Cell Death & Differentiation (2021) 28:821–824
https://doi.org/10.1038/s41418-020-00711-w

Cell Death & Differentiation

COMMENT

Antibodies, epicenter of SARS-CoV-2 immunology

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Received: 3 December 2020 / Revised: 9 December 2020 / Accepted: 14 December 2020 / Published online: 26 January 2021

Antibodies against SARS-CoV-2 are the epicenter of the efforts to understand and fight the COVID-19 pandemic. They play a central role in clearing the virus from infected patients, they are key reagents of rapid diagnostics, they are first line treatments for hospitalized COVID-19 patients and they are the main objective of COVID-19 vaccine development. However, antibodies are also blamed for worsening the disease through antibody-dependent disease enhancement (ADE), thereby putting them under the spotlight of many COVID-19 immunological studies. Here we propose a short overview of the natural antibody response induced by the infection, the efforts to isolate and produce human monoclonal antibodies for therapy and prevention, and the attempts to induce potently neutralizing antibodies by vaccination.

To prevent and cure the SARS-CoV-2 infection, a great deal of studies has been conducted to understand the capacity of the natural antibody response to neutralize this virus. Such capacity was measured by testing in vitro the ability of the antibodies to engage the S1 subunit of SARS-CoV-2 spike protein, which contains the receptor biding domain (RBD) to the human angiotensin-converting enzyme 2, and their ability to neutralize the real or pseudotyped virus. Numerous studies have reported that, while in asymptomatic subjects the antibody response is usually quite low, the intensity of the response correlates with the severity of the disease, with the highest levels being observed in hospitalized patients. It was also noticed that the quantity of circulating neutralizing antibodies rapidly declines in time, which led to the hypothesis that long term immunity to SARS-CoV-2 might be difficult to achieve. These observations are supported by studies that investigated the DNA sequences of the genes encoding the circulating antibodies and showed that neutralizing antibodies are only few mutations distant from their respective germline sequence, suggesting absence of affinity maturation in germinal centers and low capacity to induce long-lived plasma cells [1]. A report showing that COVID-19 patients have a reduced number of Bcl-6 expressing follicular helper T cells in germinal centers proposed that one of the pathogenetic mechanisms of the virus might involve avoidance of a proper affinity maturation process of the host antibodies [2]. It is important to notice that most of the reported studies followed the antibody response very early after infection, which might lead to the hypothesis that the rapid waning of the antibodies is explained by the natural decline of the peak immune response. Indeed, after an initial phase of high antibody levels, and following the normal antibody half-life of 21 days, their titer typically drops to a lower level of persistent immunity which is maintained by long-lived plasma cells (Fig. 1A). To support this hypothesis, recent longitudinal studies, where the immune response was followed over time, have reported a sustained response in some individuals and a decline in others [3]. However, the majority of subjects had detectable neutralizing responses lasting for several months. In conclusion, we believe that most of the studies published so far reported observations that we will not be able to fully understand until it is demonstrated which is the level of neutralizing antibodies that confers protection. Once the correlate of protection is identified, it will be easier to establish whether SARS-CoV-2 infection induces protective antibody levels and how long they last.

Modern medicine was born with passive immunization when Emil von Behring discovered that immune serum was able to prevent and cure diphtheria. In the absence of specific drugs and vaccines, SARS-CoV-2 antibodies present in

