Vaccines

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Time is of the essence for vaccine success

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Effective vaccines elicit neutralizing antibodies and long-lasting memory, but this can be challenging with some pathogens, such as HIV. A new study shows how a slow-delivery protein immunization strategy administered in dose-escalation format over 12 days increased the durability of germinal centers and improved immunological outcomes.

Ideally, vaccination will create antibodies of sufficient concentration, affinity, diversity and durability that they can neutralize the targeted pathogen and its variants for extended periods. However, eliciting such multifaceted responses through vaccination has been exceptionally challenging in several conditions, including HIV, even though antibodies with these characteristics naturally arise in some rare individuals[1]. Now, in a study published in *Nature*, Lee et al. reveal substantial progress toward these ends[2]. Building on previous work[3–5], they show that a particular formulation of antigen administered in a dose-escalation format over a short time frame markedly increased and sustained active germinal centers (GCs) that, remarkably, produced a memory B cell (MBC) population comprising a more broad breadth of target recognition compared to those obtained using previous approaches. That is, by mimicking aspects of natural immune responses with the vaccine, including epitope masking, feedforward stimuli and an extended duration of mutation, they were able to enhance the resultant immune response in quantity, persistence and breadth of recognition.

Vaccination is typically initiated by the delivery of a single, defined dose of antigen with an immune stimulant (adjuvant). This is followed by booster doses in the weeks, months and years thereafter to generate and maintain pathogen-specific antibodies at concentrations that negate the consequences of subsequent exposures. Although this approach has been highly successful in achieving sustained protection against many pathogens — think childhood vaccines — some diseases have escaped the best efforts at control. For example, there is no effective vaccine against HIV, and regular booster vaccinations are required against influenza virus and SARS-CoV-2. A commonality of these 'hard-to-target' pathogens is their ability to rapidly change their most immunogenic regions, thereby generating new viral strains that circumvent pre-existing immunity. However, cross-strain protective immunity against such pathogens could be achieved if the immune system were to successfully target epitopes (antigenic determinants) that the pathogen is biologically unable to change while still remaining viable. Although such epitopes exist, they are typically poorly immunogenic, meaning that vaccine-induced responses to these epitopes — if they occur at all — result in insufficient amounts of broadly neutralizing antibodies (BN-Abs)[1]. This is particularly apparent for HIV, for which extensive structural reconfiguration of antibodies is required to achieve the necessary affinity[1]. But BN-Abs to HIV do occur in nature, usually arising over a period of years in long-term non-progressing individuals, suggesting that a prolonged response may enable the immune system to overcome complex recognition issues[1]. This possibility is evidenced by a sustained inflammatory environment, long-term antigen availability and the extent of immunoglobulin V gene somatic hypermutation (SHM) in the B cells encoding such anti-HIV BN-Abs. Collectively, although specifically boosting BN-Abs is not yet achieved by vaccination, the characteristics of immune responses that might generate such antibodies have been described.

The same researchers and their colleagues have previously shown that prolonged delivery of an experimental HIV vaccine with dose escalation (referred to as long-prime) can improve vaccination outcomes[3,4]. Mechanistically this involves increasing numbers of T follicular helper cells and antigen retention[4], both crucial for GC response size and longevity[6]. They[5] and others[7] have also shown how adjuvants that specifically engage Toll-like receptors markedly affect the antibody response. Lee et al.[2] have now combined antigen, adjuvant and delivery schedule to induce long-lasting GC responses in nonhuman primates that are active for at least 6 months. The functional consequence was significantly increased diversity of epitopes being targeted by resultant MBCs and antibodies, with the latter allowing an impressive breadth of virus neutralization in infection assays using distant HIV isolates, the extent of which was related to the duration of the response. Importantly, the data suggest that conditions early in the response (the first 2 weeks) influence the long-term response in size and the diversity and quality of memory output (Fig. 1). This is good news, as it may provide a tractable way to direct immune responses using bespoke vaccine regimens. A key challenge in translating these results into human applications will be to find ways to deliver a vaccine slowly, with an increasing dose and over several days while circumventing the common aversion to repeated injections.

