depletion of a small but specific subset of DETCs that express genes encoding the key activation molecules XCL1 and 4-1BB. In addition, treatment with antibodies to Skint1 broadly affected genes encoding molecules associated with thymic selection in all DETCs. Thus, the TCR–Skint1 crosstalk has a profound effect on both DETC homeostasis and epithelial barrier function. Notably, although the number of DETCs was unaltered, their activation status was dynamically modulated by access to TCR–Skint1 signaling. Although the pool of DETCs is known to be controlled by common γ-chain cytokines provided by keratinocytes[7], precisely how constitutive TCR signaling after engagement of Skint1 'tunes' DETC activation status awaits further investigation.

McKenzie et al. then investigated the contribution of this interaction to stress responses in the skin[4]. The authors confirmed that after ultraviolet (UV) irradiation, DETCs adopted a rounded morphology and had fewer TCR foci, as previously noted after activation of DETCs[8]. Accordingly, epidermal *Skint1* mRNA and *Skint2* mRNA were downregulated. Wild-type mice upregulated transcripts encoding the granzymes GzmB and GzmF after UV irradiation. These functional molecules were lost in Tac mice, but they were restored after overexpression of Skint1, indicative of a role for Skint1 in enabling granzyme expression in DETCs and in priming DETCs against tissue perturbation. Ablation of Skint1 before UV irradiation diminished TCR signaling (as measured by a Nur77 reporter) and the expression of *Gzmb* and *Gzmf*.

To define the mechanism(s) by which Skint1 enables TCR signaling amplification, McKenzie et al. studied the costimulatory molecules modulated by Skint1 agonism[4]. 4-1BB and GITR, part of the TNFR superfamily, were upregulated by Skint1 in both thymic DETCs and peripheral DETCs. Accordingly, blocking 4-1BB or GITR diminished DETC activation and granzyme expression after UV irradiation, which identifies these costimulatory molecules as key purveyors of Skint1-mediated DETC priming. To determine the consequence of hampered activation of DETCs downstream of peripheral Skint1 depletion on UV-induced mutagenesis, McKenzie et al. assessed the DNA-damage response[4]. Consistent with previous reports of enhanced mutagenesis after UV irradiation in γδ T cell–deficient mice[8], antagonism of Skint1 increased cyclobutane pyrimidine dimers and γH2A.X foci in UV-treated mice. Epithelial pathology, ear swelling and perturbed barrier function were more severe in UV-treated mice depleted of Skint1 than in their Skint1-sufficient control counterparts. The activation of DETCs by intradermal injection of ATP and in a model of contact dermatitis was also dependent on Skint1, which emphasizes the broad function of Skint1-mediated DETC licensing and responsiveness.

The TCR-mediated sensing of normality proposed by McKenzie et al[4]. highlights a fundamental regulatory process that allows rapid immune responses at barrier sites. These data showcase the importance of steady-state monitoring of tissue integrity by resident lymphocytes and spark many questions, including how DETC–Skint1 interactions are reinstated after stress resolution, and whether other epidermal resident lymphocytes (innate lymphoid cells and resident memory CD8$^+$ T cells) participate in the sensing of normality. Additionally, mechanistic understanding of how the activation of DETCs is primed by Skint1, and how DETCs promote the expression of epithelial Skint1, could lead to better understanding of whether similar mechanisms are at play in human γδ T cells and IELs. Further work may shed light on how these processes are perturbed in other disease states, given that γδ T cells are linked to inflammatory diseases such as psoriasis. Collectively, the findings presented by McKenzie et al[4]. provide new insights into how active lymphocyte-mediated immunosurveillance and epithelial crosstalk at homeostasis 'tune' the magnitude of responses to distress. ❐

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Competing interests

The authors declare no competing interests.

Check for updates

SARS-COV-2 VACCINATION

A potential silver lining of delaying the second dose

A delayed second dose relative to the standard 3-week schedule for the BNT162b2 mRNA vaccine against SARS-CoV-2 significantly raises the levels of neutralizing antibodies against SARS-CoV-2 variants.

David R. Martinez and Eng Eong Ooi

Because of the rapid spread of SARS-CoV-2, deploying effective vaccination strategies requires not only the elicitation of protective immunity but also ensuring maximum coverage of this protective immunity in naive populations. As a result of SARS-CoV-2 vaccine shortages early in the COVID-19 pandemic, coupled with a need to deploy the first vaccine dose in naive populations, the intervals between prime and boost were not always uniform in different countries and/or regions. This

