Outsmarting the Virus

John Hewitt

Variants in the SARS-CoV-2 virus control infectivity, severity, and immunity by changing tissue tropism, innate responses, and adaptive antibody generation. Although the emergence of escape mutations can see the virus spread rapidly, regardless of vaccination or antibody status, new combination therapies that strike at its heart will complement vaccinations and provide a defense the virus cannot outsmart.

One of the first treatments developed for patients severely affected with COVID-19 involved the administration of convalescent plasma. This treatment did not progress past the testing phase after a clinical trial in the US demonstrated that there was little evidence of any impact on the virus.[1] The antibody spectrum of plasma may be diverse, but it is also complicated and, in many situations, dangerous to administer. In its stead, researchers are developing man-made monoclonal antibodies to target the virus with pinpoint accuracy. While still in experimental form, they can often be administered under an emergency use authorization.

Combination antibody therapies, such as the pairing of bamlanivimab and etesevimab, have been shown to wield considerable power against the virus. But they are already becoming obsolete in the face of escape variants conjured up by new strains. These treatments will remain part of the clinical arsenal, but a demonstrated lack of in-vitro activity against a threatening B.1.351 strain indicates that we have arrived at a new stage of viral warfare. The rapid rollout of alternative antibody formulations, such as casirivimab and imdevimab, might stall the virus temporarily—but the emergence of new variants means that the battle is far from over.[2]

The full immune system response to the complete SARS-CoV-2 virus, as opposed to just a small part of the spike protein, involves the recognition of many epitopes. These immunodominant sites vary significantly from one person to another. A recent study concluded that, on average, the immune system of each subject was able to recognize around seventeen $CD8^+$ T cell epitopes and nineteen $CD4^+$ T cell epitopes.[3] While the immune system typically mounts a strong response to the spike protein, the response from $CD4^+$ helper T cells to the spike is weak. Individuals who are slow to mount neutralizing $CD4^+$ responses capable of quickly wiping out infection, or individuals lacking a broad innate response, may harbor the virus longer, creating opportunities for resistant mutations to arise.

A few researchers have concluded that efforts to defend against the virus may be hastening inevitable selection and the spread of more infectious variants.[4] This raises another important question, namely, how infectious might this virus ultimately become? In a recent study of new strains, one lab conducted in-vitro evolution experiments to affinity maturate the receptor-binding domain from a few of the more contagious variants, such as S477N, E484K, and N501Y.[5] Technicians were able to create new variants with a spike–ACE2 receptor binding affinity that was enhanced 600-fold. Still, the overall infectiousness conferred by a given viral sequence depends on much more than just the binding affinity of particular spike protein formulations.

Last year in *Inference*, I discussed the potential pitfalls and merits of repurposing existing antivirals against SARS-CoV-2, or trying to block the spike–ACE2 receptor interaction.[6] Although viral RNA polymerase inhibitors have not yet proven effective, newer approaches that inhibit other components of the virus are now being considered. Some critical viral proteins are not attractive targets because they closely resemble our own in both form and function. Researchers have discovered that one of these enzymes, the main protease, Mpro, is unique in this regard.[7] Using computational methods to scan the library of existing approved compounds, they found that a compound known as cobicistat optimally binds to Mpro and was able to block its protease activity in experimental cell-based assays.

Other proactive approaches to treatment include retooling small-molecule tyrosine kinase inhibitors that interfere with the viral life cycle, block infection, or treat inflammatory autoimmune symptoms. To this end, highly successful targeted cancer therapies, such as ibrutinib, ruxolitinib, and imatinib, have been successfully administered to block immune reactions and acute respiratory distress. Infection with SARS-CoV-2 results in specific changes to the global phosphorylation landscape of individual cell

types. This detail is now being unmasked through massive phosphoproteomics analyses which will form the basis for the targeting of susceptible critical points in kinase second messenger systems.[8]

Among the most curious, long-lasting, and disruptive symptoms that can occur when the virus enters the human nervous system is the loss of smell and taste. This can be a persistent and difficult outcome to treat. Researchers are now getting a better handle on how the virus, or its immunogenic components, accesses different parts of the brain through nerves or by breaching the blood–brain barrier. In some cases, sensory function has been restored with a combination of dexamethasone and theophylline, or in more severe neurologic sequelae, with haloperidol.[9]

Strategies to block the initial fusion of the virus with host membranes have been successful through the daily intranasal administration of lipid-conjugated peptides. Lipopeptides corresponding to the conserved heptad repeat domain at the C terminus of the spike protein have prevented spike refolding and fusion, completely blocking infection in ferrets. These peptides form six-helix bundle-like assemblies with the extended intermediate form of the S protein trimer, disrupting the structural rearrangement of S that drives membrane fusion.[10]

Recent research has demonstrated that SARS-CoV-2 infection can be prevented and treated by the administration of a synthetic nucleoside derivative N4-hydroxycytidine known as EIDD-2801, or molnupiravir.[11] This treatment exerts its antiviral action by introducing copying errors during viral RNA replication. Similar drugs, it should be noted, have been found to be mutagenic and capable of inducing birth defects. For these reasons, any potential future rollout must proceed cautiously. As part of their study, the researchers used mice with lung tissue that had been genetically modified to express human proteins. A larger implication of this study is that bats harbor endogenous coronaviruses that can directly be transmitted to humans without any need for intermediate hosts. In such cases, infection readily occurred through type II pneumocytes present in alveoli or ciliated airway cells, and induced sustained type I interferon responses with subsequent inflammatory cytokine storm responses.

