Immune imprinting and next-generation coronavirus vaccines

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Abstract

Vaccines based on historical virus isolates provide limited protection from continuously evolving RNA viruses, such as influenza viruses or coronaviruses, which occasionally spill-over between animals and humans. Despite repeated booster immunisations, population-wide declines in neutralisation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants have occurred. This has been compared to seasonal influenza vaccinations in humans, where the breadth of immune responses induced by repeat exposures to antigenically distinct influenza viruses is confounded by pre-existing immunity – a mechanism known as imprinting. Since its emergence, SARS-CoV-2 has evolved in a population with partial immunity, acquired by infection, vaccination, or both. In this Review, we critically examine the evidence for and against immune imprinting in host humoral responses to SARS-CoV-2 and its implications for coronavirus disease 2019 (COVID-19) booster vaccine programmes.

Introduction

The decline in coronavirus disease 2019 (COVID-19) booster vaccine effectiveness against newly-evolved severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants is reminiscent of the ongoing problems with seasonal influenza vaccines. Every year, influenza vaccines are re-formulated, according to World Health Organisation (WHO) recommendations, to match the strains that might dominate circulation in the next flu season[1]. Most seasonally selected isolate-based influenza vaccines are either inactivated influenza vaccines (IIV) or live attenuated influenza vaccines (LAIV). Both types of vaccine are based on whole-virus preparations containing all structural antigens of the influenza virus. The most immunodominant of these influenza antigens are the surface glycoproteins haemagglutinin (HA) and neuraminidase (NA). HA mediates viral entry into host cells through interaction with specific cellular sialic acid receptors while NA is involved in the budding and release of progeny viruses through receptor cleavage[2]. In particular, anti-HA responses preferentially target the immunodominant, antigenically hypervariable globular head region of the protein and elicit highly strain-specific antibodies. These responses provide limited breadth of protection against antigenically diverse influenza strains[3], and therefore annual vaccine updates and booster immunisations are required (Table 1) (Figure 1). Typically, an 8–10-fold reduction in antisera titres in the gold-standard haemagglutination inhibition assay prompts an update of the seasonal influenza vaccine strain[4]. Despite frequent boosting of pre-existing immunity, acquired by the population over their lifetime of exposure to influenza, including with whole virus antigens, seasonal influenza vaccine effectiveness is limited, ranging from 10% to 60% over the past decade[5]. The multi-factorial nature of viral evolution hinders accurate vaccine strain prediction even with advanced bioinformatic tools[4,6]. However, even when vaccine and circulating strains are perfectly matched, suboptimal effectiveness occurs that is correlated to season, age group and vaccination history[5]. Two explanations for the lack of vaccine effectiveness have been proposed: host immune imprinting from lifelong exposures[7–9], or viral immune evasion through mutations in specific epitopes[10].

Unlike circulating seasonal influenza strains, which have prolonged epidemiological history in humans, such as influenza A H1N1 and H3N2 viruses acquired during pandemic spill-overs from animals in 1918 and 1968, respectively, and human-exclusive influenza B viruses first isolated in the 1940s[3], SARS-CoV-2 only recently emerged to infect humans in 2019. The first licensed COVID-19 vaccines were designed to induce host immune responses to a single viral surface spike (S) protein antigen, which binds human angiotensin-converting enzyme 2 (hACE-2) and triggers viral entry to host cells[11] (Table 1). Analogous to influenza HA head dominance, preferential antibody targeting of the receptor binding domain (RBD) of the SARS-CoV-2 S protein has been observed[12]. After introduction into humans, SARS-CoV-2 has evolved at an unprecedented rate exceeding the baseline mutation rate representative of the relatively high fidelity of its viral polymerase, resulting in the continuous emergence of different variants of concern (VOCs)[4,13]. Within the Omicron VOC, numerous sub-lineages continue to diverge, including BA.1, BA.2, BA.4 and BA.5. At present, highly immune evasive BA.2.75, BQ.1, XBB.1.5, EG.5 and BA.2.86 subvariants with competitive advantages persist worldwide[14–16]. The drastic mutational changes between Delta and early Omicron sub-lineages are comparable

to antigenic shift events in influenza A viruses[17]. Indeed, recombinational hotspots in the large RNA genomes of coronaviruses have been identified for SARS-CoV-2; as exemplified by XBB subvariants arising from co-infections of different Omicron variants, the alarming potential of SARS-CoV-2 to recombine with other coronaviruses has raised attention[18]. COVID-19 vaccines are estimated to have prevented more than 20 million deaths, but booster vaccines have not effectively blocked virus transmission. Continued circulation of SARS-CoV-2 has driven the selection of variants with improved ability to escape pre-existing vaccine-induced responses[10]. By early 2021, the reduction in neutralising antibody titres in COVID-19 mRNA-vaccinee antisera tested against the Beta VOC had already exceeded 10-fold, surpassing the threshold that is used to update seasonal influenza vaccine strains[4]. The bivalent booster vaccines comprise additional Omicron lineage S antigens, but individuals boosted with these vaccines do not induce optimal neutralising antibodies to the more recent variants[16,19–21]. As identified in influenza, immune imprinting is proposed to have a role in the failure to induce broader neutralising antibodies by boosting with heterologous S antigens[22–25].

Immune imprinting refers to the impact of viral infection, or vaccination, on the future patterns of antibody responses when re-exposed to distinct variants of the original antigen (Figure 2). The "original antigenic sin (OAS) model" suggests that an individual's early exposures to a virus limits the breadth and potency of immune responses against drifted variants[26]. Evidence has accumulated that supports both the positive and negative impacts of the first and subsequent infecting and vaccinating strains of a virus on successive antibody responses. The concept was refined to introduce a temporal, hierarchical ranking of the strains an individual is exposed to, known as the "antigenic seniority model"[7]. According to this model, earlier strains adopt a position of higher seniority, which in turn correlates with a stronger induced antibody response against these strains compared with the antibody response to subsequent exposures to other strains (Figure 3). Reported data support a role for immune imprinting in host responses to SARS-CoV-2 exposure. However, the impacts of repeated exposure to related viruses on infection outcomes or vaccine effectiveness are variable[21–25,27]. Since the emergence of SARS-CoV-2, evidence has been published that reveals comparable antigenic cross-reactivity between pre-existing antibody responses against the common cold coronaviruses (CCCoVs), severe acute respiratory syndrome virus (SARS-CoV-1) and SARS-CoV-2 in population-wide studies[27,28]. Understanding the impact of prior influenza exposure on host immune responses to subsequent influenza infections and vaccinations has been important in guiding public health measures against seasonal and pandemic influenza outbreaks. These include predicting viral evolution and seasonal vaccine strain selection, estimating seasonal severity and public health burden and ongoing efforts to design next-generation seasonal or universal influenza vaccines.

Here, we review the pre-clinical and clinical evidence for and against immune imprinting in SARS-CoV-2 and discuss implications for the development of improved booster and broadly protective vaccine strategies against influenza viruses and coronaviruses.

Prior exposure and imprinted immunity

Variability in antibody titres

Immune imprinting has been most studied for HA-specific antibody responses during sequential influenza infections and vaccinations, with recent evidence including responses to NA[29]. Imprinting has also been hypothesized to contribute to clinical outcomes of repeated Dengue fever virus[30], respiratory syncytial virus[31], cytomegalovirus[32] and coronavirus exposures[23–25]. In 1960, a pivotal study by Francis *et al.* described OAS where anti-HA antibodies generated in response to an individual's first exposure to influenza in early childhood dominated in later life[26]. Cross-sectional studies on antibody responses to different seasonal H3N2 strains that circulated between 1968-2008 in southern China exhibited an analogous and more refined hierarchical trend, where earlier exposure to a specific strain correlated with more rounds of boosting and higher titres, providing support for the antigenic seniority model[33].

The landscape of immune imprinting is complex for SARS-CoV-2. Interactions between pre-existing antibodies against CCCoVs, SARS-CoV-1, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and different VOCs convene in an immune response induced by SARS-CoV-2 infection or vaccination. In the early phase of the pandemic, population-wide pre-existing immunity to CCCoV was reported to affect the polyclonal antibody responses to SARS-CoV-2 infection and vaccination[27]. Systematic epitope profiling of COVID-19 patient sera revealed cross-reactivity to all the aforementioned coronaviruses, as well as three closely related bat coronaviruses[34]. Cross-sectional seroprevalence analysis of children and adults in France identified a positive correlation between antibody binding activity to SARS-CoV-2 and CCCoV S proteins in SARS-CoV-2 seronegative individuals[35]. Of note, anti-hCoV-OC43 responses in SARS-CoV-2 convalescent sera were markedly elevated in comparison to pre-COVID-19 controls, which could attribute to re-activation of imprinted immunity[35–39]. In contrast to the widespread prevalence of imprinted anti-CCCoV immunity, evidence of imprinted SARS-CoV-1 and MERS-CoV immunity following SARS-CoV-2 exposure is limited by the scarce epidemiological histories of these viruses in the general population. In a study investigating the nature of cross-reactivity between SARS-CoV-1 and SARS-CoV-2, cross-reactive binding antibodies against RBD and non-RBD regions of the S proteins were commonly detected, but neutralising antibody responses were rare and weak[40]. This is consistent with a study on the CR3022 epitope that is known to be neutralising for SARS-CoV-1, which, despite induction of strong binding antibody responses to SARS-CoV-2 S, failed to elicit neutralisation[41]. Another study reported that SARS-CoV-1 survivors vaccinated with 2 doses of BNT162b2 exhibited uniform boosting in anti-SARS-CoV-1 neutralisation without compromising anti-SARS-CoV-2 immunity. Sera from this group also elicited near-saturation, cross-clade, pan-sarbecovirus neutralisation against SARS-CoV-2 Wuhan-Hu-1 and VOCs up to Delta, two animal viruses from the SARS-CoV-2 clade and three viruses from the SARS-CoV-1 clade. Selective expansion of cross-reactive B cell responses in this cohort was confirmed via double-staining and monoclonal antibody competition analysis[42].

As SARS-CoV-2 rapidly evolves under selective pressure in the ongoing pandemic, successive VOCs with evolving fitness advantages continue to emerge. Through repeated waves of breakthrough infections, an increasingly complex immune history has developed in the global human population. A South African cohort study measured anti-spike IgG responses through Wuhan-Hu-1, Beta, Delta and Omicron waves and reported VOC:WT ratio of <1 in seropositive individuals, confirming the recall of imprinted responses to prior VOCs with no apparent hierarchical order[43]. Upon subsequent infection with Alpha or Delta variants, triple BNT162b2 mRNA-vaccinated individuals experienced an increase in antibody responses to the Wuhan-Hu-1 strain and lower relative responses to the VOCs, indicative of vaccine-imprinted immunity[44,45]. Omicron BA.1 breakthrough infections of mRNA-vaccinated cohorts also prompted substantial boosts of cross-reactive antibodies but limited induction of *de novo* BA.1-specific antibodies[46]. Recent Omicron BA.5 breakthrough infections continue to back-boost anti-Wuhan-Hu-1 responses, while eliciting low neutralisation of the latest sub-lineages, particularly BA.2.75.2 and BQ.1.1[47].

Attempts to update booster vaccine strains with VOC S proteins have been impeded by pre-existing immunity. Double mRNA-1273 vaccinated cohorts receiving a Beta variant-encoding booster (mRNA-1273.351) exhibited skewed responses towards the original Wuhan-Hu-1-like vaccine antigen[48]. Cohorts receiving a Beta/Delta bivalent booster (mRNA-1273.213) and a monovalent BA.1 booster (mRNA-1273.529) as a third dose yielded similar results, where cross-reactive humoral responses to Wuhan-Hu-1-like strains dominated, comparable to a Wuhan-Hu-1 booster[49]. However, *de novo* targeting of mutated, variant-specific epitopes at the molecular level was reported[49]. An alternative strategy was to include equal amounts of Wuhan-Hu-1 and VOC antigens in the booster dose. A Beta bivalent (mRNA-1273.211) vaccine still favoured biased anti-Wuhan-Hu-1 antibody responses, but with enhanced neutralisation of Beta, Delta and BA.1 subvariants superior to the Wuhan-Hu-1 booster in a Phase 2/3 study[50]. Subsequent Phase 2/3 analysis of the BA.1 bivalent booster (mRNA-1273.214) and the BA.4/BA.5 bivalent booster (mRNA-1273.222) concluded superior neutralising activity to the Wuhan-Hu-1 booster against both Wuhan-Hu-1 and matched Omicron sublieneages[20,51]. A US cohort receiving the BioNTech BA.4/BA.5 bivalent booster as a fourth dose also demonstrated enhanced neutralisation of BA.2.75.2 in addition to strains from the BA.5-derived sublineages[52]. Counter evidence was reported from other independent cohorts, where individuals receiving the latest BA.4/BA.5 bivalent mRNA vaccines as a fourth dose demonstrated no significant difference in neutralising antibody titres than those receiving the monovalent Wuhan-Hu-1 booster at ~3-4 weeks post-boost to D614G, BA.1, BA.2, BA.4/BA.5, BA.4.6, BA.2.75 and BA.2.75.2[53]. The conclusion was confirmed for BA.5 neutralisation in a separate study[54]. In BA.4/BA.5 bivalent vaccinees, neutralisation against the latest and most evasive BA.2.75.2, BQ.1.1 and XBB variants were also markedly lower than against Wuhan-Hu-1, despite comparatively better than groups receiving 1-2 doses of monovalent Wuhan-Hu-1 boosters[19,55]. In May 2023, the WHO Technical Advisory Group on COVID-19 Vaccine Composition advised the exclusion of Wuhan-Hu-1-based immunogens from future booster vaccine formulations on the basis of antigenic divergence and the possibility of imprinting[56]. For the 2023-2024 season, both the Moderna and Pfizer/BioNTech XBB.1.5 monovalent vaccines have received marketing authorisation[57,58]. Interim data from an ongoing Moderna phase

2/3 study on their latest monovalent XBB.1.5 (mRNA-1273.815) and BA.4/BA.5/XBB.1.5 bivalent (mRNA-1273.231) vaccines, administered as a 5th dose at ~8 months post-vaccination after the Wuhan/BA.4/BA.5 bivalent booster, reported comparable increases in neutralising antibody titres against the ancestral D614G strain and Omicron subvariants. Specifically, both vaccines induced similar neutralisation of BA.4/BA.5 and BQ1.1, whereas the XBB.1.5 monovalent vaccine induced numerically higher titres against the ancestral D614G strain, XBB.1.5 and XBB.1.16. Monovalent vaccinees also cross-neutralised the most recent EG.5.1 and BA.2.86 with wider mutational landscapes[57]. Of note, the highest titres were measured against the ancestral D614G strain and BA.4/BA.5, possibly indicating back-boosting to the first exposure, consistent with the OAS model, and to the latest vaccination, resembling patterns observed in seasonal influenza vaccine effectiveness studies[57,59]. Although these studies associated prior exposures to antibody levels in secondary infection, further studies are required to elucidate the potential enhancing or deleterious effects of back-boosted antibodies on subsequent protection to related strains.

