SARS-CoV-2

Beta testing the monkey model

Corbett et al. use the rhesus macaque model to evaluate the ability of the mRNA-1273 (Moderna) COVID-19 vaccine to protect against challenge with the antibody-evading Beta variant of SARS-CoV-2. Their key finding is that the vaccine prevents severe lung pathology, principally because it is able to induce a strong enough antibody resistance to overcome the variant's relative resistance.

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Non-human primate (NHP) virus-challenge models have proven very valuable for assessing the protective efficacy of COVID-19 vaccines during the past year^{1,2}. Although all animal models have limitations for extrapolation to our species, rhesus macaques have been especially useful for assessing immunity and protection against SARS-CoV-2. As these animals share immune system properties with humans, how they respond to the COVID-19 vaccines is highly informative. The mild to moderate disease course seen in SARS-CoV-2-infected rhesus macaques seems to best mimic the majority of human infections, which are generally not very serious, rather than the most severe cases that can put people in the ICU and mortuary. From the vaccine-testing perspective, the outcomes seen in macaques have generally been consistent with those of human phase 3 trial results and real-world experience with the approved vaccines. Thus, in general terms, when they are tested in macaques, the vaccines we are most familiar with—from Pfizer, Moderna, Johnson & Johnson (Janssen) and AstraZeneca—provide strong protection against disease symptoms in the lower respiratory tract (LRT) while, usually, allowing some virus replication to occur in the upper respiratory tract (URT). In other words, complete protection from infection is not always seen in the macaque model, but there is almost invariably prevention of symptomatic disease^{1,2}. Broadly, those outcomes reflect how the same vaccines perform in humans. In the current issue of *Nature Immunology*, Corbett et al.³ describe how the US Food and Drug Administration authorized mRNA-1273 vaccine fares in rhesus macaques challenged with the antibody-evading Beta variant (also known as B.1.1351) of SARS-CoV-2.

Given that the world now has multiple authorized or approved vaccines, with more soon to follow, why do we still need to use NHP models for vaccine testing? One reason is to evaluate next-generation concepts,

including through preliminary assessments of the doses and schedules that might work best in humans. An asset is the ability to sample blood and respiratory mucosal tissues to better understand how vaccines protect at the site of infection, which is not usually possible in humans. Another role for NHP experiments lies in answering the question that is the principal focus of the paper by Corbett et al.³: Can the authorized vaccines protect against SARS-CoV-2 variants that have evolved this year to dominate the pandemic in different regions of the world? These variants each have at least one property that distinguishes them from the strain that first emerged in China in late 2019 and that dominated 2020's first wave of infection: greater transmissibility, increased virulence, or resistance to neutralizing antibodies (NAbs)⁴. Globally, the Delta variant is now a major concern—it is clearly the most transmissible variant yet seen, it is quite possibly more lethal, and it also has a degree of NAb resistance that may be problematic. However, the dubious accolade for the most NAb-resistant variant seen so far goes to Beta, which surfaced in South Africa in late 2020 and caused the Janssen and, even more, AstraZeneca vaccines to fare poorly in phase 3 trials there⁵. It is prudent to assess whether Beta might best other vaccines, and the macaque model comes into play here.

Corbett et al. show that the Moderna vaccine mRNA-1273, which was co-developed with the Vaccine Research Center (VRC) of the US National Institutes of Health, can protect rhesus macaques from the lung pathologies caused by experimental infection with the Beta variant³. The focus of this study was to assess whether the dose and number of immunizations influenced immunity and protection, which are important parameters for vaccine use in humans. A key finding was that two doses of the vaccine, given in weight-adjusted amounts that are consistent with the current FDA-authorized protocol, protected against virus replication in the

LRT, as well as reducing the amount of virus detected in the URT³.

