



Relevant of neutralizing antibody during SARS-CoV-2 infection and their therapeutic usage

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SARS-CoV-2 disease cause continuity in devastation, despite rolling out of a global vaccination program. As per the WHO report more than 494 million covid cases and 6.1 million deaths have been confirmed worldwide while more than 11.2 billion total vaccine doses have been given so far. The main reasons for this destructive outcome are the emergence of new variants and the gap in vaccine coverage. SARS-CoV-2 is an enveloped beta coronavirus. It shares homology with pathogenic viruses such as SARS-CoV-1 and MERS-CoV and seasonal coronavirus such as HCoV-HKU1 and HCoV-OC43 beta-coronaviruses. Most of the Covid vaccines present to date are based on the prefusion spike glycoprotein of SARS-CoV-2 wild type Wuhan virus with recently developed vaccines targeting specifically variants of concern (VOCs). Spike protein has S1 protein, which consists of mainly two components, one is Receptor Binding Domain (RBD) which recognizes and is attached to ACE2 receptor of human lung cells, and another is N-terminal domain (NTD) which binds to Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN) and S2 protein that helps in fusion. Both are important for viral host recognition and entry into other cells including lung cells. Neutralizing antibodies are produced either by SARS-CoV-2 natural infection or via vaccination-induced immunity. The target for these neutralizing antibodies includes primarily the RBD site in the spike region but it also includes NTD and S-2 region of spike protein. Severely infected patients (both ICU and non-ICU) show higher virus-specific antibody titers than mild or asymptomatic

patients. It is found that the serum neutralization capacity is positively correlated with disease severity [1]. Among neutralizing antibody isotypes, IgM antibodies produced during the early course of infection followed by switched antibodies such as IgA provide mucosal immunity while IgG is for systemic immunity. It is observed that the IgG antibodies provide durable immunity [2]. IgM antibodies arise as soon as 4 days after infection and peak around 20 days while IgG antibodies start appearing on day 7 after infection and will reach their maximum level on around day 25 post infection. In some individuals IgM is not at all detected and only IgG can be seen in their serum. Similarly, after mRNA vaccination, individuals reported having either appearing spike specific both IgM and IgG or only IgG and no IgM [3]. The generation of neutralizing antibodies starts arising after the first few weeks' post-infection symptom onsets and is positively correlated with their recovery. The absence of neutralizing antibodies early after infection could lead to higher fatality cases in hospitalized patients and vice versa if Neutralizing antibodies developed early after infection, they would limit viral load and subsequent disease progression. These spike IgG antibodies persist for 8 months in recovered patients [4]. These antibodies are produced by B cells during humoral immune responses. Naïve B cells upon antigenic exposure get activated, proliferated, and differentiated into plasma cells and memory B cells. Plasma cells are the producer of all neutralizing antibodies and non-neutralizing antibodies detected in serum and mediate protection by binding to SARS-CoV-2 epitope and helping in the reduction of viral load. The long-term antibodies are produced either by long lived plasma cells or by quiescent memory B cells upon rechallenge. Both long lived plasma cells and memory cells are the outcomes of germinal center (GC) reaction in lymphoid follicular area and that leads to higher affinity and class switched antibody isotype. During severe SARS-CoV-2 acute infection, GC reaction gets impaired as lower

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frequency of Bcl6+ cells (pan GC marker) and Tfh cells as observed in postmortem spleen and thoracic lymph node and increase in aberrant activated B cells. Extrafollicular plasma blast got expansion [2, 5], as a result, they produce many neutralizing antibodies of low affinity. SARS-CoV-2 infection also induced autoantibodies that lead to autoimmunity. One of the reasons for generating autoimmune antibodies is regulation of anergy B cells production. Anergy of B cells are quiescence and an un-responsive state of self-reactive B cells. They do not respond to antigenic stimulation thus not secreting antibodies and serve as a preventive mechanism for generation of autoimmune antibodies [6].

Upon vaccination neutralizing, antibody titers are higher than natural infection in polyclonal serum [7]. When analyzed at monoclonal antibody level, it is reported that plasma blasts upon mRNA vaccination have a higher non-neutralizing to neutralizing ratio. This explains higher binding than neutralization features of antibodies produced during mRNA vaccination [8]. These IgG neutralizing antibodies are quite durable. As shown that post-mRNA vaccination IgG antibody titers functional activity is maintained post 6 months of their second dose. There is a moderate reduction in neutralizing titers in B.1.351 among all variants of concern tested [9]. Moreover, the neutralization titers are positively correlated with their protective efficacy as shown in both convalescent/recovered patients as well as in vaccine recipients. This shows the importance of neutralization antibody titers in fighting against SARS-CoV-2 infection [10].