Persistence of antibody production is a crucial metric of vaccine efficacy, so it is important to consider whether prolonged GC are harbingers of that outcome. Sustained GC responses have been observed following SARS-CoV-2 mRNA vaccinations[8], but the protection provided by these novel vaccines is of modest duration, notwithstanding their operational success. Both in the report of Lee et al. and following mRNA vaccination[8], overall titers of antigen-specific antibodies reached a plateau at around 4 weeks post immunization despite ongoing GC. However, unlike for primary mRNA vaccination, Lee et al. showed a steady maintenance of the antibody titer over at least 6 months, suggesting potential superiority of their approach in producing long-lived plasma cells. More experimentation will be required to determine the extent to which the protocols under examination confer increased protective immunity against infection in vivo, and for how long and with what degree of resistance against emerging viral variants, which are all crucial issues.

The detailed longitudinal analyses of the immune response by Lee at al.[2] provide clues for MBC generation and their contribution to ongoing GC responses (Fig. 1). As such, there is the possibility of MBC and plasma cell output throughout the extended response, reflected in the breadth of epitopes targeted and the high frequency of V-gene SHM found in both antibodies and MBCs. This does not

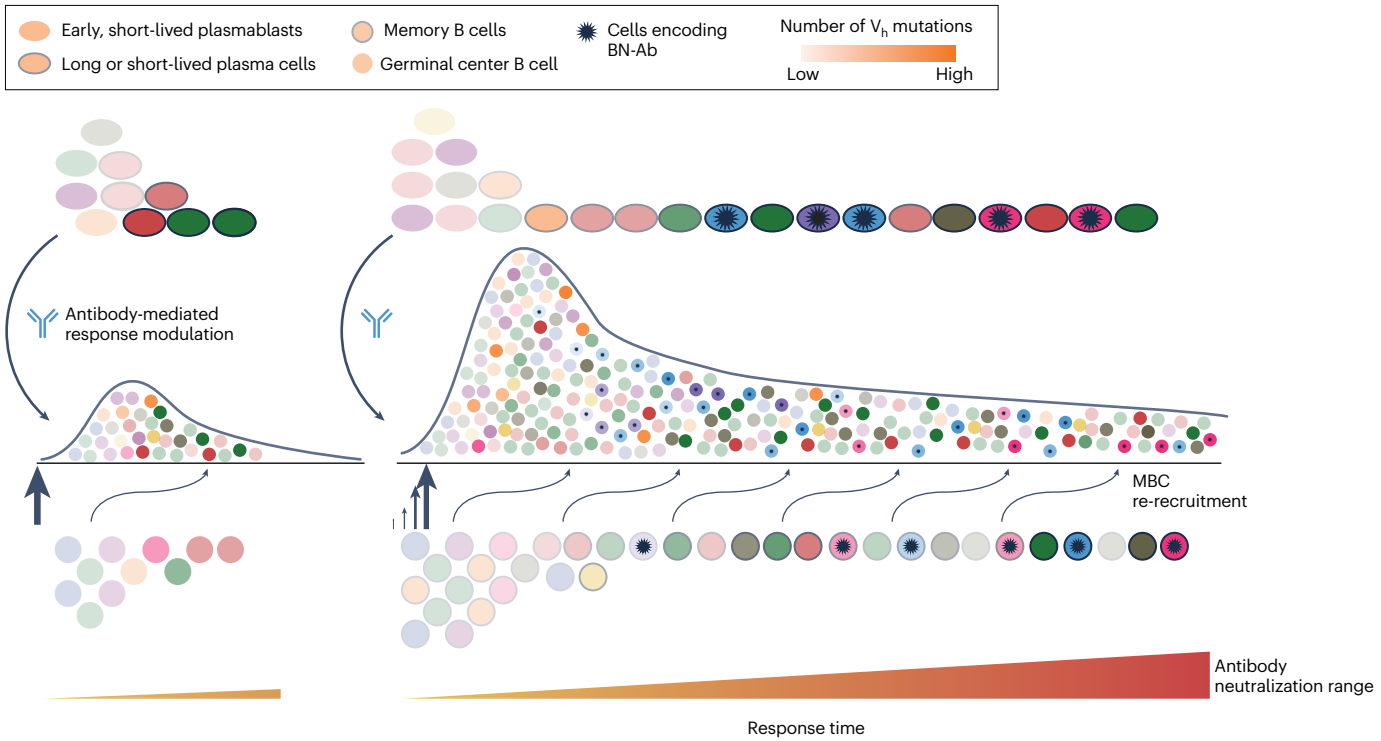

Fig. 1 | Prolonged antigen delivery increases antibody and MBC diversity through extended GC with MBC re-entry. Schematic depicting GC response magnitude (curves), output (cells at top and bottom) and their respective diversity (colors) and mutational load (color intensity) over time. Arrows indicate feedback and feedforward modulation of responses by antibodies and re-recruitment of memory B cells. In contrast to a single bolus immunization (left), long-prime results in an immune response with largely increased magnitude and diversity (right). A combination of increased T follicular helper cell priming and antigen retention — both likely mediated, at least to some extent, by PB-derived antibodies — extends the response time and allows the effective targeting of subdominant sites by GC B cells, leading to plasma cells and MBCs encoding BN-Ab (indicated by stars). V_h, antibody heavy chain variable domains.

necessarily contradict previous studies that suggest MBCs arise early in the response and are on average of lower affinity than plasma cells or antibodies[9,10] but instead highlights two interesting possibilities. One is that MBCs may reflect the continuation of affinity maturation, such that extended GC responses potentially allow highly mutated GC B cell clones to enter the MBC pool. A second is that with the detection of shared clones with high mutational load in lymph nodes at different sites and in the blood, the data of Lee et al.