A group testing the Janssen adenovirus vector vaccine Ad26.COV2.S against Beta infection of macaques recently reported broadly similar findings⁶. In both studies, disease symptoms were strongly curtailed, which was attributable to greatly reduced viral loads in the LRT and, to a lesser extent, in the URT^{3,6}. It is notoriously difficult to cross-compare studies performed by different research groups on different vaccines, as too many variables are involved. For example, the two studies did not use the same stock of the Beta variant, and the approximately two-fold lower challenge dose used by the Janssen group might be easier to protect against than the greater quantity of virus inoculated into the animals by the VRC-based researchers^{3,6}. Another difference is that the Ad26.COV2.S vaccine was given at the same dose humans receive, while mRNA-1273 worked in macaques at a weight-adjusted dose lower than that used in humans; dose adjustment makes sense when extrapolating between species. Notwithstanding these differences in how the two vaccines were administered in the two studies, mRNA-1273 is clearly superior at inducing NAb responses in macaques, which is again consistent with recent data from humans⁷.

It is encouraging that vaccination protects against the Beta variant, given that approximately ten fold higher NAb titers are needed to neutralize it in cell culture systems as compared to the earlier Wuhan and D614G viruses. Neutralization assays of different designs are also tricky to cross-compare^{1,2}. However, to the extent that this is possible, the comparison reveals that Beta seems to be more resistant to NAbs induced in macaques by Ad26.COV2.S than mRNA-1273^{3,6}. As NAbs have been found to be the principal correlate of protection in multiple animal and human studies, including the present report by Corbett et al., the implication is that the authorized vaccines induce titers high enough to cope

Fig. 1 | SARS-CoV-2 infection and vaccine protection in the URT and LRT. SARS-CoV-2 virions enter and replicate in the nose and URT of unvaccinated (left) and vaccinated (right) humans (and behave similarly in NHPs). In vaccinated people, and perhaps also in convalescent individuals (not shown), the virus particles encounter low levels of neutralizing antibodies (NAbs) that reduce virus spread into the LRT. Innate responses, such as interferons, may also contribute to viremic suppression in the URT (not shown). Some virus may still enter the lungs of vaccinated people, but at much lower levels than in the unvaccinated. In the LRT, much higher concentrations of vaccine-induced antibodies strongly suppress virus replication and prevent serious disease. When these antibodies are not present, the lungs become inflamed (red shading), leading to severe COVID-19 and sometimes death. During infection by the Beta variant, and to a lesser extent Delta, the antibodies present in the URT and LRT are less effective at suppressing virus replication, but still effective enough to protect against severe disease and death (not shown). In addition, the Delta variant has properties that enable it to replicate to far higher levels in the URT.

with some loss in NAb activity^{3,6,8–10}. A real-world study showing that the mRNA-1273 vaccine fares well in the face of the Beta variant is consistent with the macaque data¹¹. Similarly, Ad26.COV2.S retained some of its protective capabilities when tested in South Africa while Beta was on the prowl⁵. It is clear, however, that the two-dose mRNA-1273 regimen is superior to one dose of Ad26.COV2.S in protecting humans against mild or moderate COVID-19. The macaque studies do not change that perspective.

Despite the paramount importance of NAbs to vaccine-mediated protection, the possibility remains that other components of the innate and acquired immune systems also contribute, particularly in the URT. Although cellular immune responses are often invoked as protective, experimental ablation of CD8⁺ T cells in macaques did not affect the rate of virus

clearance¹². That intriguing observation warrants confirmation. How vaccines protect is important in the context of the now-burgeoning global pandemic driven by the Delta variant. As noted, Delta is the most transmissible virus we have yet encountered. The only silver lining is its ability to outcompete and squelch variants such as Beta that are thereby becoming yesterday's concern. What do we know about how vaccines perform against Delta? Although it is too early to state that definitively, there are signs of what may soon emerge.

There are now indications that Delta can break through vaccine protection at a higher rate than was seen with earlier variants, but only when the metric is asymptomatic or mild disease; very few infected vaccinated people are ending up severely ill or dying^{13,14}. The Chinese Center for Disease Control (CCDC) reports that approximately

1,000-fold higher viral loads are present on nasal swabs from people infected with Delta as compared to earlier variants¹⁵. CDC data and a preprint from Singapore both show that nasal viral loads are high and initially comparable in infected vaccinated and unvaccinated people^{13,14}. However, the Singapore study shows that viremia drops much more quickly in the vaccinated, which is why they rarely become seriously ill¹⁴. The vaccines, then, sometimes don't prevent infection in the URT but do seem to prevent much of the spill-over into the LRT where so much damage would otherwise occur (Fig. 1). The unvaccinated, of course, have no such protection. Unchecked Delta infections killed what may have been millions of people in India and, sadly, will do the same in the rest of the world.