Does imprinted immunity confer protection against SARS-CoV-2?

Immune imprinting causes either increased susceptibility or protection from subsequent infection. Collectively, interactions between seasonal[59–62], pandemic[63–68], zoonotic[69] influenza strains, acquired via infection or vaccination, have been shown to influence the clinical outcomes of subsequent exposures. However, most studies are limited by timescale and under-represent the average number of recurring infections in the adult population. Longitudinal data detailing comprehensive vaccination and exposure history is needed to unpick the relationship between different antigenic epitopes on host immune memory against influenza.

For SARS-CoV-2, the nature and extent of correlations between pre-existing antibodies to antigenically related viruses and protection against infection and disease are controversial. For susceptibility to infection, despite high seroprevalence of anti-CCCoV immunity in the global population, no apparent protection could be inferred owing to the observed rapid transmission of SARS-CoV-2 around the world. While the lack of correlation between anti-CCCoV immunity and susceptibility to SARS-CoV-2 infection was confirmed in a US cohort[38], a positive correlation was identified in a German cohort[70]. For disease outcomes, several serological studies reported cross-protection from imprinted CCCoV immunity in SARS-CoV-2 infection[28,71,72]. On the contrary, independent analysis of different cohorts of hospitalised COVID-19 patients commonly correlated pre-existing antibodies to CCCoVs with increased risk of severe disease[37,73–75]. The mode of action was hypothesised to occur through enhanced viral penetration of respiratory mucosal barriers or enhanced immunopathology via anti-CCCoV antibody-dependent cellular cytotoxicity[70]. High-dimensional flow cytometry of patient B cell repertoires highlighted an important role of extrafollicular plasmablast responses in severe disease[76]. Structural dissection of binding and neutralising antibody interactions with the RBD by Dejnirattisai *et al.* revealed an inverse correlation between pre-existing CCCoV antibody titres and *de novo* generation of SARS-CoV-2 antibodies[77]. In contrast, Kaplonek *et al.* associated pre-existing antibodies specific to the S2 subunit with mild disease, suggesting the quality of the epitope targets may influence a positive or negative outcome[78]. In light of the conflicting serological data, mathematical models were developed to better recapitulate the range of factors affecting SARS-CoV-2 infection outcomes in a CCCoV-seropositive population[79,80]. Meanwhile, due to the extremely limited epidemiological histories of SARS-CoV-1 and MERS-CoV, their impacts on SARS-CoV-2 infection and vaccination remain unclear.

Studies on updated VOC-specific booster vaccines shed light on the impact of vaccine-induced imprinting on protective immunity against SARS-CoV-2[21]. The Phase 2/3 trial of the mRNA-1273.211 Beta bivalent booster reported a reduction in symptomatic infection against circulating strains compared to the Wuhan-Hu-1 booster, from 10.7% to 3.4%[50]. Strikingly, the Phase 2/3 trial of the mRNA-1273.214 BA.1 bivalent booster reported an increase in the numerical incidence of infection in the bivalent group[51]. Relative vaccine effectiveness of the mRNA-1273.222 BA.4/BA.5 bivalent booster in protecting against symptomatic infection ranged from 28-31% at 2-3 months since the last monovalent dose, as reported in a nation-wide study conducted by the US Centers for Disease Control and Prevention (CDC)[81]. Although monovalent Wuhan-Hu-1 and bivalent VOC booster currently provide adequate protection against severe disease, continued emergence and transmission of altered variants are causing widespread breakthrough infections. These infections

repeatedly boost cross-reactive responses without inducing potent variant-specific responses. Deeper understanding is needed to correlate short-term antibody dynamics and long-term maintenance of immune memory to provide reliable prognostic data for infection and vaccination outcomes.

Factors contributing to heterogeneity

Antigenic distance

Extensive epidemiological and serological data have suggested correlations between the time of viral exposure, strain-specific antibody titres and protective immunity. However, the nature of interactions between prior and subsequent exposures demonstrates substantial heterogeneity, some of which follow trends in antigenic distance, differ in the forms of exposure or adapt over time. To explain the observed heterogeneity in protection against repeated influenza infection and seasonal vaccine effectiveness[59], Smith *et al.* proposed that the nature of immune interference between strains is dependent on antigenic distance[82]. One mechanistic explanation could be that imprinting is only relevant between drifted strains within a subtype that share multiple epitopes in the immunodominant HA head, such that the host antibody targets are highly strain-specific. The hypothesis also corroborate with real-world data collected during the 2009 H1N1 pandemic, where individuals vaccinated against this antigenically divergent and distinct strain elicited significantly greater vaccine-specific humoral immunity than recalled memory responses to prior seasonal strains[68]. Similarly, individuals born prior to the 1890s H3N8 Russian Flu pandemic, likely infected by a putative H1-like strain during initial exposure, exhibited high H3-specific antibody titres in 1950s[66]. Serological studies post-2009 H1N1 pandemic isolated broadly neutralising antibodies following infection[83] and vaccination[84], which showed low rates of somatic hypermutation and increased usage of the stem-specific *IGHV1-69* segment – characteristic of a rapid, extrafollicular response of a novel set of B cells leading to plasmablast differentiation[83,85] (Figure 4). However, as the population was subsequently exposed to closely related, drifted H1N1 seasonal strains, imprinting manifested again. During the 2013-2014 influenza season, acquisition of the K166Q mutation by the circulating strain abrogated protection by the 2009 H1N1 pandemic vaccine strain[61]. This could be due to the displacement of antibody specificities back towards strain-specific epitopes in the immunodominant HA head, which led to reduced binding to the drifted strain in middle-aged individuals[62]. This aligns with findings in the following 2014-2015 influenza season, during which vaccination with seasonal trivalent influenza vaccine led to a decrease in *IGHV1-69* usage and clonotypic diversity[84]. These observations suggest that immune imprinting would occur when the antigenic distance between the two strains is within a specific range. Below this range, neutralising responses against conserved epitopes would be sufficient to provide protection against both strains; above this range, a novel response would be induced against the second strain without interference from the first infection. At present, it remains difficult to predict the future trajectory of viral evolution. Defining the boundaries of this range for coronavirus S antigens would be critical for accurately predicting the impact of immune imprinting on SARS-CoV-2. For instance, the large antigenic distance between CCCoVs and SARS-CoV-2 may have limited the extent of imprinting, which is reflected in the heterogenous impacts on COVID-19 infection and vaccination outcomes reported across different studies[27].

Clonal evolution of memory B cell

In contrast to abrupt antigenic specificity changes upon exposure to immunologically distinct viruses, driven by naïve B cell activation, clonal evolution of the memory B cell repertoire can also progressively modify imprinting over time (Figure 4). Memory B cells emerging early during the germinal centre response typically have low levels of somatic hypermutation, which are retained in circulation for prolonged periods and recalled into the germinal centre for further rounds of affinity maturation within clonal lineages during secondary exposure. Following additional germinal centre selection, terminal differentiation of memory cells generates plasma cells that secrete antibodies with enhanced subtype-specificity to viral variants[86,87]. During SARS-CoV-2 infection, an early extrafollicular response is marked by differentiation of naïve B cells, activated by SARS-CoV-2, and memory B cells, primed by prior CCCoV infections, into antibody-secreting plasmablasts[76]. Meanwhile, the germinal centre response ensues for at least 6 months, allowing extensive memory B cell evolution to optimise their binding affinity, neutralising potency and breadth to viral variants[86,88]. Monitoring of convalescent individuals detected memory B cell clonal evolution that exceeded 12 months post-infection and emergence of broadly neutralising antibodies against Alpha, Beta, Gamma[89] and Delta VOCs[90]. In contrast, non-protective, pre-pandemic CCCoV-induced cross-reactive antibodies diminished over time[87,91,92]. Epidemiological analysis of the Qatar national cohort compared the incidence of repeat infections in individuals previously infected by a non-Omicron strain followed by an Omicron BA.1/BA.2 strain (double-primed) or only by an Omicron BA.1/BA.2 strain (Omicron-primed). During follow-up, the double-primed group exhibited lower reinfection rates, suggesting that prior encounter with an earlier, non-Omicron strain increased protection against subsequent Omicron exposure, possibly by potentiating B cell evolution and diversification[22]. Strikingly, single-cell sequencing analysis revealed alarming progression of memory B cell responses away from the SARS-CoV-2 spike towards internal viral nucleoproteins and open reading frame 8 in elderly patients, which are non-neutralising and poorly protective against both infection and disease[93]. This highlights age-dependent heterogeneity in the direction of memory B cell evolution that can lead to distinctive outcomes during acute and secondary exposures. Compared to infection, primary SARS-CoV-2 vaccination is associated with reduced persistence of clonal evolution of ~5 months, limited mutation levels in memory cells and suboptimal protection against variants. Although RBD-binding antibody response narrows in vaccinee and broadens in convalescent sera over time, vaccine-induced responses still supersede convalescent responses at 3-4 months post exposure. Additional booster dose administrations can also enhance clonal evolution of vaccine-induced immune responses towards greater breadth[94]. More recent data also suggested a role of hybrid immunity in promoting clonal evolution and variant protection[15,44,46,95–100]. Together, clonal evolution of memory B cells diminishes imprinting by simultaneously enhancing reactivity to the most recent strain and reducing anamnestic antibody responses to early historical strains. Longitudinal birth cohort studies would be needed to compare the nature and strength of immune imprint conferred by natural infection and different vaccine platforms. Such studies should consider monitoring the protective effectiveness of subsequent seasonal/booster vaccinations against

influenza and SARS-CoV-2 infection and severe disease, changes in plasma cell populations and evolution of the memory B cell repertoire[101].

Nature of exposure

Some studies emphasised the differences between exposure from infection versus vaccination on the impact of immune imprinting. Early influenza studies demonstrated heterogeneity in the nature and relative magnitudes of imprinted boosts induced by infection, vaccination or hybrid immunity[102–104]. Published data on SARS-CoV-2 vaccination-induced responses exhibited greater homogeneity in antibody profiles and resistance to variants than infection. Data from the mRNA-1273 Phase 1 clinical trial showed enhanced RBD-targeting neutralising antibody responses in vaccinees compared to convalescent sera. Vaccine-induced binding antibodies also demonstrated greater resistance to single mutations arising from antigen drift, retaining protection against triple mutants with disrupted major neutralising epitopes[105]. Consistent findings were obtained for the polyclonal serum antibodies induced by BNT162b2[106]. Human lymph node analysis by Röltgen *et al.* showed robust formation of germinal centres after BNT162b2 mRNA vaccination[45,107], which interestingly contrasted with the loss of Bcl-6-expressing T follicular helper cells and the formation of highly disrupted germinal centres observed during severe SARS-CoV-2 infection[108]. As abundant germinal centre induction and functional organisation are critical for efficient antigen presentation and the onset of adaptive responses, these findings underscore the fundamental differences between infection- and vaccine-induced immunity. In addition, the role of hybrid immunity in generating heterologous protection against VOCs has been of substantial interest. Numerous independent studies also highlighted a beneficial role of breakthrough infections in increasing the breadth of neutralising antibody responses against previous, present and future VOCs in diverse cohorts with variable vaccination and infection histories[44,46,47,96,97,100]. The hybrid immune cohort with the broadest response across different Omicron sub-lineages had the following exposure history: three doses of Wuhan-Hu-1 mRNA vaccines, a BA.1/BA.2 breakthrough infection and one dose of Wuhan/BA.5 bivalent mRNA vaccine. However, even this group demonstrated inferior Omicron-specific neutralising activity relative to Wuhan-Hu-1, which highlights the impact of imprinting as a major challenge to sustaining immunity against rapidly evolving RNA viruses[47]. Interestingly, at the other end of the spectrum, hybrid immunity has been shown to dampen subsequent Omicron-specific responses in individuals infected by Wuhan-Hu-1 before their first vaccination[96]. Such discrepancies could be parsimoniously explained by the interval between infection and vaccination, which also correlated with progressive changes in host antibody landscapes[109]. A precise definition of immune histories and longitudinal follow-ups are required to reach more informative conclusions on the impact of hybrid immunity on clinical outcomes in individual cases.

