. Anti-S-protein ELISA data were presented as Units, which again prevents cross-study comparison. The highest values recorded, on day 35, were 63,160. NAbs were measured in an RV assay and reported as $ID_{>99}$ values. Here, the peak GM values were 3906 and 3305 for the 5 and 25 μg groups, respectively, on day 35. As noted above when discussing the corresponding macaque experiment, when NAb data are presented as $ID_{>99}$ or CPE_{100} values the reported numbers are likely to be several-fold lower than the more commonly used ID_{50} values. CFC was used to measure CD4+ T-cell responses at days 0 and 28, but in only 4 participants per group. There were no responses in the placebo or protein with no-adjuvant recipients, but CD4+ T cell signals could be measured in the adjuvanted protein groups at day 28, with two protein doses inducing similar but moderate responses. Both Th1 and Th2 cytokines were released although the Th1 signals were more consistent, particularly at the lower protein dose (17). Phase 3 trials of the Novavax vaccine are thought to commence towards the end of 2020.

Taken together, and allowing for caveats about comparing data from different studies, two features of the binding-antibody and NAb data stand out (Table 4). The strongest responses were induced by the NVX-CoV2373 adjuvanted recombinant S-protein, particularly when taking into

account the presentation of $ID_{>99}$ values, which are lower than the more commonly used ID_{50} titers (17). The superior immunogenicity of the recombinant S-protein mirrors its performance in macaques (Table 2) (8, 9). The second conclusion we can draw is that binding antibody and NAb responses to the single dose Ad5 and ChAdOx1 nCoV-19 vaccines are quite weak, although a second dose of the latter did improve its performance, as judged by the limited data set available (Table 4) (11-13). The T-cell response data are too limited, and the protocols used too variable, for us to draw any conclusions about relative immunogenicity.

Summary and conclusions

Assuming that serum NAb titers are indeed the principal CoP for a human SARS-CoV-2 vaccine, we do not know what a protective NAb titer will be, we know even less about the role of T-cells in protection, there is almost no information available about mucosal immunity or immune memory, and the duration of any vaccine-induced immunity is yet another unknown. In an earlier review, we suggested that NAb titers in the low hundreds might be sufficient for protection (19). In a recent study, when 122 Seattle seamen sailed seawards, 104 of them had become SARS-CoV-2-RNA-positive from a single source of virus by the time their boat returned to port. However, three sailors who had NAb responses at the time of departure were not re-infected while at sea. Their serum PV ID_{50} NAb titers were 174, 161 and 3082. Thus, while certainly far from definitive, this study further suggests that a protective serum NAb titer may lie in the low hundreds (39). Once more, however, the use of NAb assays with different properties and sensitivities blurs any attempts at data extrapolation between studies. Now that robust, reliable and potentially high throughout assays are becoming available, perhaps these major knowledge gaps can be addressed (40, 41). Having said all that, we now find it likely that hard evidence on the comparative immunogenicity and relative worth of the leading SARS-CoV-2 vaccines will emerge from

ongoing and planned Phase 3 trials. If these vaccines turn out be inadequate, one option is to combine some of them in prime-boost formats as we have suggested previously (19). Alternatively, next-generation immunogens now at the pre-clinical stage of development may solve the problem. In that scenario, greater national and international coordination on how best to assess vaccine immunogenicity and, in NHP models, efficacy would be strongly advised.

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TABLES

Table 1: SARS-CoV-2 vaccines under evaluation in NHPs and Phase 1/2 human trials

Vaccine name[a]	Company[b]	Design principle[d]	NHP studies (citation)	Human studies (citation)
PiCoVacc	Sinovac	Inactivated virus	(1)	
BBIBP-CorV	Sinopharm/BIBP	Inactivated virus	(2)	
ChAdOx1 nCoV-19	**AstraZeneca**	ChAdeno virus - S-protein	(3)	(13)
Ad26.COV2 Various constructs	**Janssen**	Ad26 virus– S-protein	(4)	
Various constructs	Not applicable [c]	DNA – S-protein	(5)	
INO-4800	Inovio	DNA – S-protein	(6)	
mRNA-1273	**Moderna**	mRNA – S-protein	(7)	(14)
NVX-CoV2373	**Novavax**	Recombinant S-protein	(8, 9)	(17)
Unnamed	Sinopharm/WIBP	Inactivated virus		(10)
Ad5-nCoV	CanSinoBIO	Ad5 virus – S-protein		(11, 12)
BNT162b1 [e] BNT162b2	**Pfizer/BioNTech**	mRNA – RBD mRNA - full-length stabilized S-protein		(15, 16, 18)

[a] Some of these vaccines have alternative names or corporate designations. We use the one that is most common in the scientific literature.