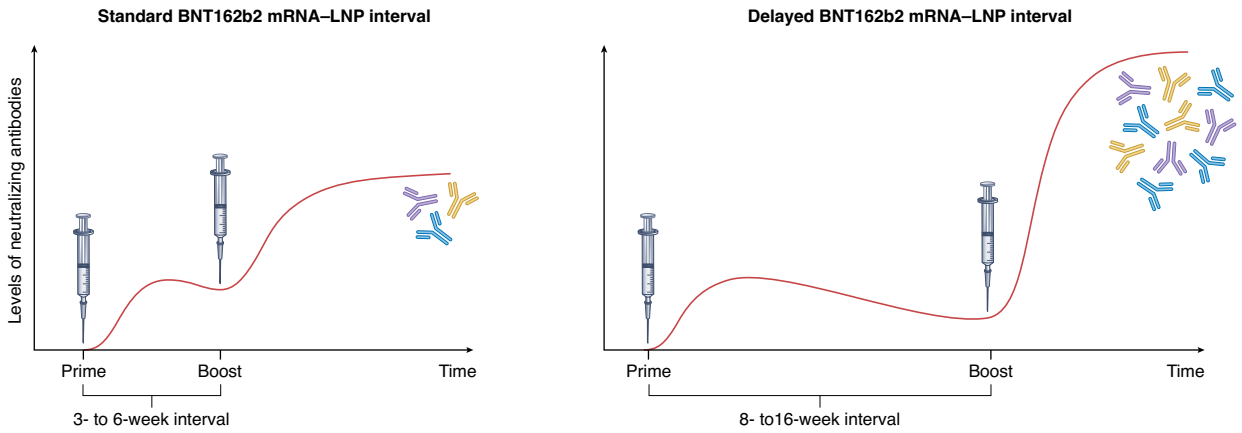

Fig. 1 | Neutralizing antibody responses in standard versus delayed vaccine dose interval in health care workers. A delayed 8- to 16-week vaccine dose interval between prime and boost leads to higher levels of neutralizing antibodies in vaccinated people than does a standard 3- to 6-week vaccine dose interval, in healthcare workers who are predominantly female.

provides a unique opportunity to evaluate if varying the time interval between the prime and boost leads to differential immune responses in naive people versus SARS-CoV-2-convalescent people. Although the current dose interval of most SARS-CoV-2 vaccines in clinical use range from 3 weeks to 4 weeks between prime and boost, extending this interval to 6–14 weeks can lead to higher levels of neutralizing antibodies and higher levels of $CD4^+$ T cells that secrete the cytokine IL-2 in naive people from the UK[1]. In the current issue of *Nature Immunology*, Hall et al. assess an extended (8- to 16-week) BNT162b2 dose interval versus a standard (3- to 6-week) dose interval in healthcare workers from Canada[2]. Relative to antibody responses after the standard interval, the delayed interval offered superior neutralizing antibody responses to SARS-CoV-2, including the Alpha, Beta and highly transmissible Delta variants (Fig. 1). However, $CD4^+$ and $CD8^+$ T cell responses to SARS-CoV-2 showed dampened trends, although most of the differences were not statistically significant, after the delayed interval, relative to such responses after the standard vaccine interval. The differences in antibody and T cell responses in the delayed versus the standard dosing interval may, however, merely reflect the differences in B cell and T cell response kinetics to BNT162b2 vaccination[3]. Another interesting observation is that the delayed interval did not lead to higher frequencies of adverse events than did the standard interval. The implications of the T cell responses after the extended interval dose in the context of long-term protection are also unknown. A key question from this study that remains is whether the adaptive immune responses elicited by the delayed dose interval will translate into increased real-world vaccine efficacy against SARS-CoV-2 variants such as Omicron or even more diverse future variants. Moreover, as this study evaluated differences in neutralizing antibody responses and T cell responses in mainly female populations, it will be critical to also evaluate if similar immune responses are observed in an extended prime and boost interval in male populations.

The development of SARS-CoV-2 vaccines, including clinical safety and efficacy testing to rapid rollout, is one of the greatest scientific, medical and public-health achievements in recent history. Several SARS-CoV-2 vaccines are safe and effective, and the mRNA–lipid nanoparticle (LNP) vaccines are arguably the most effective at protecting against severe COVID-19 and death[4,5]. Despite the high efficacy of the SARS-CoV-2 mRNA vaccines in preventing severe disease, the emergence of SARS-CoV-2 variants of concern can dampen vaccine efficacy[6]. In addition, the durability of immune responses that can protect against severe COVID-19 is uncertain.

As a result of the vaccine supply shortage during the early rollout of the SARS-CoV-2 vaccines, there was vigorous debate on the benefits versus risks of extending the interval between the first vaccine dose and second vaccine dose[7]. The rationale for the benefits included vaccinating as many people as possible with a single vaccine dose to provide partial immunity and potentially avoid severe COVID-19. This idea gained early traction because the vaccine efficacy was upward of 80–90% after a single dose and before the second dose, and even if the interval between the two doses was extended[7]. Thus, it was theorized that a single dose should at least reduce the incidence of severe disease in the vaccinated population. The opposing argument posited that the low levels of neutralizing antibodies from a single dose of mRNA–LNP vaccine could theoretically drive the emergence of variants that were partially or fully resistant to vaccine-elicited antibodies. However, a point that was less frequently discussed was the potential impact that delaying the second dose might have on vaccine-elicited immune responses. Notably, Hall et al. demonstrated a potential silver lining of delaying the second vaccine dose: the generation of higher levels of binding and neutralizing antibodies[2]. Tenfold higher levels of binding antibody responses to the receptor-binding domain of the SARS-CoV-2 spike protein were observed after the delayed dose interval. In addition, higher levels of neutralizing antibody levels against the ancestral SARS-CoV-2 and the Alpha, Beta and highly transmissible Delta variants were observed. As neutralizing antibody levels elicited by mRNA–LNP vaccines are probably a correlate of protection against COVID-19[8,9], strategies aimed at increasing neutralizing antibodies are of critical importance.

