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Opinion

COVID-19 Infection, Vaccines, and Immunity—The Antibody Response Requires Detailed Analysis

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Abstract: Current tests for antibodies specific for the SARS-CoV-2 S protein say nothing about their precise epitope specificities. These data are needed to properly assess the immune status of individuals following infection or vaccination, and the risk posed by virus variants.

Keywords: SARS-CoV-2; COVID-19; SARS-CoV-2 S protein; human neutralizing antibodies; epitope specificity; epitope mapping

Assessment of immune responses to SARS-CoV-2, the virus responsible for COVID-19, is based almost entirely on the antibody response, and apart from clinical trials, comparisons of the merits of one vaccine versus another, or of the first jab versus the second, or the immunity of individuals resulting from infection are all based on antibodies specific for the viral spike or S protein. Broadly speaking such antibodies are measured by a lateral flow test or similar in which antibody binds to the viral S protein, a test which is fast but non-quantitative and gives no indication of antibody function. Alternatively, there is the more labour-intensive neutralization test which measures the ability of antibody to inhibit virus infectivity in cell culture, under conditions that do not mimic the in vivo situation. Both types of tests give information of limited value and say nothing about the epitope specificity of antibodies present.

1. The Epitope Landscape

The virus surface protein of an enveloped virus like COVID-19 possesses a small number of antigenic sites—influenza A virus has three or four [1]—each comprising an array of epitopes composed of a small number of amino acids, with each epitope recognised by an antibody with a unique specificity (or paratope). Thus, if an individual responds to every epitope s/he would have a diverse population of antibodies each recognising a different facet of the S protein. In addition, there would be a variable amount of each antibody present, and each antibody would have different biological properties, varying in its ability to neutralize virus infectivity and the mechanism by which that was brought about (e.g., some inhibit virus attachment to the cell receptor, some inhibit post-attachment functions like fusion, while others are non-neutralizing). In addition, there are nine isotypes of human antibody defined by a unique C-terminal unvarying sequence that is not involved in epitope-binding. In theory all nine isotypes could form antibodies that are all specific for the same epitope. This is important as each isotype has evolved to protect different regions of the body and has evolved specialised functions to combat different pathogens.

However, in reality the likelihood is that different individuals produce antibodies to only a fraction of the number of epitopes present and that different individuals, being genetically heterogeneous, will respond with a different spectrum of antibodies. Unfortunately, we do not know this for sure as such information is not available and to the author's knowledge is not being sought. The best test available, the neutralization test, merely averages out the neutralizing capacity of all the antibodies possessed by an individual, and gives no information about the range of antibodies present. To take an extreme example, an individual with 1 unit of each of antibodies specific for 10 different epitopes will give

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the same neutralization titre as another individual with 10 units of antibody specific for just 1 epitope. The consequences of the latter are far-reaching and are discussed below.

2. Assessing the Risk of Viral Variants

Knowledge of the range of neutralizing antibody specificities present in a country's population is relevant to both assessing the risk posed by variant viruses that bear amino acid substitutions in the S protein, and in understanding the role of neutralizing antibody in the evolution of virus variants. Let us suppose that a mutation results in a coding change that fundamentally alters an epitope so that the relevant antibody no longer recognises it. Providing that an individual has antibodies to other unchanged neutralizing epitopes that variant will be neutralized. For example, the S protein of the COVID-19 variant B.1.1.7 has eight mutations including the N501Y substitution in the receptor-binding domain and was tested for its ability to be neutralized by a panel of 12 monoclonal antibodies (MAbs) prepared against wild-type virus: of these the neutralizing activity of ten MAbs was unchanged, one had reduced activity and the activity of one was increased [2]. Problems would arise, however, if an individual made only one neutralizing antibody and that was directed against the mutated epitope. The variant virus will not be neutralized and the individual will be re-infected. This situation could apply to people who have recovered from infection or have received one of the COVID-19 vaccines. However, a limited number of convalescent sera (n = 20) fully neutralized B.1.1.7, although most (16/20) had reduced activity against B.1.351, a variant with nine S protein mutations including a D614G substitution [3].

The significance of a very narrow antibody response comes from studies with MAbs. RNA viruses in general have a high mutation rate and a virus species such as COVID-19 comprises a mixture of differing RNA genomes called a quasi-species. If one of these variants is fitter and has a replication advantage, it will become a major component in the quasispecies.

