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Illuminating the Fc dependence of SARS-CoV-2 neutralization

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Monoclonal antibodies show efficacy in treating COVID-19, but the functional requirements for protection are unclear. In this issue of *Immunity*, Ullah et al. (2021) develop a stable SARS-CoV-2 reporter virus and use bioluminescence imaging to longitudinally monitor infection and assess neutralizing monoclonal antibody interventions in mice. They find that antibody-mediated protection depends on the Fc domain and Fc-gamma receptor-expressing immune cells.

With the emergence and persistence of clinically important SARS-CoV-2 variants, the development of mouse models that enable rapid assessment of potential therapeutic interventions, including neutralizing monoclonal antibodies (nAbs), will be impactful. *In vivo* models that rely on replication-defective SARS-CoV-2 pseudoviruses can provide a snapshot of the immune response upon encountering virus or the ability of an experimental treatment to prevent the entry step of viral replication. Other models that utilize replication-competent SARS-CoV-2 can evaluate the ability of a treatment to block any stage of the infection but are limited in utility for longitudinal studies because assessment of efficacy requires euthanasia of the animals to study viral loads in tissues. In this issue of *Immunity*, Ullah et al. (2021) have added to the array of available models of SARS-CoV-2 infections by providing a system for the tracking of viral replication and spread within multiple organs by time-lapse bioluminescence imaging (BLI) and video.

Ullah et al. describe a SARS-CoV-2 virus that expresses nanoluciferase during replication, enabling noninvasive monitoring of SARS-CoV-2 infection *in vivo* (Ullah et al., 2021). Using this reporter virus, the investigators were able to detect viral replication and spread within tissues over time in mice that express human ACE2 (K18-hACE2 mouse model), the major receptor for SARS-CoV-2. This model enabled efficient evaluation of the therapeutic potential of two SARS-CoV-2 nAbs, CV3-1 and CV3-25, which were

previously isolated from a convalescent coronavirus disease 2019 (COVID-19) patient. nAbs CV3-1 and CV3-25 were selected for neutralizing activity against the Wuhan-Hu-1 SARS-CoV-2 strain and bind to the receptor-binding domain (RBD) and S2 domain of the spike (S) protein, respectively (Jennewein et al., 2021; Stamatatos et al., 2021).

The K18-hACE2 mouse strain used in this study has been well described and utilized to model SARS-CoV-1 and SARS-CoV-2 infections (McCray et al., 2007; Winkler et al., 2020). This model is useful for evaluating the impact of potential therapeutics through approximately 6–10 days of infection, after which the animals otherwise succumb to the infection (Winkler et al., 2020; Jennewein et al., 2021; Ullah et al., 2021). In concurrence with previous studies, the investigators observed that SARS-CoV-2-infected K18-hACE2 mice exhibited robust bioluminescence signal within the brain after intranasal inoculation, likely contributing to rapid lethality in the model. While neurological complications and symptoms have been observed in COVID-19 patients, the detection of replicating SARS-CoV-2 within the human brain has been inconsistent at best. Additionally, the primary cause of death in severe COVID-19 in humans is overwhelming inflammation and damage in the lungs, not neurological sequelae. As such, while the pathophysiology of SARS-CoV-2 infection in these mice may not completely mirror the human, the early infection of the nasal cavity and lungs is

relevant to human infections in distribution and supports the K18-hACE2 mouse model as a system to efficiently assess the efficacy of prophylactic and early therapeutic agents.

One interesting observation made using this model was that luminescence signal, representing viral replication, initially increased within the upper airway before briefly decreasing to levels approaching background. While reporter viruses are notorious for losing the reporter gene signal as replication proceeds, the timing of the decrease in signal was consistent among animals and was localized solely to the upper airway, suggesting that this loss of signal was not due to loss of the reporter gene itself. Rather, it likely represented the initiation of a local antiviral immune response within the nasal cavity that transiently controlled viral replication before being overwhelmed during systemic viral spread.

In addition to characterizing the kinetics of signal from the reporter virus *in vivo*, the study further tests the effector function requirements of two nAbs using the virus. Expressed as a human IgG1 antibody, anti-RBD nAb CV3-1 was administered at a dose that provided 100% protection against lethal challenge when administered prior to or up to 3 days post-infection (dpi). However, the protection provided by delayed administration at 3 dpi was ablated when loss-of-function mutations were introduced into the Fc domain of CV3-1, revealing a reliance on Fc-mediated effector functions for therapeutic antiviral effect (Figure 1).