If the nasal swab viremia data mean that infected people exhale or sneeze out far greater amounts of Delta virus particles,

as seems likely, then we can understand why this variant spreads so efficiently. There is a clear possibility that, when a large payload of Delta virus is inhaled, it could evade or overwhelm local immunity in the URT, leading to breakthrough infections in vaccinated people, albeit of limited severity, that may be transmissible to other people. Antibodies to the spike protein are found in the URTs of mRNA vaccines, as judged by saliva assays, but in amounts that are quite low and perhaps inadequate to defeat Delta¹⁶. Systemic NABs, however, keep the infections largely restricted to the URT in vaccinated people¹⁴.

Ongoing assessments of how authorized vaccines perform against Delta in NHPs will be informative, adding to knowledge now accruing from monitoring individual humans and study cohorts. Although Beta is fading from the scene,

future variants could combine its greater NAb-resistance properties with Delta's increased transmissibility. The more we know about how these viruses intersect with the human immune system, the more prepared we will be if that happens. Given the speed at which new strains are now appearing, that new variant may be the Nu variant.

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Competing interests

The authors declare no competing interests

IMMUNOMETABOLISM

The IRX(3)some factor in macrophages

Qiu and colleagues identify IRX3 as a driver of macrophage inflammatory cytokines, which can promote metabolic dysfunction.

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Obesity is a major global healthcare concern, with over 600 million adults worldwide currently classified as living with the condition¹. Obesity has been linked to numerous comorbid conditions ranging from type 2 diabetes mellitus and cardiovascular disease to a high percentage of cancers². A core link between obesity and these comorbidities is chronic inflammation³, and thus the quest to understand the initiators and drivers of this pathogenic process in people with obesity is critically important and may lead to future therapeutics. In this issue of *Nature Immunology*, Qiu and colleagues identify the transcription factor IRX3 as a key driver of pro-inflammatory cytokine production in macrophages, which in mouse models is associated with repression of lipolysis and thermogenesis, leading to increased bodyweight and poor metabolic health⁴.

In 1993, seminal studies by Hotamisligil were the first to link inflammation to the development of obesity-related insulin

resistance⁵. Ten years later, Weisberg and colleagues put the macrophage at the front and center of obesity-driven metabolic inflammation⁶. It is now well established that the macrophage is one of the dominant drivers of metabolic inflammation in obesity.

In 2007, a genome-wide search for metabolic disease susceptibility genes led to the identification of the *FTO* (fat mass and obesity associated) gene. Common variants in *FTO* were associated with increased bodyweight, although no direct connection between *FTO* and metabolism was found⁷. In subsequent studies by Smemo and colleagues in 2014, noncoding variants of *FTO* were linked to *IRX3* expression, and a direct link to bodyweight was identified, with *IRX3*-deficient animals displaying reduced adipose tissue mass owing to increased thermogenesis⁸. However, some inconsistencies remain with respect to the *FTO*–*IRX3* circuitry, as other researchers have reported that activation of *IRX3* results in increased thermogenesis^{9,10}.

Qiu and colleagues have now shed more light on the precise molecular and cellular mechanisms by which *IRX3* controls bodyweight and metabolic health⁴. Using myeloid-specific deletion of *Irx3*, their work reveals a macrophage-intrinsic role for *IRX3* as a transcriptional regulator of pro-inflammatory cytokines that repress lipolysis and thermogenesis in adipose tissue, resulting in decreased metabolic rate that manifests in obesity and poor metabolic health (Fig. 1).

Qiu and colleagues also provide important insight into the molecular mechanism by which pro-inflammatory stimuli, such as lipopolysaccharide (LPS), can employ *IRX3* to induce expression of pro-inflammatory cytokines. LPS triggers the Toll-like receptor 4 (TLR4) pathway to positively target *IRX3* by two pathways⁴. First, stimulation of macrophages with LPS inhibits the ubiquitination of *IRX3* at a specific lysine residue, resulting in the stabilization of *IRX3* and avoidance of proteasome-mediated degradation.