Immunisation in the context of imprinting

Understanding the constraints of immune imprinting is likely to better inform future influenza and SARS-CoV-2 immunisation strategies. Vaccination in the context of imprinting requires data-informed decisions on immunogen design, expression and delivery platform and administration regimens to induce optimal, durable, and broadly protective responses against continuously evolving influenza and coronavirus variants.

Refining approaches to seasonal vaccination

Priming vaccination of naïve infants with antigenically distinct strains

Given that imprinting from first exposure influences future immune responses, variability in the timing and the strain of childhood viral infection across the global population needs to be considered to improve mass immunisation strategies. Based on the hierarchical nature of imprinting, controlled first exposure provides a unique opportunity to judiciously shape the humoral response and generate a broadly reactive B cell repertoire, which is geared to be expanded upon subsequent infections and vaccinations (Figure 4)[110]. For instance, most individuals are first infected by seasonal influenza virus between ages of 2-3 years[111], but intranasal LAIV immunisation, which effectively induces localised mucosal antibody responses and virus-specific CD4+ memory T cells in the respiratory tract, is approved for children above the age of 2 years due to wheezing reactions in younger children[112]. Safely lowering the age limit of LAIV use to 1 year would allow immunisation of the predominantly naïve population. Various SARS-CoV-2 live attenuated mucosal vaccine candidates are also under clinical investigation with the potential of expanding indication to younger children[113]. Furthermore, simultaneous administration of naïve infants with group 1 and group 2 influenza strains contained in the current seasonal vaccines is hypothesised to provide a desired cross-reactive imprint. Subsequent trials could consider including other viral subtypes to explore the prospects of increasing vaccine valency in the initial priming immunisation[110]. If proven safe and effective, multivalent strain selection would need to be carefully considered as potential priming immunisations to induce maximum breadth and avoid biased imprinting responses from seasonally circulating strains.

Sequential vaccination of adults with antigenically distinct strains

Unlike naïve infants, improving the breadth of seasonal influenza vaccination and COVID-19 booster vaccination in the adult population requires different strategies to reprogramme and redirect pre-existing immune memory. Sequential booster immunisations with antigenically distant HAs that harbour conserved, broadly neutralising epitopes in the stem region have been proposed and trialled with some success[114]. However, as substantial heterogeneity exists across the population in influenza exposure history and immune imprinting patterns, the optimal vaccine regimens can vary between different birth cohorts. Personalised influenza vaccines tailoring sequential immunisation regimens to specific cohorts have been explored but faced significant challenges[8]. On the other hand, the COVID-19 pandemic with recent population-wide exposure history to SARS-CoV-2 variants provides a unique opportunity to explore sequential immunisation regimens with strategically designed and selected antigens of the virus as an intervention to imprinting. Implementation of the

bivalent S booster has evidenced the potential to increase the protective breadth in an imprinted population[21]. A "prime and boost" strategy has also recently been explored, which uses an intranasally administered unadjuvanted S booster to establish mucosal memory responses from pre-existing immunity acquired during primary SARS-CoV-2 vaccination[113]. Moreover, new strategies to increase antigenicity beyond the S antigen could require multiple antigens to activate broader B cell and T cell effector mechanisms towards highly conserved antigenic targets. While universal vaccines are under development, new formulations of current seasonal influenza vaccines and SARS-CoV-2 booster vaccines based on mRNA delivery are being continuously trialled to modulate population immunity, from which the impact on imprinting will become evident.

Future-proofing viral vaccines

Novel antigen technologies emerging out of universal influenza and coronavirus vaccine research initiatives will guide next-generation vaccine solutions. For instance, priming with broadly reactive immunogens capable of expanding the immune footprint beyond the constraints of current imprinted responses, may improve variant-specific boosting strategies and vaccine effectiveness longitudinally. Current approaches targeting broadly protective epitopes for both naïve and exposed populations include subunit antigen structures that focus immune responses to highly conserved sites, chimeric and *in silico* optimised recombinant immunogens and multivalent self-assembling nanoparticle immunogens.

Subunit immunogens

A classic immune-focusing strategy relies on avoiding immunodominant, hypervariable regions of antigens to prevent recall of imprinted immunity against the region and re-direct host responses towards broadly protective epitopes. A diverse range of modified headless HA vaccine candidates have demonstrated superior stem-reactive, broadly neutralising antibody responses to full-length HAs in mice[115]. In particular, animals immunised with headless H1N1 HAs were protected against lethal challenge by heterosubtypic avian H5N1 viruses[115]. Recently, more advanced attempts harnessed the self-assembling protein nanoparticle platform for multivalent display of HA stems in ordered arrays and yielded potent heterosubtypic protection in a phase 1 clinical trial[116,117]. On the other hand, the immunodominant SARS-CoV-2 RBD has been selectively used as a subunit antigen during early pandemic response[118,119]. Subsequent monitoring of the S protein has identified progressive evolution of the S2 domain to acquire more rigidity and hence resistance to future conformational changes, providing potential opportunities for SARS-CoV-2 S stem-only approaches that is analogous to influenza headless HAs[120,121].

Chimeric immunogens

Another approach to re-focus the imprinted immune response towards conserved epitopes in an immuno-subdominant domain is by replacing the adjoining immunodominant domain with a series of heterologous structures to generate chimeric immunogens. Chimeric HAs (cHAs) have been generated from fusion of non-

human globular head structures with conserved stem domains. Sequential prime-boost immunisation with these cHAs was hypothesised to eliminate head-specific cross-reactive immunity and favour recall of pre-existing memory B cells targeting conserved epitopes in the immuno-subdominant stem. Cumulative back-boosting should strengthen the anti-stem response with every vaccination. In a recent Phase 1 clinical trial, cHAs composed of exotic avian influenza head fused to H1 HA stems showed promising safety and broad protection against group 1 IAVs[122]. Latest fine-tuning of this approach focused on minimising head replacement by only switching key antigenic sites with corresponding exotic epitopes. These mosaic cHA vaccines demonstrated improved structural and functional integrity and protected mice against heterosubtypic challenge by distinct influenza A and B viruses[123,124]. Another approach directly masked immunodominant head epitopes through hyperglycosylation[125]. Based on these principles, chimeric coronavirus spike designs are also under development. An immunogen encompassing S N-terminal domains (NTDs), RBDs, and S2 domains of epidemic, pandemic and zoonotic origins protected mice from lethal challenge by diverse coronaviruses within the sarbecovirus subgenus[126]. A Delta-Omicron chimeric RBD dimer was also shown to protect against matched VOCs[127].

In-silico designed immunogens

Antigens harbouring selected epitopes enable more fine-tuned manipulation of immune imprinting to generate desired protective responses. Multiple specific epitopes can be recombined to generate mosaic immunogens. For example, Thompson *et al*. identified a set of epitopes with limited variability within the H1N1 lineage and grafted these epitopes on non-human influenza HAs. Mice vaccinated with these antigens showed broader protection in comparison to wild-type strain[128]. Through comprehensive phylogenetic analysis and sequence comparison, Ross *et al*. generated computationally optimised broadly reactive antigen (COBRA) HA candidates with consensus sequences representing conserved immune epitopes[129,130]. This multi-consensus sequence layering approach harnesses natural evolutionary pressure to select desirable epitopes on virus-like particles in an effort to induce broad protection[131]. Another distinct approach involves an iterative pipeline of digital structure prediction of variants across viral families and subgenera to design a library of highly conserved viral antigen targets, followed by optimisation of key viral antigens through *in vitro* selection. Best-in-class, digitally designed, immune optimised and selected vaccine antigens (DIOSynVax) are combined and successfully delivered by a variety of different platforms, such as DNA, mRNA, or viral vectors. This approach has been used for influenza and coronaviruses and a broad pan-sarbecovirus candidate is currently in clinical trial[132,133].

Multivalent nanoparticle immunogens

Self-assembling protein nanoparticles, both of natural and synthetic origins, have been adapted for multivalent antigen display and targeted immune tissue delivery as a promising universal vaccine design platform for enhancing protective breadth and potency. Co-display of heterotypic antigens on a single continuous surface has been shown to target B cell responses towards conserved epitopes and increase resistance to strain-specific antigenic variation[134]. The high potency of nanoparticle-induced, broadly protective responses could provide a

route to overwriting existing imprints. *Helicobacter pylori* ferritin has been modified for the development of multivalent influenza and coronavirus vaccines. Next-generation mosaic ferritin nanoparticles expressing arrays of heterotypic H1N1 HA demonstrated comparable neutralising potency at much lower doses and increased breadth of protection against mismatched H1N1 viruses spanning 90 years in mice[135]. Bivalent and trivalent ferritin nanoparticle vaccines containing mixtures of SARS-CoV-2 D614G, Beta and Delta RBDs offered cross-protection to diverse VOCs in macaques[136,137]. Another popular natural nanoparticle platform is the *Brucella abortus* lumazine synthase (LS). SARS-CoV-2 RBD LS nanoparticles induced potent neutralising activity against several VOCs and SARS-CoV-1[138]. In addition to natural nanoparticles, Baker *et al*. generated a pool of *de novo* synthesised vectors with tailored geometries for displaying prototype pathogen glycoproteins[139]. The I53_dn5 vector has been used to develop quadrivalent influenza nanoparticle vaccines by expressing 60 copies of HAs composed of the four seasonal quadrivalent influenza vaccine strains in equimolar ratios[140]. The I53_50B vector displaying Wuhan-Hu-1 RBD has also been approved for use after demonstrating non-inferior safety and efficacy profiles to the licensed ChAdOx1 vaccine in Phase 3 clinical trial[141]. Quadrivalent mosaic I53_50 nanoparticles displaying heterotypic copies of RBD[142] or pre-fusion stabilised S[143] conferred heterologous protection in animal models. Alternatively, the mi3 vector, synthetically engineered from a *Thermotoga maritima* aldolase and linked via the SpyTag/SpyCatcher conjugation system by Howarth *et al.*[144,145], was conjugated to eight coronavirus RBDs, including WA1 or Beta, RaTG13, SHC014, Rs4081, pang17, RmYN02, Rf1 and WIV1. The WA1 mosaic octavalent nanoparticles induced heterologous responses to SARS-CoV-1 and bat coronaviruses Yun 11, BM-4831 and BtKY72 in mice. The Beta octavalent nanoparticles fully protected against Beta and SARS-CoV-1 infection and disease in mice, while also offering partial protection in non-human primates[146,147]. The latest multiviral quartet approach developed by Hills *et al.* further enabled mosaic display of strings of four RBDs using the SpyTag-SpyCatcher-mi3 system, which required fewer assembling components and induced higher binding antibody titres against all the matched strains of the octavalent mosaic nanoparticles and superior neutralisation of Wuhan-Hu-1, Delta and SARS-CoV-1 in mice[148].

Reshaping host immune memory

While the development of novel, universal vaccine immunogens capable of inducing broadly protective and long-lasting immunity remains the focus of vaccine research, substantial interest arose in strategies that directly modulate the host immune system (Box 1). Building on recent characterisation of prolonged germinal centre response post-infection[86], active manipulation of the nature and intensity of this competitive selection process presents novel opportunities to establish controlled, desirable immune responses to overcome imprinting at the host level (Figure 4). Although not pragmatic at population level, such studies provide insight and important proof of concept. Approaches include: 1) selective expansion of immuno-subdominant B cells through guided evolution; 2) selective reduction of immuno-dominant B cells through targeted elimination; 3) enhancement of B cell function through DC activation.

Selective expansion of immuno-subdominant B cells through guided evolution

Distinct subpopulations of memory B cells can undergo extensive clonal evolution and progressively develop specificity against viral variants. In mice, the $CD80^{-}PDL2^{-}$ double-negative memory B cell subset preferentially re-enters the germinal centre for additional cycles of somatic hypermutation and affinity maturation upon re-exposure to viral variants, while the $CD80^{+}PDL2^{+}$ double-positive memory B cells directly differentiate into plasma cells. Selective activation of the double-negative memory B cell subset has the potential to increase the overall plasticity of the memory B cell repertoire and facilitate adaption of SARS-CoV-2 vaccine-induced immune responses to variants[149]. The concept is expected to be translatable to human studies, where similar subset distinctions within the memory B cell pool have been reported after SARS-CoV-2 infection[86,93]. Mass cytometry screening data and functional analysis of human peripheral B cell landscapes highlighted $CD45RB^{+}CD27^{+}CD73^{+}CD95^{+}$ and $CD19^{hi}CD11c^{+}$ memory B cell subsets as prospective biomarkers for optimised vaccine-induced immunity against SARS-CoV-2[150]. Definitive characterisation of the transcription networks and cytokine profiles that underlie functional distinctions between memory B cell subsets will pinpoint specific drivers of enhanced clonal evolution and provide routes to their targeted activation. In addition to increasing variant-specific responses, guided evolution towards immuno-subdominant, broadly neutralising epitopes can further enhance the robustness of humoral immunity. Understanding the mechanistic basis of progressive displacement of CCCoV-specific or non-neutralising antibodies post-SARS-CoV-2 infection will enable directed manipulation of memory B cell clonal evolution away from prior imprints, providing a future strategy for enhancing the breadth of protective vaccine responses[86,87,89,91,92].