[2] are consistent with MBCs re-entering ongoing GCs for further affinity maturation and selection. In contrast to the potentially insignificant participation of MBCs reported during secondary GC responses[11], re-recruitment here, in particular within the same anatomical site[12], may commonly occur. That is, the observed rarity of MBCs with low mutational load could either be due to their gradual consumption through repeated re-entry or reflect a 'dilution' through continued MBC output. It will be important to investigate the details of how long-lasting immune responses affect MBC composition, as maintaining a high breath of specificities within the MBC compartment, including germline-encoded ones, is thought to provide a crucial 'second line of defense' against pathogens[13]. Equally, MBCs 'taking a pause' before rejoining ongoing GC might allow the GCs to maintain appropriate diversity.

Independent of the time after the long-prime, booster vaccinations significantly increased antibody titers. But unlike the steady antibody amount achieved by the primary immunization, this increase appeared temporary, consistent with a rapid, transient plasmablast response. In keeping with studies on the recruitment of MBCs into GCs in the presence of antigen-specific antibodies, the GC response itself was seemingly unaffected by booster vaccination, independent of the time or previous immunization regimen, as was the diversity of epitopes targeted. Again, whether the boost following long-prime actually resulted in increases in long-lived plasma cell output and antibody titers was not established by this study. However, the data suggest that boosting during an ongoing GC response results in a new wave of MBC output, including cells recognizing epitopes conserved between strains. The absence of an effect on the still-ongoing GC response also indicates that there is little detriment in 'too early' booster vaccination, although again, this will require confirmation. Another possible advantage of long-prime could be that by extending MBC and antibody reactivities to non-dominant epitopes, it might reduce the impact of 'original antigenic sin' following recall. This could be one step closer toward achieving the longstanding goals of adequately promoting antibody responses to epitopes mediating broad neutralization against HIV or influenza, with high enough titers for pan-strain protection.

In summary, the results presented by Lee et al.[2] strongly suggest that prolonging GC persistence in an immune response through a judicious combination of antigen, adjuvant and scheduling is a very promising strategy for improving vaccine efficacy and breadth. The details of the response, particularly the regulatory mechanisms that determine these outcomes, will be important to define, and we can

hope that these will provide further insights into the processes that, collectively, produce life-long protective immunity.

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Competing interests

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