While the many new and promising therapeutic options for SARS-CoV-2 are too numerous to describe here, one area likely to benefit from further exploration is the effect of host variants on susceptibility. Many genes were already known to affect susceptibility and immune responses, and are themselves targets for treatments. Variants in the interferon receptor IFNAR2 and tyrosine kinase TYK2 have been identified as key controllers of COVID outcomes. Polymorphism in the transmembrane protein TMEM41B is present in East Asians at a rate of 20%, and has been shown to be protective for many types of viruses. As this protein is essential for SARS replication, its inhibition is being explored as the basis for a future therapy. In addition to this protein, one recent study found over 100 other potential drug targets that merited investigation.[12]

Other studies have shown that the furin cleavage site is traded off against heparan sulfate binding upon cell culture adaptation.[13] The origin of the furin cleavage site is at the center of the debate about the first emergence of the virus. The acquisition of a novel form of furin cleavage site in the virus is yet to be explained by any theory centered on a naturally evolving zoonotic virus. Adding furin sites is a patented process routinely done in several labs to confer new functions to viruses.[14]

One intriguing line of research has turned up several key variants derived from a Neanderthal heritage. Led by Svante Pääbo from the Max Planck Institute, researchers discovered a Neanderthal haplotype protective against severe COVID-19 on chromosome 12.[15] The haplotype contains

> parts or all of the three genes *OAS1*, *OAS2*, and *OAS3*, which encode oligoadenylate synthetases. These enzymes are induced by interferons and activated by double-stranded RNA. They produce short-chain polyadenylates, which, in turn, activate ribonuclease *L*, an enzyme that degrades intracellular double-stranded RNA and activates other antiviral mechanisms in cells infected by viruses.[16]

Pääbo's group previously found that another exclusively Neanderthal variant present in the promoter region of the DPP4 gene at chr2q24.2 also controls COVID susceptibility.[17] DPP4 is a widely expressed extracellular dipeptidyl peptidase involved in immune function and glucose metabolism. It is also known to be the receptor used by the MERS coronavirus.

News of a more subtle defense against the virus has recently been published on *medRxiv* by Peter Klein et al.[18] In contrast to the tyrosine kinase therapies, which tame overactive immune systems or block host pathways needed for viral maturation, the new therapy targets a serine/threonine host kinase that acts on viral proteins directly. This enzyme, glycogen synthase kinase 3 (GSK-3) is co-opted by the virus to phosphorylate specific points on its nucleocapsid (N) protein. Without this modification, viral transcription and replication are impaired.

Klein had previously demonstrated that the SARS-CoV-2 N protein contained GSK-3 consensus sites, known as an arginine–serine (RS) domain, that were identical to those found in the SARS-CoV-1 N protein.[19] Lithium chloride was already known to block GSK-3 phosphorylation of the SARS-CoV-1 N protein, which suggested it might have some efficacy for treating SARS-CoV-2. Since many people already take lithium for a variety of reasons, such as bipolar disorder, schizophrenia, and major depression. Klein's group was able to look at existing data for patients with COVID and infer that lithium protected them.

There are several other already approved small-molecule inhibitors for GSK-3 in need of further research to determine an optimal clinical approach. This kind of drug discovery usually begins by determining how compounds behave when introduced in vitro to different cell lines that have been engineered to express the target. One compound known as CHIR99021 is highly selective for GSK-3 and was found to block effectively viral replication in the human lung cell line Calu-3. Similarly, the drug enzastaurin has the benefit of being clinically well-tolerated and was found to inhibit infection in A549-ACE2 cells and to inhibit the viral-mediated cytopathic effect in Vero E6 cells. While these findings are encouraging, the results are usually cell-line specific and often depend on which lab is doing the study.

The experimental treatment molnupiravir, currently being developed by Merck, has attracted a lot of attention after a new study found that the drug not only halved the chances of a patient dying from COVID, but also halved the chances of hospitalization. By contrast, currently approved antivirals like remdesivir, frequently given alongside steroids like dexamethasone, have been assigned only for post-hospitalization care. If mutagenicity fears for pregnant women can be allayed, the availability of a preventative oral medication—remdesivir must be injected—for widespread distribution would likely have an enormous impact. To that end, Merck has been ramping up production for a projected 10 million courses of molnupiravir, at $700 per course.[20]

Determination of the optimal treatment regimen, that is, the dose, frequency, and duration for the course, depends on many factors, including the time to maximal plasma concentration and the half-life for elimination. With a rapid uptake and approximately seven-hour half-life, molnupiravir can safely be given twice daily across the treatment window with no apparent accumulation in the body.

For the dosage level under consideration, molnupiravir seems to be well behaved in its exclusive specificity for RNA machinery, and has not exhibited any tendency for DNA substitutions that could lead to birth defects. As with any nucleoside analog that operates via the introduction of errors into the viral code, it is important to be mindful of the potential for subthreshold effects. Even if the high mutation rates that the drug induces are avoided in specific outposts of the virus, there is yet the possibility that some evolution of the virus could occur as a direct result of therapeutic intervention.

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