Selective reduction of immuno-dominant B cells through targeted elimination

Opposed to expanding desired, immuno-subdominant memory B cells through guided evolution, other groups explored the prospects of reducing non-desirable, immunodominant B cells via targeted elimination as an alternative method of removing immune imprints and increasing the breadth of humoral immunity. Antigen-driven apoptosis during the germinal centre reaction has been well-established as a mechanism of maintaining B cell self-tolerance[151]. Injection of soluble, heterologous antigen during a primary immune response can induce rapid germinal centre B cell death in an affinity- and avidity-dependent manner[152,153]. In a recent study, Silva *et al.* immunised mice with variable forms of the soluble antigen 4-hydroxy-3-nitrophenylacetyl (HNP)-ovalbumin (OVA). Administration of soluble antigens encompassing exclusively the immunodominant HNP epitope promoted targeted apoptosis of cognate germinal centre B cells. Following selective elimination of HNP-specific B cells from the germinal centre, progressive expansion of subdominant OVA-specific B cells was observed, followed by significant increases in the quantity and persistence of serum antibodies, long-lived plasma cells and memory cells. Nevertheless, whether these functional immunological features can be translated into improved vaccine efficacy is pending further investigation. The authors envisioned future developments to include combination with targeted delivery systems and inhibitory agents to reduce the antigen load required for sufficient abrogation immunodominant germinal centre B cells[154]. However, the long-term impacts of

artificial tolerance induction remain elusive, which raises concerns over the development of immunopathology upon subsequent exposures to related antigens and ethical feasibility in human vaccination trials.

In silico simulation by Meyer-Hermann suggested a similar method to adjust skewness in the B cell repertoire – passive transfer of high-affinity antibodies as competitive inhibitors to immunodominant memory B cells with identical specificities. The model hypothesised that transferred antibodies enter peripheral lymphoid organs and compete with germinal centre B cells for antigens presented by follicular dendritic cells in a concentration- and affinity-dependent manner. Specific binding of transferred antibodies to immunodominant epitopes reduces their relative availability to otherwise occluded, conserved epitopes for germinal centre B cell recognition, thereby favours the induction of immuno-subdominant, cross-reactive antibody responses. In this case, vaccination regimens can be complemented with immunodominant antibody cocktails to redirect affinity maturation towards conserved epitopes. Precise cocktail composition can be further tailored to individuals based their pre-existing B cell repertoires, as shaped by exposure history[155]. To this end, repression of immuno-dominant B cells, through soluble antigen administration or passive antibody transfer are distinctive strategies for alleviating the competitive pressure from germinal centre selection and expanding desirable B cell subpopulations that provide novel, broadly protective responses in the presence of pre-existing immunity.

Enhancing B cell function through dendritic cell activation

Virus-specific B cell responses can also be indirectly enhanced by upregulating antigen uptake, processing and presentation by dendritic cells. Recent advancements in modern vaccinology platforms have enabled several routes of targeted antigen delivery to lymph nodes. Immunisation via footpads draining to single lymph nodes increased local antigen concentration, which induced broadly neutralising antibodies to the HA stem in mice[156]. Translation of the "albumin hitchhiking" approach used for lymph node visualisation in medical oncology has enabled design of recombinant amphiphile conjugate immunogens, which are composed of an antigen cargo that is linked to a lipophilic albumin binding tail via an interconnecting, solubility-promoting polar polymer chain. Through enhanced lymph node targeting, structurally optimised amphiphile vaccines demonstrated superior T cell priming activities and efficacies in mice[157]. Upon arrival at lymph nodes, the antigen must persist and effectively activate dendritic cells to induce a sustained adaptive immune response. Amphiphile vaccine antigens bound to endogenous albumin are prevented from dissemination into circulation and effectively retained in lymphoid tissues[157]. Slow-release vaccines can also be employed for sustained antigen release[156]. Immunogens engineered to target dendritic cell-specific surface biomarkers, such as DC-SIGN, DEC-205 and Clec9A, have also been shown to improve vaccine immunogenicity *in vivo*[158]. Co-administration of adjuvants during sequential exposures to closely related H1N1 PR/8 and FM/1 influenza strains enhanced antigen presentation by dendritic cells, thereby alleviating the effects of imprinting and enhancing protection against the second virus. Protection conferred by adjuvant administration during primary exposure implies preferential activation of durable, cross-reactive antibody responses that persisted beyond the time interval between infections[159].

Outlook

Over the past 3 years, the epidemiology of SARS-CoV-2 has shifted from a pandemic phase, with rapid spread in a naïve human population, to an endemic phase, with re-infections by increasingly transmissible variants in a hybrid immune human population. Owing to the recent zoonotic origin of SARS-CoV-2, the evidence for immune imprinting is confounded by the scope and quality of available antibody[23], T cell[27] and trained immunity data[160]. Variability in the definitions and quantitation of imprinting, study designs and reference parameters are commonly seen in influenza and SARS-CoV-2 literature. The non-linear relationship between antibody titres and seroprotection limits the robustness of immunological data for extrapolating clinical outcomes[161]. Most antibody reactivity studies lack controls due to the scarcity of comprehensive, longitudinal immune history of individuals, creating another hurdle for drawing conclusive correlations towards disease severity[161,162]. Recent evaluation of observational studies to predict COVID-19 booster vaccine effectiveness suggested that imprinting could fully attribute to the intrinsic selection bias in the analytical process. Removal of such bias requires quantitative characterisation of individual susceptibility that is not currently feasible in real-world settings[163].

We note that inherent differences between influenza and SARS-CoV-2 virology, immunology and epidemiology mean that caution is warranted when comparing them in the context of imprinting. These include the aforementioned compressed and recent timelines of SARS-CoV-2 circulation in the human population since 2019 and the enormous diversity its variants have acquired during widespread dissemination of infection in both humans and susceptible animal species. The unexpectedly high rate of SARS-CoV-2 evolution in the global population with varying levels of immunity has facilitated accumulation of abundant immune escape and evasion mutations, as evidenced by the latest Omicron subvariants. Additionally, some of the most widely used SARS-CoV-2 mRNA booster vaccines still incorporate the single S antigen from the very early SARS-CoV-2 genomes sequenced during the initial Wuhan outbreak. These contrast with the array of whole virus antigens used in inactivated seasonal influenza vaccines. Thus, while immune imprinting may have occurred to a certain degree amongst some of the human coronaviruses, and with SARS-CoV-2 infection- and vaccine-induced immune responses, the genetic and antigenic diversity of recent SARS-CoV-2 variants may produce novel epitopes that are beyond the theoretical constraints of imprinting. The latest evidence suggests that heterologous boosting with VOC S antigens could diversify antibody responses and their ability to neutralise SARS-CoV-2 variants[49,52]. In addition, repeated boosting with Omicron S antigens has been shown to alleviate imprints from prior exposure to the ancestral strain through affinity maturation towards neutralising epitopes in the RBD[97,164]. Systematic studies examining different types of SARS-CoV-2 vaccine combinations, and the order in which they were administered, have begun to identify lymphocyte signatures that correlate with immunogenicity[165]. Clinical trials are required to determine the optimal immune responses and antigens targets that correlate with the best vaccine protection.

Next-generation SARS-CoV-2 immunisation approaches may benefit from avoiding full-length, wild-type S protein immunogens that pose the risk of boosting responses to historical epitopes rather than the desired newer epitopes[56]. Prospective future strategies include immune-focused targeting of novel RBD antigens of future variants, the use of conserved structures of the immuno-subdominant S2 subunit, such as the critical fusion domain, and the development of *in silico* designed, mosaic immunogens and self-assembling nanoparticle vaccines. Fortunately, T cell epitope responses have remained relatively conserved, which do not prevent infection but rather contain, control and eliminate infected cells. Effective T cell responses in the population, accompanied by changes in the pathobiology of the current Omicron variants that impact the upper respiratory tract, are factors believed to have contributed to reduced disease severity, hospitalisations and fatality rates despite declining vaccine effectiveness[27,166]. Third-generation vaccines are likely to include other conserved T cell antigen targets, such as nucleoproteins, or other pan-sarbecovirus or β-coronavirus targets to cover the larger subgenera of viruses that represent future human spill-over threats. Furthermore, current intravenous COVID-19 booster vaccines are used predominantly to reduce disease severity. To effectively block virus transmission in the global population and further variant adaptation in future, mucosal vaccines that induce protective immune effector responses in the nasopharynx would be highly desirable[113].

The COVID-19 pandemic triggered a step-change in enabling new platform vaccine technologies, global immune monitoring and genomic surveillance capacity at scale. Combined with innovative antigen technologies, next-generation vaccine research offers substantial opportunities to future-proof influenza and coronavirus vaccines.

Table 1. Influenza virus and SARS-CoV-2 compared

1a. Comparison of viruses

Virus	**Influenza**	**SARS-CoV-2**
Genome	Negative-sense, single-stranded RNA Segmented 14kB Multiple co-circulating antigenically distinct subtypes	Positive-sense, single-stranded RNA Non-segmented 30kB Multiple co-circulating lineages with successive antibody evasion properties
Receptor-binding glycoprotein	Haemagglutinin Receptor-binding pocket	Spike Receptor-binding motif in receptor binding domain
Human target receptors	Sialic acid (2,6 or 2,3)	Human angiotensin converting enzyme-2 and other candidate receptors
Size of binding site	Small (239Å^2)	Large (924Å^2)
Hypervariable and immunodominant regions	Haemagglutinin head	Receptor binding domain
Antigenic evolution	Drift Shift via reassortment	Drift Shift via recombination
Zoonotic reservoirs	Domestic birds, migrating birds, swines, seals, whales, bats, equines, mammals	Horseshoe bats (primary reservoir), minks (reverse zoonotic), intermediary hosts (deer, pangolins, civets)
Immune-escape strategies	Mutations in surface glycoproteins	Mutations in surface glycoproteins Increased affinity to host receptors Promiscuity in priming protease Use of altered proteolytic cleavage
Other viral surface proteins	Neuraminidase, Matrix 2	Envelope protein, Membrane protein
Seasonality	Peak during winter months in respective hemispheres	Less apparent

1b. Comparison of booster vaccines

	Influenza	**SARS-CoV-2**
Dominant licensed platform	Whole inactivated vaccines Live attenuated vaccines	mRNA vaccines
Antigen target	Haemagglutinin	Spike
Antigen content	Haemagglutinin, Neuraminidase, Matrix 2, internal proteins	Spike
Frequency of update	Seasonal and hemisphere specific, based on WHO recommendations	Irregular updates, driven by Variants of Concern and waning immunity
Valency	Trivalent (H1N1, H3N2, Victoria) Quadrivalent (H1N1, H3N2, Victoria, Yamagata)	Monovalent (Wuhan-Hu-1, XBB.1.5) Bivalent (Wuhan-Hu-1/BA.1, Wuhan-Hu-1/BA.4/BA.5, BA.4/BA.5/XBB.1.5)

