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Immune imprinting and next-generation coronavirus vaccines

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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 immunizations, population-wide declines in the neutralization 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. Here 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.

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 reformulated according to World Health Organization (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, and 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], so annual vaccine updates and booster immunizations are required (Table 1 and Fig. 1). Typically, an eight- to tenfold reduction in antisera titres in the gold-standard HA 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 a long 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

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Table 1 | Comparison of influenza virus and SARS-CoV-2

Comparison of viruses		
Virus	**Influenza**	**SARS-CoV-2**
Genome	Negative-sense, single-stranded RNA	Positive-sense, single-stranded RNA
	Segmented	Non-segmented
	14 kB	30 kB
	Multiple co-circulating antigenically distinct subtypes	Multiple co-circulating lineages with successive antibody evasion properties
Receptor-binding glycoprotein	HA	Spike
	Receptor-binding pocket	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 (239 Å^2)	Large (924 Å^2)
Hypervariable and immunodominant regions	HA head	Spike receptor-binding domain
Antigenic evolution	Drift	Drift
	Shift via reassortment	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 sites
Other viral surface proteins	NA, matrix 2	Envelope protein, membrane protein
Seasonality	Peak during winter months in respective hemispheres	Less apparent
Comparison of booster vaccines		
	Influenza	**SARS-CoV-2**
Dominant licensed platform	Whole inactivated vaccines	mRNA vaccines
	Live attenuated vaccines	
Antigen target	HA	Spike
Antigen content	HA, NA, 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)	Monovalent (Wuhan-Hu-1 or XBB.1.5)
	Quadrivalent (H1N1, H3N2, Victoria, Yamagata)	Bivalent (Wuhan-Hu-1, BA.1; Wuhan-Hu-1, BA.4/BA.5; BA.4/BA.5, XBB.1.5)

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 sublineages 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 sublineages 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 the 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 neutralizing antibody titres in COVID-19 mRNA-vaccinee antisera tested against the Beta VOC had already exceeded tenfold, 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 neutralizing 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 neutralizing 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 (Fig. 2). The 'original antigenic sin (OAS) model' suggests that an individual's early exposures to a virus limit 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 to which an individual is exposed, 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 (Fig. 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.

In this Review, we examine 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 and 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 be attributed to the 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 neutralizing antibody responses were rare and weak[40]. This is consistent with a study on the CR3022 epitope that is known to be neutralizing for SARS-CoV-1, which, despite induction of strong binding antibody responses to SARS-CoV-2 S, failed to elicit neutralization[41]. Another study reported that SARS-CoV-1 survivors vaccinated with two doses of BNT162b2 exhibited uniform boosting in anti-SARS-CoV-1 neutralization without compromising anti-SARS-CoV-2 immunity. Sera from this group also elicited near-saturation, cross-clade, pan-sarbecovirus neutralization 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 immunoglobulin G (IgG) responses through Wuhan-Hu-1, Beta, Delta and Omicron waves, and reported a 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 neutralization of the latest sublineages, 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

Fig. 1 | Immune responses to influenza virus and SARS-CoV-2 in the context of infection and vaccination. a, Cartoon representation of the structure of influenza A H1N1 HA head and the RBD region of the SARS-CoV-2 S protein binding to sialic acid and human angiotensin-converting enzyme 2 (hACE-2), respectively. HA is coloured in light blue, S in green, sialic acid and hACE-2 in yellow, and interacting regions in dark blue. The receptor-binding site of SARS-CoV-2 is more than three times greater than that of 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 immune responses allow for viral infection in naive as well as previously infected hosts. TMPRSS2, transmembrane serine protease 2. **c**, Antibodies preventing attachment to the cellular receptor in 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 to which the immune system is exposed differs between natural infection and vaccination depending on the vaccine platform employed. Responses are restricted to the S 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 HA, not NA*. **e**, T cell responses are important for viral clearance and symptom severity, and are less susceptible to imprinting. IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α. **f**, Fc receptor (FcR)-based immunity (antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP)) plays an important role in immunity to both viruses. **g**, Temporal antigenic evolution of the influenza H1N1, and SARS-CoV-2. Surface representation of the ectodomain of the HA of influenza, and spike protein of SARS-CoV-2. The tree represents the phylogenetic relationship between different strains of influenza, and SARS-CoV-2 (not drawn to scale). The differences in amino acids with respect to the oldest strain is highlighted in red. Structure visualization and image rendering were performed using PyMOL.