[b] The five companies highlighted in bold in this and subsequent tables are part of the US government's Operation Warp Speed program. As this program rapidly evolves, readers should consult appropriate websites (e.g., https://medicalcountermeasures.gov/app/barda/coronavirus/COVID19.aspx) for updated information. In some cases, the companies have academic partners. For example, the Moderna vaccine is being co-developed with the NIH's Vaccine Research Center and the AstraZeneca product (also known as AZD1222) involves Oxford University. BIBP = Beijing Institute of

Biological Products; WIBP = Wuhan Institute of Biological Products. Both these organizations are part of the Sinopharm consortium.

[c] The DNA vaccines tested in the macaque study are not known to be part of a clinical development program; we include this paper in the review because it has a macaque challenge component and is therefore relevant to the comparison with other such studies.

[d] The SARS-CoV-2 components of these vaccines are all based on the S-protein or, in the case of the Pfizer/BioNTech BNT162b1 vaccine, the S-protein's RBD. The three inactivated virus vaccines all include S-proteins together with other viral components. For details of the immunogens, including modifications made to the S-proteins, the primary papers should be consulted.

[e] Although both vaccines were studied at Phase 1 only BNT162b2 was advanced into Phase 2/3 trials.

Table 2. Vaccine immunogenicity in NHP studies

Vaccine name (citation)	Vaccine dose[a]	Binding antibody titer[b]	NAb titer[c]	T-cell response[d]
Sinovac PiCoVacc (1)	6 μg x 3	GM EP ~12,800	RV GM ID_{50} ~50	ND [e]
Sinopharm/BIPP BBIBP-CorV (2)	8 μg x 2	ND	RV GM ID_{50} ~230	ND
AstraZeneca ChAdOx1 nCoV-19 (3)	2.5 x 10^{10} VP x 2	Median EP ~28,000 [f]	RV median ID_{50} ~280 [f]	ND
Janssen Ad26.COV 2S.PP (4)	1 x 10^{11} VP x 1	Median EP ~ 4,000	PV median ID_{50} 408 RV median ID_{50} 113	Median ~80 (day 28)
DNA, full-length S-protein (5)	5 mg x 2	Median EP ~ 140	PV median ID_{50} ~200 RV median ID_{50} ~40	Median ~80 (day 35)
Inovio INO-4800 (6)	1 mg x 2	GM EP ~130,000	PV GM ID_{50} ~1000	AM ~140 (day 42) AM ~30 (day 84)
Moderna mRNA-1273 (7)	100 μg x 2	Log AUC = 4-5	PV GM ID_{50} 1862 RV GM ID_{50} 3481	ND
Novavax NVX-CoV2373 (8)	5 μg x 2	GM EC_{50} 174,000	RV GM $ID_{>99}$ 17,000	ND
Novavax NVX-CoV2373 (9)	25 μg x 2	GM EC_{50} 469,739	RV GM CPE_{100} 23,040	ND

[a] Only results for the optimal dose, i.e., the strongest responses without unacceptable side effects, are recorded. When the number of immunizations differed between groups, the one inducing the strongest response was chosen. VP = virus particle.

[b] Antibody binding was measured in S-protein IgG ELISA 2 weeks after the last immunization and listed as EC_{50} or endpoint (EP) values; GM = geometric mean; AUC = area under the curve.

[c] Neutralization was quantified in PV or RV assays, as indicated. The potency was measured as ID_{50} or $ID_{>99}$ values (CPE_{100} in ref. 9 is the approximate the equivalent of $ID_{>99}$ in ref. 8).

[d] T-cell responses measured in ELISPOT IFN-gamma assays SFC/10^6 cells (PBMC) after stimulation with different SARS-CoV-2 S-derived peptides. The days between immunization (day 0) and sampling are also listed (in brackets). AM = arithmetic mean.