3. Antibody Responses Can Be Very Narrow

A virus antibody-resistant variant or escape mutant emerges as a result of selection pressure from a single neutralizing MAb but, if the virus (such as influenza A) is incubated simultaneously with two neutralizing MAbs directed against different non-overlapping epitopes, no resistant variant emerges. The latter happens only when the second MAb is diluted to a critical low concentration [4]. The reason for this lies in the mutation rate, which is approximately 1 nucleotide change in every 100,000 nucleotides synthesised per round of influenza genome replication or 10^{-5}. It follows that the chance of a single virus particle escaping neutralization by MAbs to two discrete epitopes is 10^{-10} and is vanishingly unlikely. However, if an antibody-resistant mutant/variant to one of the MAbs already exists in the population, then it has only to undergo a single mutation to escape the second MAb. Thus, a mixture of three MAbs to discrete epitopes is the minimum required to prevent the emergence of a neutralizing antibody-resistant mutant. The same logic underlies the use of a cocktail of three HIV-1 antivirals, each acting on a different virus target, to prevent drug-resistant mutants arising.

As mentioned earlier it is generally assumed that a normal immune response to an infection or a vaccine comprises a plethora of antibodies specific to different epitopes in approximately equal amounts. If so, no antibody-selected variants should ever arise. With a 'weak immune response' (to two or more discrete neutralizing epitopes) there might be a failure to prevent infection and a breakthrough of replication by the whole virus population, but no variants would be produced. However, the fallacy lies in the assumption that no individual produces an antibody response which is dominated by antibody to just one epitope. In experiments in which mice and rabbits were conventionally immunized with two or more doses of an inactivated influenza A virus vaccine it was found that sera from 11% mice and 75% rabbits mixed with virus and grown in embryonated eggs, selected virus that escaped neutralization. In other words, these serum antibody responses were

functionally monoclonal [4,5]. Serial bleeds taken after vaccination showed that there was either a biased response from the get-go or it evolved only later [6]. Antiserum-resistant variant virus no longer reacted with one of a panel of MAbs, and nucleotide sequencing showed that it was a genuine antibody-resistant mutant. The HA-specific antibody responses in mice and rabbits were biased to different antigenic sites. The situation with COVID-19 is exacerbated by the appearance of virus variants with a changed epitope landscape, so that what was a broad antibody response to the original virus becomes a narrow response to the variant.

Few data are available for the human population although a restricted antibody response to influenza infection has been observed in children [7,8] and adults [9,10]. A relatively broad response was found in infected adults [8,11]. However, we as individuals are genetically heterogeneous and it is unlikely that everyone will make the same spectrum of antibodies [12,13]. The antibody response after administration with any of the approved inactivated influenza vaccines is largely HA-specific and narrow in character [11,14], but whether it is as narrow as was found above in mice and rabbits is not known. Naturally occurring, clinically significant influenza A virus antigenic drift variants that can infect people who have previously been infected, have four or more changes in two or more antigenic sites, and take around 4 years to arise [1]. Such data imply that the antibody response in some individuals is functionally monoclonal and is driving the evolution of the virus.

4. We Need an Epitope Specificity Test for Antibodies

To determine person-to-person variation in the COVID-19 antibody response, it would be necessary to determine the epitope specificity of the antibodies stimulated in each individual. This is a problem that runs all through virology and radical new approaches are needed to solve it. Currently, mapping of COVID-19-specific antibodies is pretty crude: antibodies can be assigned to sub-regions of the S protein [15], or more specifically by binding to overlapping peptides derived from regions of interest on the S protein [16,17], but neutralization activity can only be inferred, or shown using relevant peptides to deplete the neutralizing activity of sera [15]. A serious problem is that neutralization epitopes often comprise non-contiguous/conformational regions within the S protein monomer or at the interfaces in the trimer, that cannot easily be mimicked by synthetic peptides. An alternative approach would be to make a panel of multiple escape mutants by sequential exposure to COVID-19-specific MAbs. These would then be utilised to analyse the epitope specificity of antibody samples, as used for influenza virus-specific sera [4–6]. At the same time epitope mapping of COVID-19 S protein would allow an informed evaluation of new virus variants, as interpretation of the relevance of a new S protein sequence/structure to the antibody landscape is largely guesswork.

In summary, we lack information about the epitope-specificity of antibodies that are made in response to COVID-19 infection and vaccines. For protection from variants, we need individuals to make neutralizing antibodies which are specific for a range of different epitopes. To understand the evolution of virus variants, we need to know if we have individuals in the population who, whether from infection or vaccination, make an extremely narrow antibody response that is effectively monoclonal and can drive the selection of new variants. Thus, in assessing vaccine efficacy, immunity from infection, variation between ethnic groups etc we are missing important data. To tackle this problem, we need first of all to establish the mindset that we have a serious problem here that needs answering, and then devise the means to quantitatively test for neutralizing antibodies that react to different epitopes using appropriate high throughput tests.