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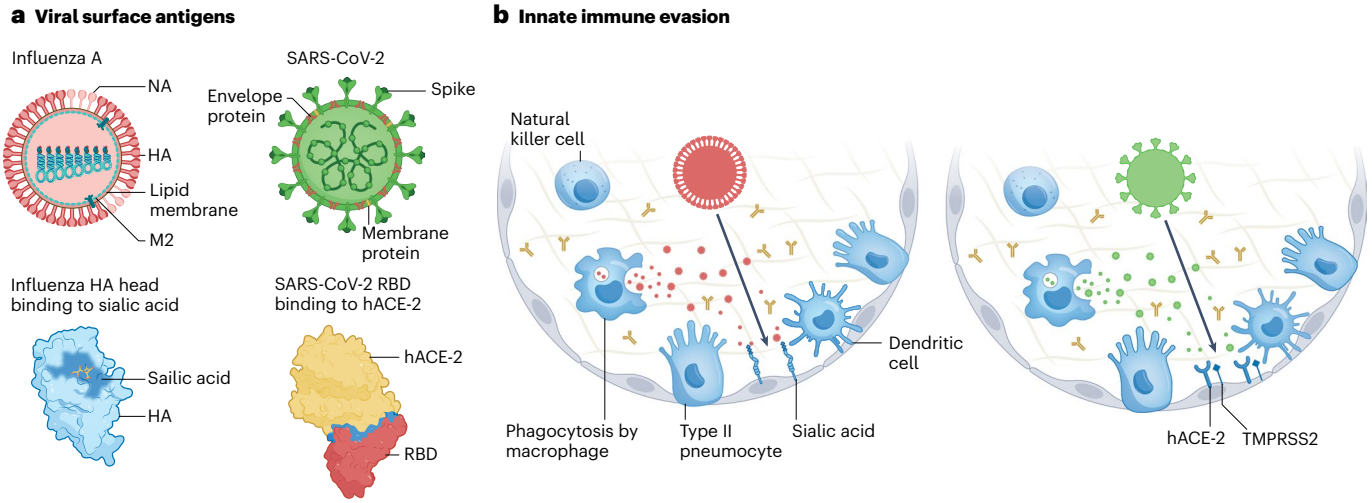
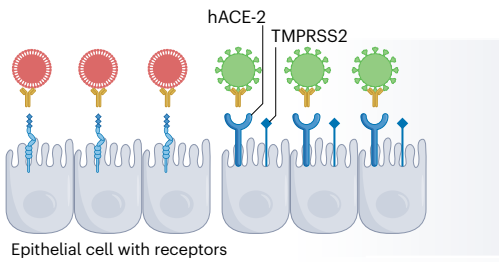
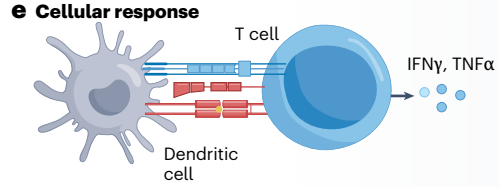
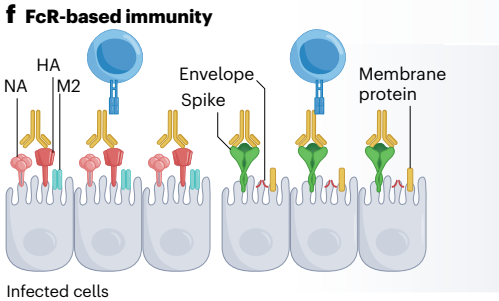