[e] ND = not done (no data were presented in the paper).

[f] Data are for the 2 dose (prime boost) group.

Table 3. Antibody responses at the time of challenge and degree of protection in NHP studies

Vaccine name (citation)	Binding antibody titer near time of challenge[a]	NAb titer near time of challenge[b]	Dose and route of challenge[c]	Time from last immunization to challenge	Viral load reductions[d]
Sinovacc PiCoVacc (1)	GM EP ~12,800 1 day BC	RV GM ID_{50} ~50 1 day BC	1 x 10^6 $TCID_{50}$ IT	22 days	TS AM ~1.8 AS AM ~4.7
Sinopharm/BIBP BBIBP-CorV (2)	ND [e]	RV GM ID_{50} ~230 Day of challenge	1 x 10^6 $TCID_{50}$ IT	14 days	TS AM ~5.0 [f] AS AM ~2.9
AstraZeneca ChAdOx1 nCoV-19 (3)	Median EP ~6,300 [g] 28 days BC	RV median ID_{50} ~60 28 days BC	(1.6+0.8+0.8+0.2) x 10^6 $TCID_{50}$ IT-IN-OR-OC [h]	14 days	BAL median ~1.7 INS median ~1.5
Janssen Ad26.COV 2S.PP (4)	Median EP ~ 4,000 14 days BC	PV median ID_{50} 408 RV median ID_{50} 113 14 days BC	1 x 10^5 $TCID_{50}$ IT-IN	42 days	BAL median 3.2 0/6 detectable INS median 3.9 1/6 detectable
Full-length S-protein (5)	Median EP ~ 160 7 days BC	PV median ID_{50} ~40 RV median ID_{50} ~200 7 days BC	1.2 x 10^8 VP = 1.1 x 10^4 PFU IT-IN	21 days	BAL median 3.1 INS median 3.7
Inovio INO-4800 (6)	GM EP ~ 3,200 14 days BC	PV GM ID_{50} ~260 14 days BC	1.1 x10^4 PFU IT-IN	77 days	BAL median ~1.5 INS median ~0.20
Moderna mRNA-1273 (7)	Log AUC = 4-5 14 days BC	PV GM ID_{50} 1862 RV GM ID_{50} 3481 14 days BC	7.6 x10^5 PFU 10^6 $TCID_{50}$ IT-IN	28 days	BAL median ~4.0 INS median ~3.0
Novavax NVX-CoV2373 (9)	GM EC_{50} 469,739 Day of challenge	RV GM CPE_{100} 23,040 Day of challenge	1.04 x 10^4 PFU IT-IN	35 days	BAL median ~2.6 (0/4 detectable) INS median ~2.6 (0/4 detectable)

[a] Antibody binding was measured in S-protein IgG ELISA and listed as EC_{50} or endpoint (EP) values; GM = geometric mean; AUC = area under the curve. The data are derived from the timepoint (listed in days) closest to the time of challenge. BC = before challenge

[b] Neutralization was quantified in PV or RV assays, as indicated and the potency measured as ID_{50} values.

[c] Challenge dose (in plaque-forming units (PFU) or tissue culture infectious dose yielding infection in 50% of wells ($TCID_{50}$) and route of challenge; only in ref. 7 were both PFU and $TCID_{50}$ given. IT = intratracheal; IN = intranasal; OR = oral; OC = ocular. VP = virus particles.

[d] Protection was measured as median log reductions in subgenomic RNA copies/ml (except for reference 2 where viral RNA data are listed). The viral load (VL) data were derived from bronchoalveaolar lavages (BAL), intranasal swabs (INS), throat swabs (TS) or anal swabs (AS), at times when VLs were approximately at their peak levels post-challenge. In some studies, more substantial protective effects could be detected after the peak values began to decline (see the primary papers for details).
AM = arithmetic mean of the VL log values, which is equivalent to the GM values of the antilog values.

[e] ND = not done (no data were presented in the paper).

[f] Since viral RNA declined without any discernable peak in the control animals, only RNA measurements for day 7 (the last time point sampled) are listed.

[g] Data are for the 2 dose (prime boost) group.

[h] The macaques were challenged simultaneously via 4 different routes (IT-IN-OR-OC) with the various doses listed in the same order in the brackets.