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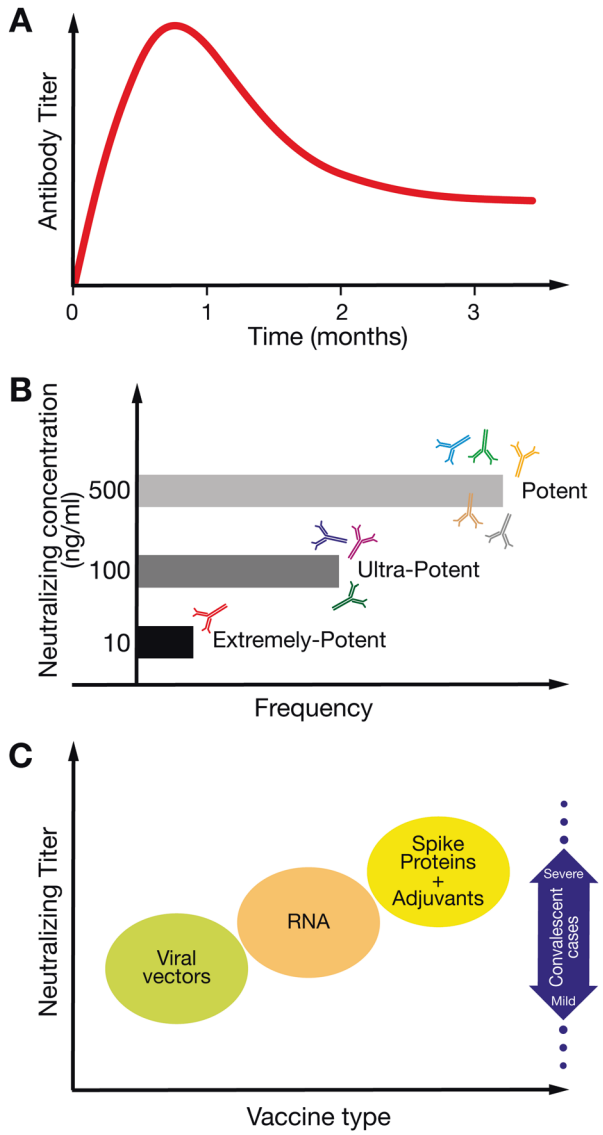

Fig. 1 Antibodies to understand and fight COVID-19. A Typical antibody response curve showing an initial peak followed by long-term durability. **B** Frequency and potency of SARS-CoV-2 neutralizing mAbs. **C** Schematic representation showing the antibody levels observed in convalescent patients compared to those induced by COVID-19 vaccines in phase 1 clinical trials [12–16]. Importantly, neutralizing titers have been obtained with non-standardized assays and the correlation between neutralizing antibodies and vaccine efficacy is still unknown.

the plasma of convalescent patients that recovered from infection were also the most obvious tool to cure COVID-19. The first exploitation of antibodies in the COVID-19 pandemic was the transfusion of plasma with high titers of neutralizing antibodies from convalescent to hospitalized patients. Several trials have demonstrated some benefit and plasma therapy was the first treatment authorized by the FDA for emergency use [4]. However, due to the complexity and risks linked to the administration of hyper-immune plasma, a massive effort in dozens of labs around the world let to the isolation of human monoclonal antibodies (mAbs) from convalescent patients. Before the COVID-19 pandemic, little attention had been paid to the use of mAbs to treat infections despite more than 100 had been licensed as therapeutics for cancer, autoimmunity and inflammation. This was mostly because of their high cost, which did not make them suitable for a broader use. A game changer in the field of mAbs for infectious diseases was the cloning of potent neutralizing antibodies from memory B cells of convalescent subjects following SARS infection [5]. Since then, several technologies were developed for isolating and cloning mAbs from B cells. They were extensively used for instance in the HIV research, where mAbs helped the discovery of highly conserved epitopes, thus driving vaccine design and the development of passive treatments for prevention of infection and therapy [6]. This progress allows today the routine isolation of mAbs which are 1000 fold more potent than those identified two decades ago, and which require 1000 fold less doses to be effective, which translates into the possibility to apply the same approaches to develop affordable therapies also for other infectious diseases. When the COVID-19 pandemic started in January 2020, the field was mature. Several academic laboratories and pharmaceutical companies focused on the development of mAbs specific for the SARS-CoV-2 virus. Potent, ultrapotent and extremely potent mAbs targeting the SARS-CoV-2 spike protein RBD have been promptly described which targeted the RBD of the spike protein and neutralized the virus at concentrations of 100 ng/ml or below, with only very rare antibodies able to neutralize the virus at concentrations below 10 ng/ml [7] (Fig. 1B). These mAbs protected animals from infection when administered prior to virus challenge and allowed faster recovery when used as therapeutics [8]. Several mAbs entered clinical testing within the first six months of the pandemic and have already started to deliver preliminary results. When administered early after infection, accelerated viral clearance and reduced hospitalization by 75% was reported [9]. Based on preliminary clinical data, two of them received emergency use authorization by the FDA. At this point it is clear that mAbs are a good preventive and therapeutic tool for SARS-CoV-2. However, there are still many pending questions. We do not know yet whether the antibodies can be used to treat severely ill hospitalized patients. Indeed, it remains to be proved whether the antibody Fc portions may need to be engineered to remove their effector functions, which may worsen the disease outcome by promoting increased inflammation via ADE [10]. Moreover, little is known on whether mAbs can promote mutations that may select the so-called “escape mutants.” These mutants can be selected in vitro when the virus (or pseudovirus) is grown in the presence of antibodies, but it is not clear whether this will be relevant in the therapeutic setting, where the mAb will