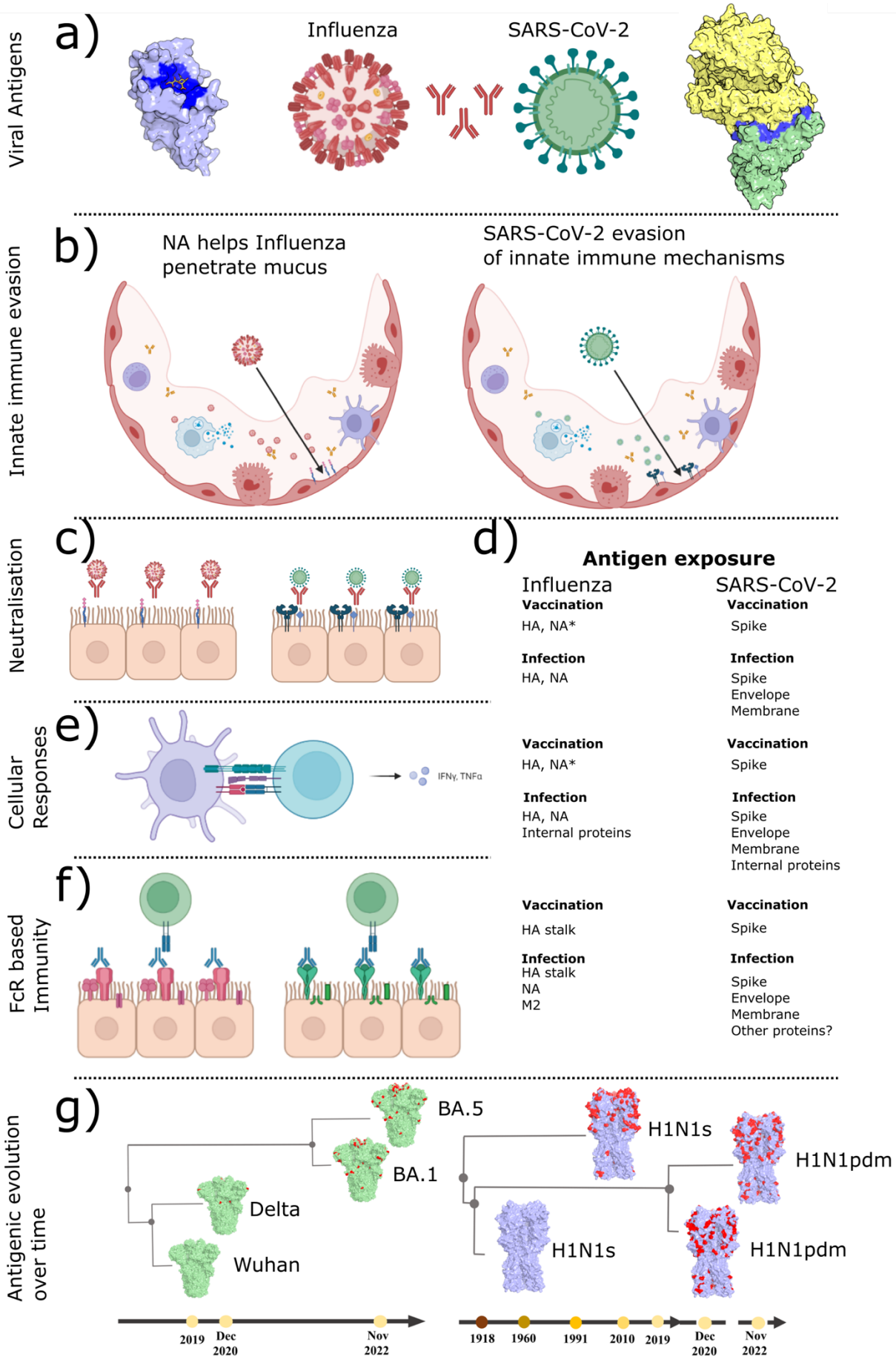
Viral Antigens
a)
Influenza
SARS-CoV-2
Innate immune evasion
b)
NA helps Influenza penetrate mucus
SARS-CoV-2 evasion of innate immune mechanisms
Neutralisation
c)
d)
Antigen exposure
Influenza
Vaccination
HA, NA*
Infection
HA, NA
Vaccination
HA, NA*
Infection
HA, NA
Internal proteins
Vaccination
HA stalk
Infection
HA stalk
NA
M2
SARS-CoV-2
Vaccination
Spike
Infection
Spike
Envelope
Membrane
Vaccination
Spike
Infection
Spike
Envelope
Membrane
Internal proteins
Vaccination
Spike
Infection
Spike
Envelope
Membrane
Other proteins?
Cellular Responses
e)
IFNγ, TNFα
FcR based Immunity
f)
Antigenic evolution over time
g)
BA.5
BA.1
Delta
Wuhan
2019
Dec 2020
Nov 2022
H1N1s
H1N1pdm
H1N1s
H1N1pdm
1918
1960
1991
2010
2019
Dec 2020
Nov 2022

Figure 1. Immune responses to influenza virus and SARS-CoV-2 in the context of infection and vaccination. **A.** Influenza A H1N1 haemagglutinin and SARS-CoV-2 spike bind to sialic acid and human angiotensin converting enzyme-2 (hACE-2) respectively. Haemagglutinin is coloured in lilac, spike is coloured in green, sialic acid and ACE-2 are coloured in yellow and interacting regions are coloured in blue. The receptor binding site of SARS-CoV-2 is more than three times greater than the Influenza A H1N1. As the receptor binding sites correspond to the regions under the highest selective pressures from host antibodies, this difference has a wider impact on the greater number of immune escaping variants of SARS-CoV-2 that accumulate over time. **B.** Penetration of the respiratory mucosa and associated innate and adaptive immune responses allow for viral infection in naïve as well as previously infected hosts. TMPRSS2, transmembrane serine protease 2. **C.** Antibodies preventing attachment to the cellular receptor for both viruses are crucial for immunity, and immune history will affect the qualities of antibodies raised to an antigen and conserved epitopes presented to the immune system on subsequent exposure. **D.** The number of antigens the immune system is exposed to differs between natural infection and vaccination depending on the vaccine platform employed. Responses are restricted to the spike protein in SARS-CoV-2 vaccine recipients, whereas influenza whole inactivated vaccines provide all viral proteins despite only being quality checked and calibrated based on the activity of the haemagglutinin, not neuraminidase (*). **E.** T-cell responses are important for viral clearance, symptom severity and are less susceptible to imprinting. IFN-γ, interferon-γ; TNF-α, tumour necrosis factor-α. **F.** Fc receptor (FcR) based immunity (antibody-dependent cellular cytotoxicity (ADCC) & antibody-dependent cellular phagocytosis (ADCP)) plays an important role in immunity to both viruses. **G**. Neutralising and binding antibody levels at the time of exposure impact the outcome of infection, transmission and by proxy the ability of immune evasive mutants to evolve, giving rise to antigenically new strains or variants of interest/concern. Structure visualisation and image rendering were performed using PyMOL.

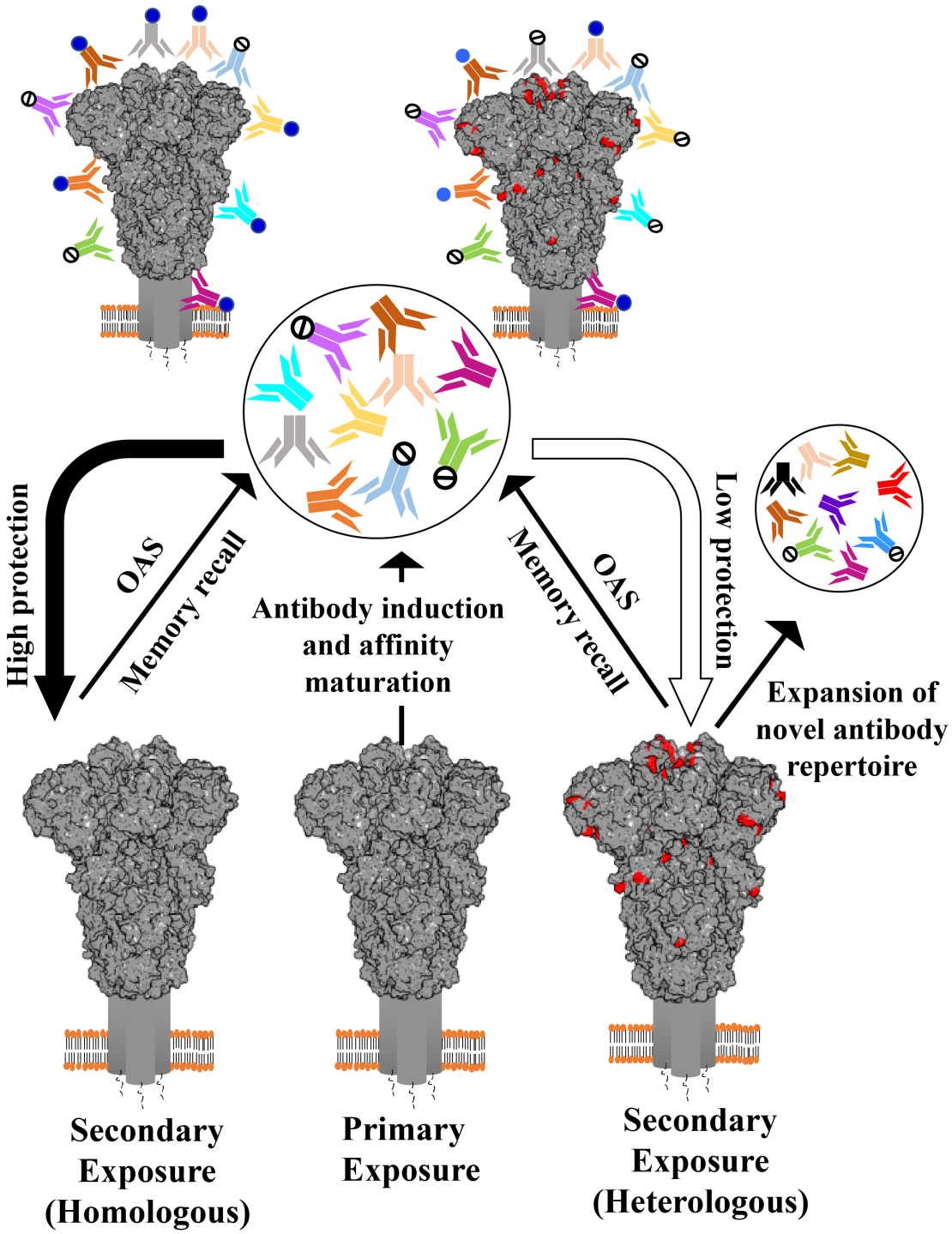

Figure 2. Primary exposure to the virus generates a pool of antibodies with both neutralising and non-neutralising (stop symbol at the end of antibody structure) paratopes. On secondary exposure to a homologous strain, the previously matured antibody pool is recalled and provides better protection to the homologous strain. On secondary exposure to a heterologous strain, the previously matured antibody pool is recalled again but this pool, predominantly consists of non-neutralising or low-affinity binding antibodies, provides low to no protection to the new strain. A pool of novel antibody is also generated in addition to the existing pool of antibodies, on exposure to heterologous strain. The top panel shows the binding of the antibodies to each strain, low-affinity antibodies are tagged with light blue filled circle, high-affinity binding antibodies are tagged with dark blue filled circle, and non-neutralising antibodies are tagged with stop symbol. Structure visualisation and image rendering were performed using PyMOL.

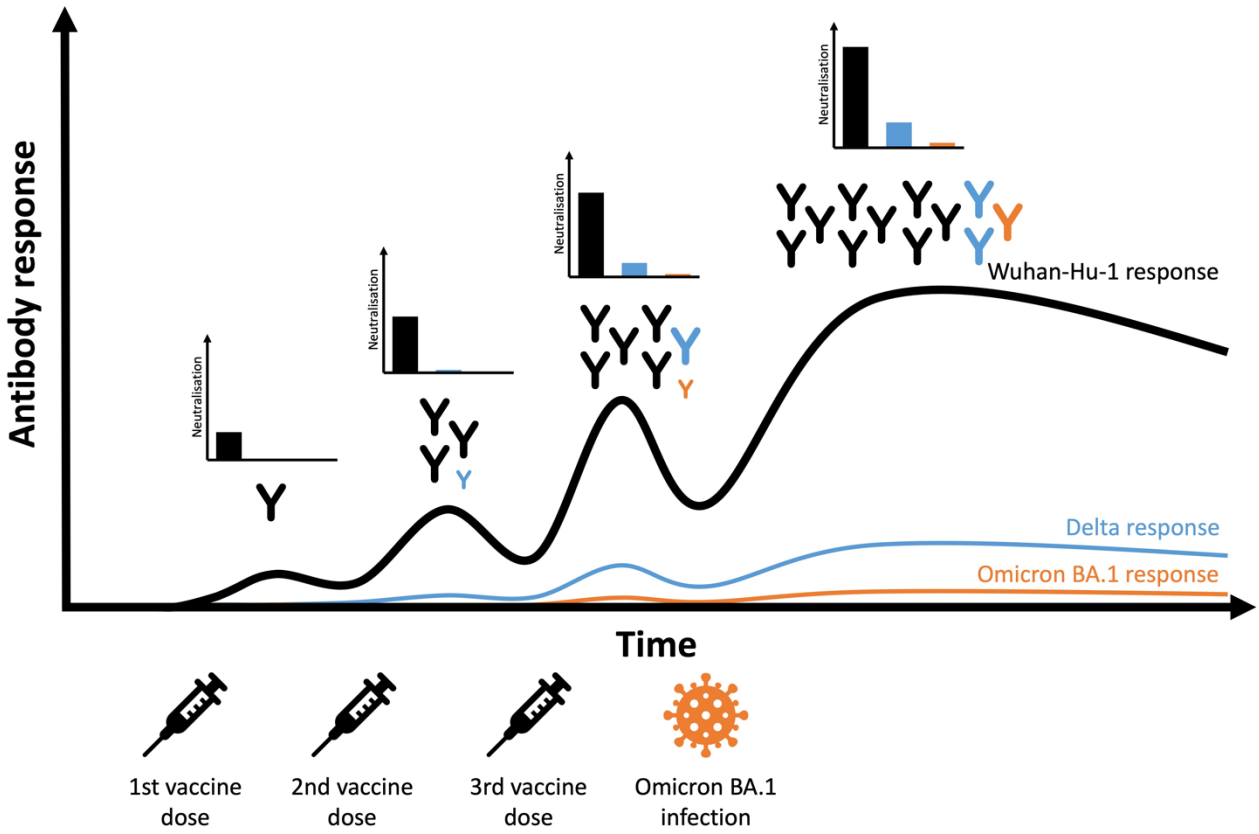

Figure 3. The antigenic seniority model: hierarchical nature of imprinting on the antibody responses to SARS-CoV-2 and its variants. Repeated vaccination of a Wuhan-Hu-1 spike-based vaccines primes and imprints Wuhan-Hu-1 specific antibody responses, with virus neutralisation potential increasing with each homologous boost. The breadth of the response also increases with each homologous boost in the form of cross-reactive and *de novo* VOC-specific responses, but these are at much lower orders of magnitude. Subsequent infection with a distantly related VOC, such as Omicron BA.1, greatly back-boosts the Wuhan-Hu-1 spike responses, whilst also boosting some responses to cross-reactive and conserved epitopes. The *de novo* generation of BA.1-specific responses is minor in comparison to the memory recall of the original antigenic imprinting of Wuhan-Hu-1 spike. Successive exposures to related antigens back-boosts pre-existing antibodies to the ancestral strain. Over time, increasing breadth is acquired in the host antibody repertoire against subsequent viral variants.