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References

1. Wilson, I.A.; Cox, N.J. Structural basis of immune recognition of influenza virus hemagglutinin. *Ann. Rev. Immunol.* **1990**, *8*, 737–771. [CrossRef] [PubMed]
2. Cheng, L.; Song, S.; Zhou, B.; Ge, X.; Yu, J.; Zhang, M.; Ju, B.; Zhang, Z. Impact of the N501Y substitution of SARS-CoV-2 spike on neutralizing monoclonal antibodies targeting diverse epitopes. *Virol. J.* **2021**, *87*, 1–6.
3. Wang, P.; Nair, M.S.; Liu, L.; Iketani, S.; Luo, Y.; Guo, Y.; Wang, M.; Yu, J.; Zhang, B.; Kwong, P.D.; et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *Nature* **2021**, *593*, 130–135. [CrossRef] [PubMed]
4. Lambkin, R.; McLain, L.; Jones, S.E.; Aldridge, S.L.; Dimmock, N.J. Neutralization escape mutants of type A influenza virus are readily selected by antiserum from mice immunized with whole virus: A possible mechanism for antigenic drift. *J. Gen. Virol.* **1994**, *75*, 3493–3502. [CrossRef] [PubMed]
5. Lambkin, R.; Dimmock, N.J. All rabbits immunized with type A influenza virions have a serum antibody haemagglutination-inhibition antibody response biased to a single epitope in antigenic site B. *J. Gen. Virol.* **1995**, *76*, 889–897. [CrossRef] [PubMed]
6. Lambkin, R.; Dimmock, N.J. Longitudinal study of an epitope-biased haemagglutination-inhibition antibody response in rabbits immunized with type A influenza virus. *Vaccine* **1996**, *14*, 212–218. [CrossRef]
7. Haaheim, L.R. Haemagglutinin-inhibition antibodies in human sera to an antigenic mutant of influenza A/Texas/1/77 (H3N2) virus obtained in vitro. *Acta Pathol. Microbiol. Immunol. Scand. B* **1980**, *88*, 351–353.
8. Meade, P.; Kuan, G.; Strohmeier, S.; Maier, H.E.; Amanat, F.; Balmaseda, A.; Ito, K.; Kirkpatrick, E.; Javier, A.; Gresh, L.; et al. Influenza virus infection induces a narrow antibody response in children but a broad recall response in adults. *mBio* **2020**, *11*, e03243. [CrossRef] [PubMed]
9. Natali, A.; Oxford, J.S.; Schild, G.C. Frequency of naturally ocurring antibody to influenza virus antigenic variants selected in vitro with monoclonal antibody. *J. Hyg. (Camb.)* **1981**, *87*, 185–190. [CrossRef] [PubMed]
10. Wang, M.-L.; Skehel, J.J.; Wiley, D.C. Comparative analyses of the specificities of anti-influenza hemagglutinin antibodies in human sera. *J. Virol.* **1986**, *57*, 124–128. [CrossRef]
11. Kubo, M.; Miyauchi, K. Breadth of antibody responses during influenza virus infection and vaccination. *Trends Immunol.* **2020**, *51*, 394–405. [CrossRef] [PubMed]
12. Lambkin, R.; Novelli, P.; Oxford, J.; Gelder, C. Human genetics and responses to influenza vaccination. *Am. J. Pharm.* **2004**, *4*, 293–298. [CrossRef] [PubMed]
13. Vidarsson, G.; Dekkers, G.; Rispens, T. IgG subclasses and allotypes: From structure to effector functions. *Front. Immunol.* **2014**, *5*, 520. [CrossRef] [PubMed]
14. Krammer, F. The human antibody response to influenza A virus infection and vaccination. *Nat. Rev. Immunol.* **2019**, *19*, 383–397. [CrossRef] [PubMed]
15. Zost, S.J.; Gilchuk, P.; Chen, R.E.; Case, J.B.; Reidy, J.X.; Trivette, A.; Nargi, R.S.; Sutton, R.E.; Suryadevara, N.; Chen, E.C.; et al. Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nat. Med.* **2020**, *26*, 1422–1427. [CrossRef] [PubMed]
16. Poh, C.M.; Guillaume, C.; Wang, B.; Naqiah, A.S.; Lee, C.Y.-P.; Chee, R.S.-L.; Fong, S.-W.; Yeo, N.K.-W.; Lee, W.-H.; Torres-Ruesta, A.; et al. Two linear epitopes on the SARS-CoV-2 spike protein that elicit neutralising antibodies in COVID-19 patients. *Nat. Commun.* **2020**, *11*, 2806. [CrossRef] [PubMed]
17. Shrock, E.; Fujimura, E.; Kula, T.; Timms, R.T.; Lee, I.-H.; Leng, Y.; Robinson, M.L.; Sie, B.M.; Li, M.Z.; Chen, Y.; et al. Viral epitope profiling of COVID-19 patients reveals cross-reactivity and correlates of severity. *Science* **2020**, *370*, 1058. [CrossRef] [PubMed]