Although the finding of higher levels of immunity associated with protection from severe disease is certainly welcome news, it is important to also consider the nuances of this approach, especially in the setting of highly divergent variants such as Omicron or more genetically diverse variants that could emerge in the future. The ambiguity about the T cell responses elicited after delayed dosing, due to both limited sampling and the type of assay used, needs to be clarified with more-detailed studies, including granularity in the different T cell subsets. This issue is important, as it

raises questions about protection from severe disease once neutralizing antibodies have waned to levels below those needed to block infection. In fact, studies have demonstrated that in the context of lower levels of neutralizing antibody responses, $CD8^+$ T cells are critical for more rapid control of SARS-CoV-2 in animal models[10]. Although delaying the second dose results in clearly higher levels of neutralizing antibodies, the possibility of lowered T cell responses in this regimen should also be considered. Other key questions remain. What is the durability of these higher neutralizing antibody responses after the delayed dose? Does delaying the second dose also lead to a slower decrease in the levels of neutralizing antibody responses? Will these higher levels of neutralizing antibodies more rapidly curb the upper-respiratory airway transmission of divergent variants such as Omicron? It will also be important to evaluate the immunological basis for the improved humoral responses with delayed second dose and whether this effect would also apply to other mRNA vaccine strategies[11–13] and protein-based constructs[14]. As previous studies have determined that germinal-center responses persist for several (7–15) weeks after vaccination in humans[15], it will also be important to determine if delaying vaccine doses potentially leads to longer-lasting germinal-center responses and, as a result, more-mature and more-potent neutralizing antibodies at the monoclonal antibody level. This knowledge could have important implications for future pandemic vaccines. Ultimately, the fine tuning of vaccines for optimal immunity against SARS-CoV-2 and its variants will probably form part of the solution to one day end the COVID-19 pandemic. ❐

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Competing interests

The authors declare no competing interests.

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VIRAL INFECTION

SARS-CoV-2 learned the 'Alpha'bet of immune evasion

Comparative analysis of SARS-CoV-2 isolates uncovers important mutations outside the spike gene that help the Alpha variant to operate under the radar of innate immune surveillance.

GuanQun Liu and Michaela U. Gack

The continuous emergence of SARS-CoV-2 variants of concern (VOCs), from Alpha to Omicron, underscores the extraordinary capability of the virus to adapt to the human immune system. Extensive research has elucidated how changes in the viral spike protein, which mediates entry into cells, promote human-to-human spread and viral escape from antibody responses. By contrast, the role of mutations outside the spike protein in virus pathogenesis remains scarcely explored. In *Nature*, Krogan and colleagues[1] crack the code of non-spike mutations found in the Alpha variant of SARS-CoV-2 by showing that some of these mutations ramp up the expression of viral innate immune antagonists, allowing escape from intrinsic immune defenses.

Alpha (Pango lineage: B.1.1.7 and Q lineages), which was first identified in the UK and declared a VOC in December 2020, gained a substantial transmission advantage over earlier SARS-CoV-2 strains. While this superior performance is primarily due to specific spike mutations that enhance affinity to the viral entry receptor, angiotensin-converting enzyme 2 (ACE2), it has been unknown whether Alpha had learned new tricks once inside human cells. Intriguingly, Krogan and colleagues[1] find that Alpha replicates on the sly and stimulates antiviral responses much less efficiently than two first-wave isolates, indicating that Alpha is equipped with new mechanisms of immune evasion.

Type I and III interferons (IFNs) have key roles in virus restriction by upregulating a myriad of IFN-stimulated genes (ISGs) with antiviral or immunomodulatory properties. As a countermeasure, SARS-CoV-2 has evolved ways to suppress or dysregulate IFN responses, a phenomenon observed both in vitro and also in patients critically ill with COVID-19[2,3]. IFN induction is initiated by pattern-recognition receptors such as the retinoic acid–inducible gene I (RIG-I)-like receptors (RLRs), which detect RNA species of viral and host origins and are primary sensors of coronavirus infection[4]. Downstream of RLRs, TANK-binding kinase 1 (TBK1) and other kinases are activated, which then phosphorylate transcription factors (for example, IRF3 and IRF7) that drive the expression of IFNs and proinflammatory cytokines. Phosphoproteomics analysis revealed curtailed activities of these kinases early during infection with Alpha[1], consistent with low IFN and ISG induction. Conversely, activation of these kinases at a later time was higher in cells infected with Alpha than in cells infected with the earlier