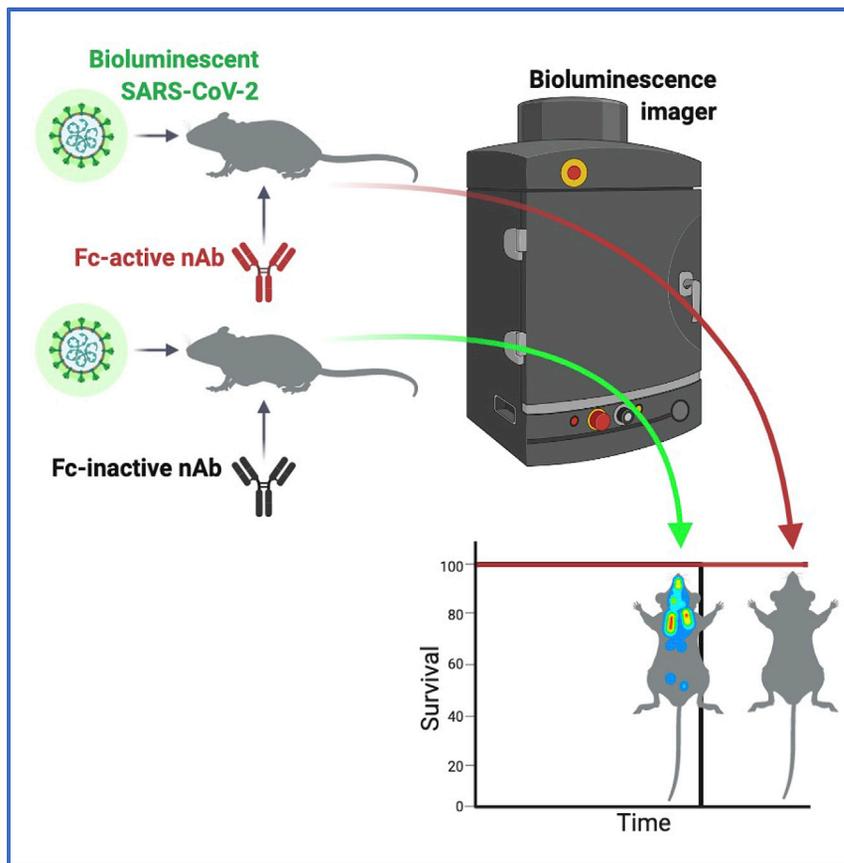


Figure 1. Bioluminescence imaging revealed that therapeutic activity of neutralizing antibodies requires an active Fc domain

K18-hACE2 mice were inoculated with a nanoluciferase-expressing SARS-CoV-2 virus. Three days post-infection, mice were treated with either the wild-type IgG1 nAb CV3-1 (Fc-active nAb) or a LALA-mutated CV3-1 (Fc-inactive nAb). Imaging revealed that Fc-inactive nAb administration failed to reduce viral replication, in contrast to complete protection that was offered by Fc-active nAb administration.

CV-1-mediated protection was dependent on immune cells that express Fc-gamma receptors (Fc γ Rs); specifically, depletion of neutrophils, monocytes, and natural killer cells reduced protective activity, highlighting the importance of Fc-mediated effector functions in limiting viral replication. Ullah et al. (2021) next tested the activity of human IgG1 nAb CV3-25. This nAb has less potent neutralizing activity *in vitro* but targets a more conserved region of the S protein—the S2 domain—and shows cross-neutralizing activity with the South African B.1.351 variant (Jennewein et al., 2021). Though CV3-25 administration, even prophylactically, showed little protective activity when expressed as a wild-type IgG1, introducing mutations to the Fc of CV3-25 that enhance activating Fc γ R binding dramatically increased the *in vivo*

efficacy of this nAb. Thus, the investigators demonstrated that protection offered by a less potent but more broadly neutralizing nAb could be improved through Fc engineering for enhanced binding to activating Fc γ Rs. This supports other studies demonstrating Fc-Fc γ R-dependent protection by anti-SARS-CoV-2 antibodies (Ravetch et al., 2021; Winkler et al., 2021). A caveat to these studies was the use of human IgGs in mice that express murine Fc γ Rs along with Fc mutations that enhance binding to human activating Fc γ Rs but that are not well characterized for activity in the mouse system. Murine Fc γ Rs differ from their human orthologs, in both their affinities for IgG subclasses and their expression levels and distribution on immune cells. The most interpretable studies of Fc-Fc γ R interactions in mice use murine IgGs in animals express-

ing murine Fc γ Rs or human IgGs in mice expressing humanized Fc γ Rs.

Aside from the activities of monoclonal antibodies, studies assessing the humoral response in severe COVID-19 patients have observed an enrichment of SARS-CoV-2-reactive IgG1 exhibiting a specific endogenous Fc modification, namely afucosylation (Chakraborty et al., 2021a). Afucosylated antibodies lack a core fucose within their N-linked Fc glycans, a modification that increases IgG1 binding affinity for the activating receptor Fc γ R11a. Immune complexes composed of afucosylated polyclonal antibodies and SARS-CoV-2 S protein are activating because of the enhanced binding to Fc γ R11a, triggering cellular inflammation in the lungs and production of inflammatory cytokines that mirror some findings in severe COVID-19 (Shields et al., 2002; Chakraborty et al., 2021b). There is an interesting distinction between the increased protection and absence of inflammation provided by Fc-enhanced nAbs and the enrichment of afucosylated antibodies in patients with severe COVID-19. Further studies are required to dissect the role that afucosylated, polyclonal immune complexes may have in promoting a pathological, early inflammatory response to the infection compared to the protection that can clearly be afforded by monoclonal antibodies that are engineered for enhanced activating Fc γ R affinity (Ravetch et al., 2021).

This new study by Ullah et al. (2021) demonstrates a convenient and accessible means of assessing the pre-clinical efficacy of anti-SARS-CoV-2 therapeutics and highlights the critical roles for both the antibody Fab specificity and the Fc domain of therapeutic IgG antibodies. Using BLI, the investigators demonstrate that an antibody with relatively weaker neutralizing capability but broader binding activity can be improved through Fc engineering to limit virus spread. While the cellular players and exact mechanism of human IgG1-mediated protection may differ in the K18-hACE2 model used in this study and in humans, the work demonstrates the utility of the nanoluciferase SARS-CoV-2 reporter virus and confirms the important observation that anti-SARS-CoV-2 antibody therapeutics should be Fc engineered for optimal activity *in vivo*.

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