Neutralizing antibodies can be used in serological tests and therapeutic purposes as in plasma therapy or commercially available monoclonal antibodies to treat patients suffering from SARS-CoV-2 breakthrough infection. The detection of functional neutralization antibody titers is measured either by live virus neutralization assay (FRNT₅₀) in BSL3 facility or by Pseudo virus neutralization assays using reporter gene (PRNT₅₀) in BSL2 facility. SARS-CoV-2 Antibody binding assays are done either by classical ELISA or high throughput Electro-luminescence based meso-scale discovery assay using samples such as plasma, serum, or nasal swabs. These serological tests are different from the diagnostic test such as RT-PCR. Diagnostic tests quantify viral titers to measure the intensity of ongoing infection while serological tests measure antibody titers which measure level and duration of recovery [2]. Examples of commercially available neutralizing antibody kits for quantifying SARS-CoV-2 IgG ELISA include VIDAS IgG ELISA, Euroimmun QuantiVac ELISA IgG, and Microblot-Array COVID-19 IgG assays to detect natural infection. These assays are useful in sero-conversion to monitor neutralizing antibody response in population and durability of antibody response [11]. Regarding therapeutic usage of antibodies, there is some success observed using plasma therapy which is based on transfer of polyclonal antibodies, derived from

recovered patients or monoclonal antibodies that were generated in a laboratory setting using tissue culture. The success is higher if transferred in the early phase of SARS-CoV-2 infection course [12]. There are four classes of antibodies developed commercially to use as a therapeutic agents to combat SARS-CoV-2 infection. Class 1 antibodies represent the most dominant type of antibody type that mainly compete with ACE2 for RBD binding and only recognize 'up' RBD conformation, encoded by VH3-53 and VH3-66 germline segments. It is reported that both binding and neutralization capacity of Class 1 antibodies gets reduced by N501Y, E484K, and K417N mutations in RBD region thus being found to be less effective in the case of B.1.1.7, B.1.351, and P.1 SARS-CoV-2 variants. An example of this class includes COVOX-222. There are other examples of this group, Fab 1–57 and Fab 2–7 antibodies, that also bind to the RBD site but are excluded from evolutionary pressure of mutation. Krammer et al. isolated 54,042–4 monoclonal antibodies by using Libra-seq advanced technology from convalescent patients which had the potential to neutralize Variants such as B.1.17, B.1.351, P1, B.1.141, B.1.258. Class 2 type of monoclonal antibodies bind to RBD in both 'up' and 'down' conformations and compete with ACE2 for RBD binding. The example includes C144, C121, COVA2-15, COVA2-37, MD65 and LY-CoV555 in this category. LY-CoV555 or bamlanivimab but not MD65 has been shown to abrogate in their binding capacity against B.1.351 variant due to E484K mutation. Class 3 type of monoclonal antibodies bind to the outside RBD region compared to Class 1 and Class 2 binding epitope close to an N-glycan attached to residue N343. So, this group of antibodies can be used in combination with class 1 or class 2 group of antibodies and target different sites of spike protein and provide synergistic protection against SARS-CoV-2 infection. For example, LY-CoV1404 shows effectiveness against B.1.1.7, B.1.351, B.1.427, P.1, and B.1.526 variants. Other examples of this group include VIR-7831 and VIR-7832. The Class 4 antibody, usually with poor neutralizing activity, binds to the conserved region and targets a cryptic epitope that faces the interior of the Spike protein on 'up' RBDs. Some Class 4 Abs also show cross reactivity and neutralize a broad spectrum of β -coronaviruses. So, they are important against the novel SARS-CoV-2 mutants [13]. There is an urgent need for the development of broad and cross-neutralizing antibodies which can target the conserved region of SARS-CoV-2 and are potentially useful in the upcoming future therapeutic agent. Besides clinical usage of neutralizing antibodies and controlling viral load during SARS-CoV-2 infection, one of the potential side effects of therapeutic usage of neutralizing antibodies is exacerbating SARS-CoV-2 pathogenesis by antibody dependent enhancement (ADE) mechanism. ADE mechanism, in which antibody coupled with a virus is endocytosed into Fc receptors such as Fc γ RIIa or Fc γ RIIIa

expressing phagocytic cells macrophage. This process enhances viral load by increasing viral replication. Another mechanism that leads to excessive inflammation, is enhanced immune activation followed by immunopathology caused by formation of an immune complex by either non-neutralizing or sub-neutralizing antibodies [14]. So the analysis of ADE mediated risk during development of therapeutic antibodies should be assessed carefully.