Table 4. Vaccine immunogenicity in human phase 1 and/or phase 2 trials

Vaccine name (citation)	Design	Vaccine dose[a]	Binding antibody titer[b]	NAb titer[c]	T-cell response[d]
Sinopharm/WIBP Unnamed (10), Phase 1	Inactivated virus	10 μg x 3 [e]	GM EP (whole virus) 311	RV GM ID_{50} 297	ND [f]
Sinopharm/WIBP Unnamed (10), Phase 2	Inactivated virus	5 μg x 2	GM EP (whole virus) 215	RV GM ID_{50} 247	ND [f]
CanSinoBIO Ad5-nCoV (11)	Ad5 virus	1.5 x 10^{11} VP x 1	GM titer 596.4	RV GM titer 34 PV GM titer 45.6	GM ~580 (day 14)
CanSinoBIO Ad5-nCoV (12)	Ad5 virus	1.0 x 10^{11} VP x 1	GM titer (RBD) 656.5	RV GM titer 19.5 PV GM titer 61.4	ND
AstraZeneca ChAdOx1 nCoV-19 (13)	ChAdeno virus	5 x 10^{10} VP x 1	Median EU 157.1	RV median ID_{50} 201 PV median ID_{50} 87.9	Median 856 (day 14) Median 424 (day 56)
AstraZeneca ChAdOx1 nCoV-19 (13)	ChAdeno virus	5 x 10^{10} VP x 2	Median EU 997.5	RV median ID_{50} 372 PV median ID_{50} 450.9	Median 1642.3(day14) Median 528.7 (day 35) Median 614 (day 56)
Moderna mRNA-1273 (14)	mRNA	250 μg x 2	GM EP 1,192,154	PV GM ID_{50} 270.2	ND
Novavax NVX-CoV2373 (17)	Protein	5 μg x 2	GM EU 63,160	RV GM $ID_{>99}$ 3305	ND
Pfizer/BioNTech BNT162b1 (15)	mRNA RBD	30 μg x 2	GM EU (RBD) 16,166	RV GM ID_{50} 267	ND
Pfizer/BioNTech BNT162b1 (16)	mRNA RBD	50 μg x 2	GM EU (RBD) 25,006	RV GM ID_{50} 578 PV GM ID_{50} 3100	CD4+ median ~2000 CD8+ median ~2600 (day 29)
Pfizer/BioNTech BNT162b1[g] (18)	mRNA RBD	30 μg x 2	GM EU (S1) 6580-23,516	RV GM ID_{50} 101-267	ND
Pfizer/BioNTech BNT162b2 [g] (18)	mRNA S-protein	30 μg x 2	GM EU (S1) 7895-9136	RV GM ID_{50} 149-361	ND

[a] The number of immunizations is also given. VP = viral particles.

[b] Antibody binding was measured in S-protein (except when RBD, S1 protein, or inactivated, purified whole virus was used instead, as stated) in IgG ELISAs and listed as endpoint (EP) values. EU indicates relative ELISA units (see primary papers for details); titer = unspecified method of the titer determination. The samples were obtained at a time corresponding approximately to the peak response after the final (or only) immunization. GM = geometric mean.

[c] Neutralization was quantified in PV or RV assays as indicated. The potency was measured as ID_{50} or $ID_{>99}$ values 2 weeks after the final (or only) immunization, which corresponds approximately to the peak response. The values reported for references 11 and 12 are given simply as geometric mean titers, as in the original text where the cut-off was not defined.

[d] T-cell responses were measured in ELISPOT IFN-gamma assays and recorded as SFC/10^6 cells (PBMC except where subpopulations of CD4+ and CD8+ are indicated) after stimulation with different SARS-CoV-2 S-protein-derived peptides. The days between immunization (day 0) and sampling are also listed (in brackets).

[e] The binding antibody and NAb titer ranges were similar in the lower dose (2.5 and 5.0 μg) groups 14 days after the third dose. In other studies one dose stood out as giving stronger responses and was chosen for tabulation.

[f] ND = not done (no data were presented in the paper).

[g] The ranges listed for BNT162b1 and BNT162b2 are the GM values for the age groups 65-85 years (lower value) and 18-55 years (higher value).

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