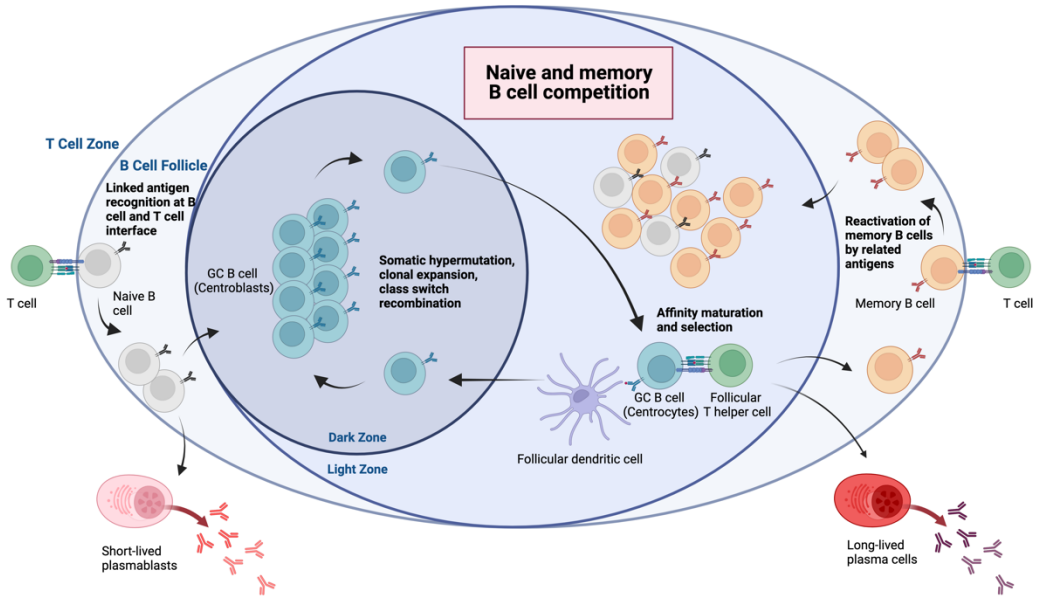

Figure 4. Germinal centre dynamics of imprinting: distraction, exhaustion and competitive inhibition
B cell responses to viral antigens are tailored to generate plasmablasts and plasma cells, which produce antibodies that neutralise, opsonise and activate complement against specific antigens on the invading pathogen. Long-lived memory cells are capable of rapid effector differentiation upon secondary exposure to the same antigen[167]. A typical T cell-dependent B cell response in peripheral lymphoid organs includes linked antigen recognition, the extrafollicular response leading to formation of the primary focus and plasmablast production, the germinal centre (GC) response leading to formation of the secondary follicle and effector plasma cell differentiation. The germinal centre response involves complex cellular interactions between resident stromal and follicular dendritic cells (DCs) and naïve B and T cells arriving from circulation and afferent lymph. Through rounds of somatic hypermutation, affinity maturation and class-switch recombination, B cells capable of producing the highest-affinity and class-switched antibodies are selected for expansion, generating a diverse, monoclonal pool of B effector cells that provides more specific and effective humoral immunity to the host. **Distraction and exhaustion:** Failure to form functional germinal centres is associated with catastrophic disease progression and outcomes[86]. Ongoing immune activation, cytokine release and non-protective antibody production can result in the exhaustion of immune cell populations and impaired disease resolution[168]. During severe COVID-19 disease, inflammatory cytokine TGFβ stimulates the chronic inflammatory state with continuous plasmablast release and persistence in circulation but reduced antibody specificity to viral proteins in a process denoted immune distraction[169]. In the presence of higher frequencies of stimulatory T helper cells, there is also excessive expansion, activation and exhaustion of memory B cells with reduced somatic hypermutation, class-switching and functional capacities[170]. **Competitive inhibition:** When a virus is encountered for the first time, an immunologically naïve host mounts a rapid, innate immune response as a primary line of defence, while allowing the slower induction of the primary adaptive response. The resulting time lag between infection and efficient control permits relatively unrestricted viral replication and generation of high viral antigen loads for naïve B cell recognition in peripheral lymphoid organs. As anamnestic responses accumulate in subsequent infections, the total antigen load decreases with the rapid onset of host defences. In the germinal centre reaction, naïve B cells targeting novel epitopes are also subjected to increasing competition with memory B cells targeting conserved epitopes for limited cognate antigen and follicular T helper cell (Tfh)-derived signals. Tfh cells are critical for B cell survival, proliferation and selection in germinal centres. Intrinsically, memory B cells exhibit significantly lower activation thresholds than naïve B cells, characterised by higher affinity antigen recognition via optimised B cell receptor (BCR) and elevated expression of major histocompatibility complex class II (MHC II) and co-stimulatory molecules. As the number of B cells within any one germinal centre is limited, rapid antigen acquisition and presentation by memory B cells occur at the expense of naïve B cell activation through competitive inhibition. Variable gene expression profiles between naïve and memory B cells also contribute to elevated effector differentiation activity of the latter, further facilitating a potent recall response upon antigen re-encouter[167].

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Author information

C.Q.H and J.L.H conceived the main ideas of the article. C.Q.H. researched data for and wrote the article. C.Q.H., S.V., G.W.C. and A.C.Y.C developed the figures with input from J.L.H.. All authors contributed substantially to discussion of the content, reviewed and/or edited the manuscript before submission.

Ethical declarations

Competing interests

J.L.H. is an employee of the University of Cambridge and the founder and CEO of DIOSynVax Ltd.. J.L.H., S.V. and G.W.C. are inventors of patents on influenza and coronavirus vaccines.

[i]References

[i] 1. Global Influenza Surveillance and Response System (GISRS). https://www.who.int/initiatives/global-influenza-surveillance-and-response-system.

2. Gaymard, A., Le Briand, N., Frobert, E., Lina, B. & Escuret, V. Functional balance between neuraminidase and haemagglutinin in influenza viruses. *Clinical Microbiology and Infection* **22**, 975–983 (2016).

3. Petrova, V. N. & Russell, C. A. The evolution of seasonal influenza viruses. *Nat Rev Microbiol* **16**, 47–60 (2018).

4. Telenti, A. *et al.* After the pandemic: perspectives on the future trajectory of COVID-19. *Nature* **596**, 495–504 (2021).

5. Lewnard, J. A. & Cobey, S. Immune History and Influenza Vaccine Effectiveness. *Vaccines (Basel)* **6**, E28 (2018).

6. Smith, D. J. *et al.* Mapping the antigenic and genetic evolution of influenza virus. *Science* **305**, 371–376 (2004).

7. Henry, C., Palm, A.-K. E., Krammer, F. & Wilson, P. C. From Original Antigenic Sin to the Universal Influenza Virus Vaccine. *Trends in Immunology* **39**, 70–79 (2018).

8. Biswas, A., Chakrabarti, A. K. & Dutta, S. Current challenges: from the path of "original antigenic sin" towards the development of universal flu vaccines. *International Reviews of Immunology* **39**, 21–36 (2020).

9. Yewdell, J. W. & Santos, J. J. S. Original Antigenic Sin: How Original? How Sinful? *Cold Spring Harb Perspect Med* **11**, a038786 (2021).

10. Carabelli, A. M. *et al.* SARS-CoV-2 variant biology: immune escape, transmission and fitness. *Nat Rev Microbiol* 1–16 (2023) doi:10.1038/s41579-022-00841-7.

11. Lan, J. *et al.* Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* **581**, 215–220 (2020).

12. Piccoli, L. *et al.* Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell* **183**, 1024-1042.e21 (2020).

13. Duchene, S. *et al.* Temporal signal and the phylodynamic threshold of SARS-CoV-2. *Virus Evolution* **6**, veaa061 (2020).
14. Tracking SARS-CoV-2 variants. https://www.who.int/activities/tracking-SARS-CoV-2-variants.
15. Cao, Y. *et al.* BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by Omicron infection. *Nature* **608**, 593–602 (2022).
16. Wang, Q. *et al.* Alarming antibody evasion properties of rising SARS-CoV-2 BQ and XBB subvariants. *Cell* (2022) doi:10.1016/j.cell.2022.12.018.
17. Simon-Loriere, E. & Schwartz, O. Towards SARS-CoV-2 serotypes? *Nat Rev Microbiol* **20**, 187–188 (2022).
18. Goldstein, S. A., Brown, J., Pedersen, B. S., Quinlan, A. R. & Elde, N. C. Extensive Recombination-driven Coronavirus Diversification Expands the Pool of Potential Pandemic Pathogens. *Genome Biology and Evolution* **14**, evac161 (2022).
19. Kurhade, C. *et al.* Low neutralization of SARS-CoV-2 Omicron BA.2.75.2, BQ.1.1 and XBB.1 by parental mRNA vaccine or a BA.5 bivalent booster. *Nat Med* 1–4 (2022) doi:10.1038/s41591-022-02162-x.
20. Chalkias, S. *et al.* Safety and Immunogenicity of Omicron BA.4/BA.5 Bivalent Vaccine Against Covid-19. 2022.12.11.22283166 Preprint at https://doi.org/10.1101/2022.12.11.22283166 (2022).
21. Khoury, D. S. *et al.* Predicting the efficacy of variant-modified COVID-19 vaccine boosters. *Nat Med* **29**, 574–578 (2023).
22. Chemaitelly, H. *et al.* Immune Imprinting and Protection against Repeat Reinfection with SARS-CoV-2. *New England Journal of Medicine* **387**, 1716–1718 (2022).
23. Aguilar-Bretones, M., Fouchier, R. A. M., Koopmans, M. P. G. & Nierop, G. P. van. Impact of antigenic evolution and original antigenic sin on SARS-CoV-2 immunity. *J Clin Invest* **133**, (2023).
24. Offit, P. A. Bivalent Covid-19 Vaccines — A Cautionary Tale. *New England Journal of Medicine* **0**, null (2023).
25. Hoehl, S. & Ciesek, S. Recalling ancestral SARS-CoV-2 variants: is it an original sin with benefits? *The Lancet Infectious Diseases* **0**, (2023).

26. Francis, T. On the Doctrine of Original Antigenic Sin. *Proceedings of the American Philosophical Society* **104**, 572–578 (1960).
27. Murray, S. M. *et al.* The impact of pre-existing cross-reactive immunity on SARS-CoV-2 infection and vaccine responses. *Nat Rev Immunol* **23**, 304–316 (2023).
28. Ng, K. W. *et al.* Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science* **370**, 1339–1343 (2020).
29. Rajendran, M. *et al.* Analysis of Anti-Influenza Virus Neuraminidase Antibodies in Children, Adults, and the Elderly by ELISA and Enzyme Inhibition: Evidence for Original Antigenic Sin. *mBio* **8**, e02281-16 (2017).
30. Rothman, A. L. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. *Nat Rev Immunol* **11**, 532–543 (2011).
31. Tripp, R. A. & Power, U. F. Original Antigenic Sin and Respiratory Syncytial Virus Vaccines. *Vaccines* **7**, 107 (2019).
32. Baraniak, I., Kern, F., Holenya, P., Griffiths, P. & Reeves, M. Original Antigenic Sin Shapes the Immunological Repertoire Evoked by Human Cytomegalovirus Glycoprotein B/MF59 Vaccine in Seropositive Recipients. *The Journal of Infectious Diseases* **220**, 228–232 (2019).
33. Lessler, J. *et al.* Evidence for Antigenic Seniority in Influenza A (H3N2) Antibody Responses in Southern China. *PLOS Pathogens* **8**, e1002802 (2012).
34. Shrock, E. *et al.* Viral epitope profiling of COVID-19 patients reveals cross-reactivity and correlates of severity. *Science* **370**, eabd4250 (2020).
35. Woudenberg, T. *et al.* Humoral immunity to SARS-CoV-2 and seasonal coronaviruses in children and adults in north-eastern France. *eBioMedicine* **70**, (2021).
36. Aguilar-Bretones, M. *et al.* Seasonal coronavirus–specific B cells with limited SARS-CoV-2 cross-reactivity dominate the IgG response in severe COVID-19. *J Clin Invest* **131**, (2021).
37. McNaughton, A. L. *et al.* Fatal COVID-19 outcomes are associated with an antibody response targeting epitopes shared with endemic coronaviruses. *JCI Insight* **7**, e156372.
38. Anderson, E. M. *et al.* Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection. *Cell* **184**, 1858-1864.e10 (2021).