The Omicron variant is a recent emerging threat of SARS-CoV-2 variants which raised serious concern because most therapeutic interventions such as a successful global vaccine via two dose regime administration, and usage of most monoclonal antibodies show immune evasion against omicron. This variant originated in Botswana and Gauteng province in South Africa in November 2021. This variant is currently predominant among all existing variants. Reports confirmed that this variant has replaced the Delta variant globally. There is a striking reduction of neutralization titers against Omicron variant B.1.1.529 (BA) post first series of vaccination. There is the emergence of omicron sub-lineages as BA.1, (BA.1 + R346K), and BA.2 which show highly polymorphism and have more than 30 spike mutations [15]. Due to extensive mutation in their spike region, the Omicron variant causes a rapid surge in re-infection cases in vaccinated individuals or covid-recovered individuals. Moderate protection has been seen in the case of two doses of vaccination plus mild infection or two dose of vaccination plus a third booster dose of vaccination. Hence it seems to have three layers of immune response for better protection against Omicron [16, 17]. A Clinical trial study reported immunogenicity and safety for fourth dose of mRNA vaccine post four month of their third vaccine dose. Forth dose exhibited approximately ten times higher antibody binding and neutralization titers against Omicron variant and show no adverse effects [18]. Most antibodies that were approved and were in clinical use show resistance against the Omicron variant [19, 20]. When tested by a panel of clinically recommended anti-RBD monoclonal antibodies, it has reported that Eli Lilly (LY-CoV555 and LY-CoV016), Regeneron (REGN10933 and REGN10987), Celltrion (CT-P59) shows a sharp reduction in their neutralization potential so escaping antibody mediated protection whereas AstraZeneca (COV2-2196 and COV2-2130) and Vir Biotechnology (S309 or Sotrovimab) efficacies are moderately affected [20]. A recent report also suggested that S309 or Sotrovimab and DZIF10c provide better protection against omicron infection than other monoclonal antibodies [21]. Furthermore, FDA also suggested limited benefits of two monoclonal antibody usage first is combined use of bamlanivimab and etesevimab and second is REGEN-COV (casirivimab and imdevimab) against Omicron. FDA has recently approved bebtelovimab

(LY-CoV1404) by Eli Lilly, under emergency use authorization against Omicron infection therapeutic reagent. This monoclonal antibody has been shown to retain both binding and neutralization potential in all circulating variants of concerns including Omicron [22]. Another study showed retaining of partial neutralization capacity with Vir-7831 and a combination of AZD8895 + AZD1061 monoclonal antibodies against this variant [23]. mRNA vaccination booster causes significant enhancement in neutralization titers against Omicron in both naïve and recovered individuals. Interestingly, the third dose of mRNA vaccine also enhances the breadth of response and improved cross reactivity to all circulating variants of concerns [24]. Usage of ultrapotent antibodies and bispecific antibodies are better alternative options for antibody mediated therapy. Ultrapotent neutralizing antibodies can be effective against most SARS-CoV-2 variants. Their neutralization titers (IC50) value is in nanomolar (ng/mL) range and is isolated from either convalescent human serum or from memory B cells [25]. While bispecific antibodies are constructed by combining two monoclonal antibodies so having two specificities potentially neutralizes better than its parental monoclonal antibodies. This strategy can also be used in targeting SARS-CoV2 variants of concerns [26].

Global vaccine availability and filling the gap in vaccine coverage are utterly needed to achieve herd immunity and prevent community infectious disease transmission by future SARS-CoV-2 variants. Successful clinical trials and better scientific conversation can speed up the process of vaccination and mass scale awareness. For example, FDA in the USA approved the administration of the Pfizer vaccine in children (5–11) and adolescents (12–17) under emergency use of authorization showing promising effects in filling the gaps in vaccine coverage. Children are broadly spared from covid infection but can cause a source of natural infection. Vaccine induced neutralization titers are either equivalent or higher in children compared to adults [27].

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