work together with the natural cocktail of antibodies produced by the patients. An obvious way to reduce the risk of selecting escape mutants is to use a cocktail of antibodies, which however has the downside to make the development of the treatments more complex and more expensive.

Concomitantly to mAb development, more than 320 laboratories worldwide have been developing vaccines with the aim of eliciting neutralizing antibodies against the SARS-CoV-2 virus. During the COVID-19 pandemic, the field experienced a massive acceleration, fueled by international collaborations, substantial public-private investments and close connections with the regulators. With few exceptions where an inactivated virus is used, all vaccines were made from synthetic genes encoding the whole spike protein or its RBD. In most cases, the protein had been engineered by introducing two prolines which had been shown to stabilize the spike protein in the prefusion conformation [11]. COVID-19 vaccine candidates can be divided in three main categories based on the use of the synthetic gene. The first ones are the RNA vaccines, fully synthetic vaccines encoding the spike protein. In the second type of vaccines the synthetic gene is expressed by a viral vector such as a non-replicating adenovirus or measles virus. In the third case, the spike protein is purified after synthetic gene expression in eukaryotic cells, such as Chinese Hamster Ovary cells, insect cells or tobacco cells. Spanning these three categories of vaccines, over 40 candidates are now being tested for safety and efficacy in clinical trials, with over 10 in phase III studies involving more than—on average—35,000 subjects per vaccine. Some of them are already providing preliminary phase III data showing efficacy of 95% for RNA vaccines and in the range of 62–90% for vaccines based on non-replicating adenoviruses. Immunogenicity data of these vaccines after Phase I-II clinical trials have been published [12–16]. The neutralizing titers reported in the papers cannot be compared directly because they were obtained using non standardized assays. Nonetheless, since most of the studies also reported neutralizing data from convalescent patients, we can infer their relative potency by comparing the relative neutralization titer and the immunogenicity of each vaccine against the range of neutralizing titers observed in convalescent patients. This analysis shows that, in broad terms, all vaccines induce antibody titers within the range observed in convalescent patients, confirming that they are all likely to induce protective immunity (Fig. 1C). Notably, antibody titers induced by the different vaccine platforms seem to be different. Indeed, viral vectors appear to induce titers which are in the middle range with respect to those found in convalescent patients, while RNA and vaccines based on recombinant proteins plus adjuvants seem to induce antibody titers in the high range or exceeding those found in convalescent patients. Importantly, we still do not know how neutralizing antibody titer correlates with efficacy, so the next months and follow up studies will be critical to learn more about the primary efficacy of these vaccines, the duration of protection, and whether vaccines can also protect from infection and transmission. Hopefully, we will also be able to establish which are the antibody titers which correlate with protection thus allowing the licensure of new vaccines without the need for efficacy trials.

Acknowledgements We wish to thank Giorgio Corsi for the artwork and Catherine Mallia for the editorial assistance.

Compliance with ethical standards

Conflict of interest SP, MP, and RR are permanent employees of the GSK group of companies. All authors contributed to the development of this manuscript and gave final approval before submission. This work was sponsored by GlaxoSmithKline Biologicals SA.

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