39. Prévost, J. *et al.* Cross-Sectional Evaluation of Humoral Responses against SARS-CoV-2 Spike. *CR Med* **1**, (2020).

40. Lv, H. *et al.* Cross-reactive Antibody Response between SARS-CoV-2 and SARS-CoV Infections. *Cell Reports* **31**, 107725 (2020).

41. Yuan, M. *et al.* A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. *Science* **368**, 630–633 (2020).

42. Tan, C.-W. *et al.* Pan-Sarbecovirus Neutralizing Antibodies in BNT162b2-Immunized SARS-CoV-1 Survivors. *New England Journal of Medicine* **385**, 1401–1406 (2021).

43. Zar, H. J. *et al.* Natural and hybrid immunity following four COVID-19 waves: A prospective cohort study of mothers in South Africa. *eClinicalMedicine* **53**, (2022).

44. Goel, R. R. *et al.* Efficient recall of Omicron-reactive B cell memory after a third dose of SARS-CoV-2 mRNA vaccine. *Cell* **185**, 1875-1887.e8 (2022).

45. Röltgen, K. *et al.* Immune imprinting, breadth of variant recognition, and germinal center response in human SARS-CoV-2 infection and vaccination. *Cell* **185**, 1025-1040.e14 (2022).

46. Kaku, C. I. *et al.* Recall of preexisting cross-reactive B cell memory after Omicron BA.1 breakthrough infection. *Science Immunology* **7**, eabq3511 (2022).

47. Hoffmann, M. *et al.* Effect of hybrid immunity and bivalent booster vaccination on omicron sublineage neutralisation. *The Lancet Infectious Diseases* **23**, 25–28 (2023).

48. Chu, L. *et al.* Immune response to SARS-CoV-2 after a booster of mRNA-1273: an open-label phase 2 trial. *Nat Med* **28**, 1042–1049 (2022).

49. Alsoussi, W. B. *et al.* SARS-CoV-2 Omicron boosting induces de novo B cell response in humans. *Nature* **617**, 592–598 (2023).

50. Chalkias, S. *et al. Safety, Immunogenicity and Antibody Persistence of a Bivalent Beta-Containing Booster Vaccine*. https://www.researchsquare.com/article/rs-1555201/v1 (2022) doi:10.21203/rs.3.rs-1555201/v1.

51. Chalkias, S. *et al.* A Bivalent Omicron-Containing Booster Vaccine against Covid-19. *New England Journal of Medicine* **387**, 1279–1291 (2022).

52. Zou, J. *et al.* Neutralization of BA.4–BA.5, BA.4.6, BA.2.75.2, BQ.1.1, and XBB.1 with Bivalent Vaccine. *New England Journal of Medicine* **388**, 854–857 (2023).
53. Wang, Q. *et al.* Antibody Response to Omicron BA.4–BA.5 Bivalent Booster. *New England Journal of Medicine* **388**, 567–569 (2023).
54. Collier, A. Y. *et al.* Immunogenicity of BA.5 Bivalent mRNA Vaccine Boosters. *N Engl J Med* **388**, 565–567 (2023).
55. Davis-Gardner, M. E. *et al.* Neutralization against BA.2.75.2, BQ.1.1, and XBB from mRNA Bivalent Booster. *N Engl J Med* **388**, 183–185 (2023).
56. Statement on the antigen composition of COVID-19 vaccines. https://www.who.int/news/item/18-05-2023-statement-on-the-antigen-composition-of-covid-19-vaccines.
57. Chalkias, S. *et al.* Safety and Immunogenicity of XBB.1.5-Containing mRNA Vaccines. 2023.08.22.23293434 Preprint at https://doi.org/10.1101/2023.08.22.23293434 (2023).
58. Pfizer and BioNTech Receive Positive CHMP Opinion for Omicron XBB.1.5-adapted COVID-19 Vaccine in the European Union | Pfizer. https://www.pfizer.com/news/press-release/press-release-detail/pfizer-and-biontech-receive-positive-chmp-opinion-omicron-2.
59. Jones-Gray, E., Robinson, E. J., Kucharski, A. J., Fox, A. & Sullivan, S. G. Does repeated influenza vaccination attenuate effectiveness? A systematic review and meta-analysis. *The Lancet Respiratory Medicine* **11**, 27–44 (2023).
60. Gostic, K. M. *et al.* Childhood immune imprinting to influenza A shapes birth year-specific risk during seasonal H1N1 and H3N2 epidemics. *PLOS Pathogens* **15**, e1008109 (2019).
61. Petrie, J. G. *et al.* Antibodies Against the Current Influenza A(H1N1) Vaccine Strain Do Not Protect Some Individuals From Infection With Contemporary Circulating Influenza A(H1N1) Virus Strains. *J Infect Dis* **214**, 1947–1951 (2016).
62. Linderman, S. L. *et al.* Potential antigenic explanation for atypical H1N1 infections among middle-aged adults during the 2013–2014 influenza season. *Proceedings of the National Academy of Sciences* **111**, 15798–15803 (2014).
63. Gagnon, A. *et al.* Age-specific mortality during the 1918 influenza pandemic: unravelling the mystery of high young adult mortality. *PLoS One* **8**, e69586 (2013).

64. Ma, J., Dushoff, J. & Earn, D. J. D. Age-specific mortality risk from pandemic influenza. *J Theor Biol* **288**, 29–34 (2011).

65. Gagnon, A. *et al.* Pandemic Paradox: Early Life H2N2 Pandemic Influenza Infection Enhanced Susceptibility to Death during the 2009 H1N1 Pandemic. *mBio* **9**, e02091-17 (2018).

66. Gagnon, A., Acosta, J. E., Madrenas, J. & Miller, M. S. Is Antigenic Sin Always "Original?" Re-examining the Evidence Regarding Circulation of a Human H1 Influenza Virus Immediately Prior to the 1918 Spanish Flu. *PLOS Pathogens* **11**, e1004615 (2015).

67. Simonsen, L. *et al.* Global mortality estimates for the 2009 Influenza Pandemic from the GLaMOR project: a modeling study. *PLoS Med* **10**, e1001558 (2013).

68. O'Donnell, C. D. *et al.* Humans and Ferrets with Prior H1N1 Influenza Virus Infections Do Not Exhibit Evidence of Original Antigenic Sin after Infection or Vaccination with the 2009 Pandemic H1N1 Influenza Virus. *Clinical and Vaccine Immunology* **21**, 737–746 (2014).

69. Gostic, K. M., Ambrose, M., Worobey, M. & Lloyd-Smith, J. O. Potent protection against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting. *Science* **354**, 722–726 (2016).

70. Wratil, P. R. *et al.* Evidence for increased SARS-CoV-2 susceptibility and COVID-19 severity related to pre-existing immunity to seasonal coronaviruses. *Cell Rep* **37**, 110169 (2021).

71. Aran, D., Beachler, D. C., Lanes, S. & Overhage, J. M. Prior presumed coronavirus infection reduces COVID-19 risk: A cohort study. *Journal of Infection* **81**, 923–930 (2020).

72. Sagar, M. *et al.* Recent endemic coronavirus infection is associated with less-severe COVID-19. *J Clin Invest* **131**, (2021).

73. Smit, W. L. *et al.* Heterologous Immune Responses of Serum IgG and Secretory IgA Against the Spike Protein of Endemic Coronaviruses During Severe COVID-19. *Frontiers in Immunology* **13**, (2022).

74. Aydillo, T. *et al.* Immunological imprinting of the antibody response in COVID-19 patients. *Nat Commun* **12**, 3781 (2021).

75. Guo, L. *et al.* Cross-reactive antibody against human coronavirus OC43 spike protein correlates with disease severity in COVID-19 patients: a retrospective study. *Emerging Microbes & Infections* **10**, 664–676 (2021).

76. Woodruff, M. C. *et al.* Extrafollicular B cell responses correlate with neutralizing antibodies and morbidity in COVID-19. *Nat Immunol* **21**, 1506–1516 (2020).

77. Dejnirattisai, W. *et al.* The antigenic anatomy of SARS-CoV-2 receptor binding domain. *Cell* **184**, 2183-2200.e22 (2021).

78. Kaplonek, P. *et al.* Early cross-coronavirus reactive signatures of humoral immunity against COVID-19. *Science Immunology* **6**, eabj2901 (2021).

79. Pinotti, F. *et al.* Potential impact of individual exposure histories to endemic human coronaviruses on age-dependent severity of COVID-19. *BMC Medicine* **19**, 19 (2021).

80. Thompson, R. N. *et al.* The impact of cross-reactive immunity on the emergence of SARS-CoV-2 variants. *Frontiers in Immunology* **13**, (2023).

81. Link-Gelles, R. Effectiveness of Bivalent mRNA Vaccines in Preventing Symptomatic SARS-CoV-2 Infection — Increasing Community Access to Testing Program, United States, September–November 2022. *MMWR Morb Mortal Wkly Rep* **71**, (2022).

82. Smith, D. J., Forrest, S., Ackley, D. H. & Perelson, A. S. Variable efficacy of repeated annual influenza vaccination. *Proc Natl Acad Sci U S A* **96**, 14001–14006 (1999).

83. Wrammert, J. *et al.* Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection. *J Exp Med* **208**, 181–193 (2011).

84. Cortina-Ceballos, B. *et al.* Longitudinal analysis of the peripheral B cell repertoire reveals unique effects of immunization with a new influenza virus strain. *Genome Medicine* **7**, 124 (2015).

85. Avnir, Y. *et al.* Molecular signatures of hemagglutinin stem-directed heterosubtypic human neutralizing antibodies against influenza A viruses. *PLoS Pathog* **10**, e1004103 (2014).

86. Laidlaw, B. J. & Ellebedy, A. H. The germinal centre B cell response to SARS-CoV-2. *Nat Rev Immunol* **22**, 7–18 (2022).

87. Gaebler, C. *et al.* Evolution of antibody immunity to SARS-CoV-2. *Nature* **591**, 639–644 (2021).

88. Kim, W. *et al.* Germinal centre-driven maturation of B cell response to mRNA vaccination. *Nature* **604**, 141–145 (2022).

89. Wang, Z. *et al.* Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature* **595**, 426–431 (2021).

90. Wang, Z. *et al.* Memory B cell responses to Omicron subvariants after SARS-CoV-2 mRNA breakthrough infection in humans. *Journal of Experimental Medicine* **219**, e20221006 (2022).

91. Moriyama, S. *et al.* Temporal maturation of neutralizing antibodies in COVID-19 convalescent individuals improves potency and breadth to circulating SARS-CoV-2 variants. *Immunity* **54**, 1841-1852.e4 (2021).

92. Sokal, A. *et al.* Maturation and persistence of the anti-SARS-CoV-2 memory B cell response. *Cell* **184**, 1201-1213.e14 (2021).

93. Dugan, H. L. *et al.* Profiling B cell immunodominance after SARS-CoV-2 infection reveals antibody evolution to non-neutralizing viral targets. *Immunity* **54**, 1290-1303.e7 (2021).

94. Muecksch, F. *et al.* Increased memory B cell potency and breadth after a SARS-CoV-2 mRNA boost. *Nature* **607**, 128–134 (2022).

95. Park, Y.-J. *et al.* Imprinted antibody responses against SARS-CoV-2 Omicron sublineages. *Science* **378**, 619–627 (2022).

96. Reynolds, C. J. *et al.* Immune boosting by B.1.1.529 (Omicron) depends on previous SARS-CoV-2 exposure. *Science* **377**, eabq1841 (2022).

97. Cao, Y. *et al.* Imprinted SARS-CoV-2 humoral immunity induces convergent Omicron RBD evolution. *Nature* **614**, 521–529 (2023).

98. Quandt, J. *et al.* Omicron BA.1 breakthrough infection drives cross-variant neutralization and memory B cell formation against conserved epitopes. *Science Immunology* **7**, eabq2427 (2022).

99. Kaku, C. I. *et al.* Evolution of antibody immunity following Omicron BA.1 breakthrough infection. *Nat Commun* **14**, 2751 (2023).

100. Reynolds, C. J. *et al.* Heterologous infection and vaccination shapes immunity against SARS-CoV-2 variants. *Science* **375**, 183–192 (2022).

101. Butler, D. Long-term studies will track indelible marks of first flu. *Nature* **569**, 464–465 (2019).

102. Kim, J. H., Skountzou, I., Compans, R. & Jacob, J. Original Antigenic Sin Responses to Influenza Viruses. *The Journal of Immunology* **183**, 3294–3301 (2009).

103. Fox, A. *et al.* Opposing Effects of Prior Infection versus Prior Vaccination on Vaccine Immunogenicity against Influenza A(H3N2) Viruses. *Viruses* **14**, 470 (2022).

104. Saito, N. *et al.* Negative impact of prior influenza vaccination on current influenza vaccination among people infected and not infected in prior season: A test-negative case-control study in Japan. *Vaccine* **35**, 687–693 (2017).

105. Greaney, A. J. *et al.* Antibodies elicited by mRNA-1273 vaccination bind more broadly to the receptor binding domain than do those from SARS-CoV-2 infection. *Science Translational Medicine* **13**, eabi9915 (2021).

106. Amanat, F. *et al.* SARS-CoV-2 mRNA vaccination induces functionally diverse antibodies to NTD, RBD, and S2. *Cell* **184**, 3936-3948.e10 (2021).

107. Pillai, S. SARS-CoV-2 vaccination washes away original antigenic sin. *Trends in Immunology* **43**, 271–273 (2022).

108. Kaneko, N. *et al.* Loss of Bcl-6-Expressing T Follicular Helper Cells and Germinal Centers in COVID-19. *Cell* **183**, 143-157.e13 (2020).

109. Buckner, C. M. *et al.* Interval between prior SARS-CoV-2 infection and booster vaccination impacts magnitude and quality of antibody and B cell responses. *Cell* **185**, 4333-4346.e14 (2022).

110. Worobey, M., Plotkin, S. & Hensley, S. E. Influenza Vaccines Delivered in Early Childhood Could Turn Antigenic Sin into Antigenic Blessings. *Cold Spring Harb Perspect Med* **10**, a038471 (2020).

111. Bodewes, R. *et al.* Prevalence of Antibodies against Seasonal Influenza A and B Viruses in Children in Netherlands. *Clin Vaccine Immunol* **18**, 469–476 (2011).

112. Belshe, R. B. *et al.* Live attenuated versus inactivated influenza vaccine in infants and young children. *N Engl J Med* **356**, 685–696 (2007).

113. Pilapitiya, D., Wheatley, A. K. & Tan, H.-X. Mucosal vaccines for SARS-CoV-2: triumph of hope over experience. *eBioMedicine* **92**, (2023).

114. Meade, P. *et al.* Antigenic Landscape Analysis of Individuals Vaccinated with a Universal Influenza Virus Vaccine Candidate Reveals Induction of Cross-Subtype Immunity. *Journal of Virology* **97**, e01070-22 (2022).

115. Krammer, F. The Quest for a Universal Flu Vaccine: Headless HA 2.0. *Cell Host & Microbe* **18**, 395–397 (2015).

116. Widge, A. T. *et al.* An influenza hemagglutinin stem nanoparticle vaccine induces cross-group 1 neutralizing antibodies in healthy adults. *Science Translational Medicine* **15**, eade4790 (2023).

117. Andrews, S. F. *et al.* An influenza H1 hemagglutinin stem-only immunogen elicits a broadly cross-reactive B cell response in humans. *Science Translational Medicine* **15**, eade4976 (2023).

118. Dai, L. *et al.* Efficacy and Safety of the RBD-Dimer–Based Covid-19 Vaccine ZF2001 in Adults. *New England Journal of Medicine* **386**, 2097–2111 (2022).

119. Hernández-Bernal, F. *et al.* Safety, tolerability, and immunogenicity of a SARS-CoV-2 recombinant spike RBD protein vaccine: A randomised, double-blind, placebo-controlled, phase 1-2 clinical trial (ABDALA Study). *eClinicalMedicine* **46**, (2022).

120. Ng, K. W. *et al.* SARS-CoV-2 S2–targeted vaccination elicits broadly neutralizing antibodies. *Science Translational Medicine* **14**, eabn3715 (2022).

121. Braet, S. M. *et al.* Timeline of changes in spike conformational dynamics in emergent SARS-CoV-2 variants reveal progressive stabilization of trimer stalk with altered NTD dynamics. *eLife* **12**, e82584 (2023).

122. Nachbagauer, R. *et al.* A chimeric hemagglutinin-based universal influenza virus vaccine approach induces broad and long-lasting immunity in a randomized, placebo-controlled phase I trial. *Nat Med* **27**, 106–114 (2021).

123. Broecker, F. *et al.* A mosaic hemagglutinin-based influenza virus vaccine candidate protects mice from challenge with divergent H3N2 strains. *npj Vaccines* **4**, 1–9 (2019).

124. Sun, W. *et al.* Development of Influenza B Universal Vaccine Candidates Using the "Mosaic" Hemagglutinin Approach. *Journal of Virology* **93**, (2019).

125. Eggink, D., Goff, P. H. & Palese, P. Guiding the Immune Response against Influenza Virus Hemagglutinin toward the Conserved Stalk Domain by Hyperglycosylation of the Globular Head Domain. *J Virol* **88**, 699–704 (2014).

126. Martinez, D. R. *et al.* Chimeric spike mRNA vaccines protect against Sarbecovirus challenge in mice. *Science* **373**, 991–998 (2021).

127. Xu, K. *et al.* Protective prototype-Beta and Delta-Omicron chimeric RBD-dimer vaccines against SARS-CoV-2. *Cell* **185**, 2265-2278.e14 (2022).

128. Thompson, C. P. *et al.* A naturally protective epitope of limited variability as an influenza vaccine target. *Nature Communications* **9**, 3859 (2018).

129. Ross, T. M. *et al.* A computationally designed H5 antigen shows immunological breadth of coverage and protects against drifting avian strains. *Vaccine* **37**, 2369–2376 (2019).

130. Allen, J. D. & Ross, T. M. Bivalent H1 and H3 COBRA Recombinant Hemagglutinin Vaccines Elicit Seroprotective Antibodies against H1N1 and H3N2 Influenza Viruses from 2009 to 2019. *Journal of Virology* **96**, e01652-21 (2022).

131. Allen, J. D. & Ross, T. M. Next generation methodology for updating HA vaccines against emerging human seasonal influenza A(H3N2) viruses. *Sci Rep* **11**, 4554 (2021).

132. Carnell, G. *et al.* Glycan masking of a non-neutralising epitope enhances neutralising antibodies targeting the RBD of SARS-CoV-2 and its variants. *Frontiers in Immunology* **14**, 1118523 (2023).

133. Heeney, J. *et al.* Gene delivery of a single,structurally engineered Coronavirus vaccine antigen elicits SARS-CoV-2 Omicron and pan-Sarbecovirus neutralisatio. Preprint at https://doi.org/10.21203/rs.3.rs-995273/v1 (2022).

134. Fries, C. N. *et al.* Advances in nanomaterial vaccine strategies to address infectious diseases impacting global health. *Nat. Nanotechnol.* **16**, 1–14 (2021).

135. Kanekiyo, M. *et al.* Mosaic nanoparticle display of diverse influenza virus hemagglutinins elicits broad B cell responses. *Nature Immunology* **20**, 362–372 (2019).

136. Yuan, Y. *et al.* A bivalent nanoparticle vaccine exhibits potent cross-protection against the variants of SARS-CoV-2. *Cell Reports* **38**, (2022).

137. Chen, R. *et al.* Development of Receptor Binding Domain (RBD)-Conjugated Nanoparticle Vaccines with Broad Neutralization against SARS-CoV-2 Delta and Other Variants. *Adv Sci (Weinh)* **9**, 2105378 (2022).

138. Geng, Q. *et al.* Novel virus-like nanoparticle vaccine effectively protects animal model from SARS-CoV-2 infection. *PLoS Pathog* **17**, e1009897 (2021).

139. Ueda, G. *et al.* Tailored design of protein nanoparticle scaffolds for multivalent presentation of viral glycoprotein antigens. *eLife* **9**, e57659 (2020).

140. Boyoglu-Barnum, S. *et al.* Quadrivalent influenza nanoparticle vaccines induce broad protection. *Nature* **592**, 623–628 (2021).

141. SK bioscience and GSK's adjuvanted COVID-19 vaccine candidate meets coprimary objectives in a phase III study; Biologics License Application submitted for SKYCovione^{TM}(GBP510/GSK adjuvant) in South Korea | GSK. https://www.gsk.com/en-gb/media/press-releases/sk-bioscience-and-gsk-s-adjuvanted-covid-19-vaccine-candidate-meets-coprimary-objectives-in-a-phase-iii-study/ (2022).

142. Walls, A. C. *et al.* Elicitation of broadly protective sarbecovirus immunity by receptor-binding domain nanoparticle vaccines. *Cell* **184**, 5432-5447.e16 (2021).

143. Kang, Y.-F. *et al.* Quadrivalent mosaic HexaPro-bearing nanoparticle vaccine protects against infection of SARS-CoV-2 variants. *Nat Commun* **13**, 2674 (2022).

144. Bruun, T. U. J., Andersson, A.-M. C., Draper, S. J. & Howarth, M. Engineering a Rugged Nanoscaffold To Enhance Plug-and-Display Vaccination. *ACS Nano* **12**, 8855–8866 (2018).

145. Rahikainen, R. *et al.* Overcoming Symmetry Mismatch in Vaccine Nanoassembly through Spontaneous Amidation. *Angewandte Chemie International Edition* **60**, 321–330 (2021).

146. Cohen, A. A. *et al.* Mosaic nanoparticles elicit cross-reactive immune responses to zoonotic coronaviruses in mice. *Science* **371**, 735–741 (2021).

147. Cohen, A. A. *et al.* Mosaic RBD nanoparticles protect against challenge by diverse sarbecoviruses in animal models. *Science* **0**, eabq0839 (2022).

148. Hills, R. A. *et al.* Multiviral Quartet Nanocages Elicit Broad Anti-Coronavirus Responses for Proactive Vaccinology. 2023.02.24.529520 Preprint at https://doi.org/10.1101/2023.02.24.529520 (2023).

149. Zuccarino-Catania, G. V. *et al.* CD80 and PD-L2 define functionally distinct memory B cell subsets that are independent of antibody isotype. *Nat Immunol* **15**, 631–637 (2014).

150. McGrath, J. J. C., Li, L. & Wilson, P. C. Memory B cell diversity: insights for optimized vaccine design. *Trends Immunol* (2022) doi:10.1016/j.it.2022.03.005.

151. Chan, T. D. *et al.* Elimination of Germinal-Center-Derived Self-Reactive B Cells Is Governed by the Location and Concentration of Self-Antigen. *Immunity* **37**, 893–904 (2012).

152. Shokat, K. M. & Goodnow, C. C. Antigen-induced B-cell death and elimination during germinal-centre immune responses. *Nature* **375**, 334–338 (1995).

153. Pulendran, B., Kannourakis, G., Nouri, S., Smith, K. G. & Nossal, G. J. Soluble antigen can cause enhanced apoptosis of germinal-centre B cells. *Nature* **375**, 331–334 (1995).

154. Silva, M. *et al.* Targeted Elimination of Immunodominant B Cells Drives the Germinal Center Reaction toward Subdominant Epitopes. *Cell Reports* **21**, 3672–3680 (2017).

155. Meyer-Hermann, M. Injection of Antibodies against Immunodominant Epitopes Tunes Germinal Centers to Generate Broadly Neutralizing Antibodies. *Cell Reports* **29**, 1066-1073.e5 (2019).

156. Angeletti, D. *et al.* Outflanking immunodominance to target subdominant broadly neutralizing epitopes. *Proceedings of the National Academy of Sciences* **116**, 13474–13479 (2019).

157. Liu, H. *et al.* Structure-based programming of lymph-node targeting in molecular vaccines. *Nature* **507**, 519–522 (2014).

158. Caminschi, I. & Shortman, K. Boosting antibody responses by targeting antigens to dendritic cells. *Trends Immunol* **33**, 71–77 (2012).

159. Kim, J. H., Davis, W. G., Sambhara, S. & Jacob, J. Strategies to alleviate original antigenic sin responses to influenza viruses. *Proceedings of the National Academy of Sciences* **109**, 13751–13756 (2012).

160. Netea, M. G. *et al.* Defining trained immunity and its role in health and disease. *Nat Rev Immunol* **20**, 375–388 (2020).

161. Koutsakos, M. & Ellebedy, A. H. Immunological imprinting: Understanding COVID-19. *Immunity* **56**, 909–913 (2023).

162. Monge, S., Pastor-Barriuso, R. & Hernán, M. A. The imprinting effect of covid-19 vaccines: an expected selection bias in observational studies. *BMJ* **381**, e074404 (2023).

163. Barda, N. The ups and downs of observational vaccine research. *The Lancet Infectious Diseases* **23**, 767–768 (2023).

164. Yisimayi, A. *et al.* Repeated Omicron exposures override ancestral SARS-CoV-2 immune imprinting. 2023.05.01.538516 Preprint at https://doi.org/10.1101/2023.05.01.538516 (2023).

165. Nuñez, N. G. *et al.* High-dimensional analysis of 16 SARS-CoV-2 vaccine combinations reveals lymphocyte signatures correlating with immunogenicity. *Nat Immunol* 1–14 (2023) doi:10.1038/s41590-023-01499-w.

166. Muik, A. *et al.* Progressive loss of conserved spike protein neutralizing antibody sites in Omicron sublineages is balanced by preserved T-cell recognition epitopes. 2022.12.15.520569 Preprint at https://doi.org/10.1101/2022.12.15.520569 (2022).

167. Inoue, T. & Kurosaki, T. Memory B cells. *Nat Rev Immunol* 1–13 (2023) doi:10.1038/s41577-023-00897-3.

168. Mohammed, R. N. *et al.* A comprehensive review about immune responses and exhaustion during coronavirus disease (COVID-19). *Cell Communication and Signaling* **20**, 79 (2022).

169. Ferreira-Gomes, M. *et al.* SARS-CoV-2 in severe COVID-19 induces a TGF-β-dominated chronic immune response that does not target itself. *Nat Commun* **12**, 1961 (2021).

170. Pušnik, J. *et al.* Memory B cells targeting SARS-CoV-2 spike protein and their dependence on CD4+ T cell help. *Cell Reports* **35**, (2021).