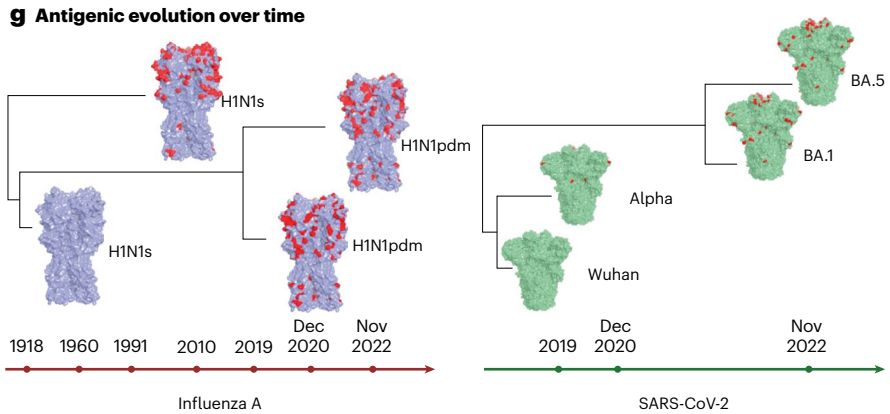






d Antigen exposure

	Influenza	SARS-CoV-2
Vaccination	HA, NA*	Spike
Infection	HA, NA	Spike, envelope, membrane
Vaccination	HA, NA*	Spike
Infection	HA, NA, internal proteins	Spike, envelope, membrane, internal proteins
Vaccination	HA stem	Spike
Infection	HA stem, NA, M2	Spike, envelope, membrane, other proteins



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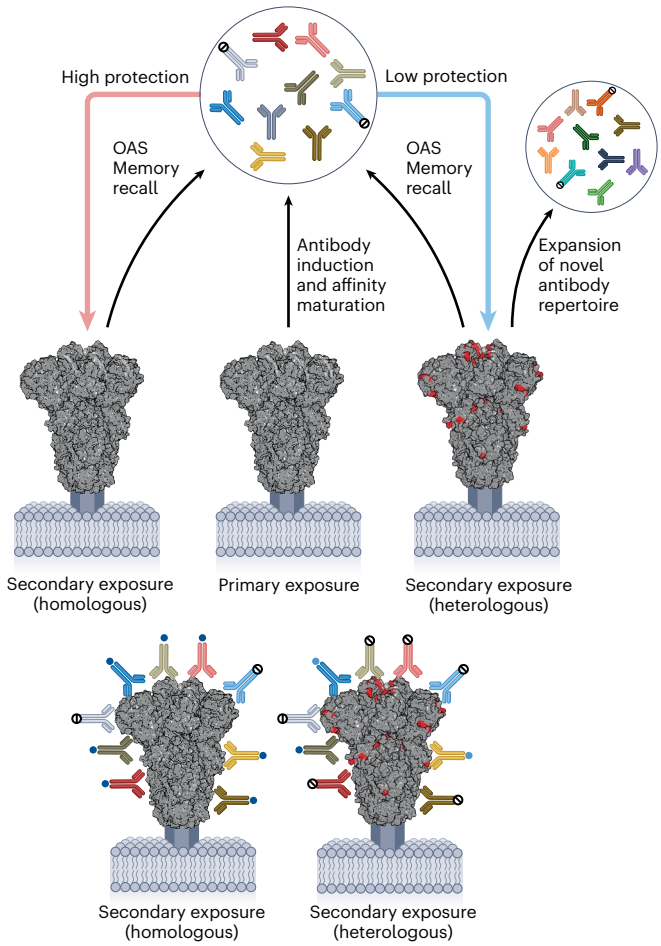

Fig. 2 | Primary exposure to a virus generates a pool of antibodies with both neutralizing and non-neutralizing paratopes. On secondary exposure to a homologous strain, the previously matured antibody pool is recalled and provides better protection to the homologous strain. On secondary exposure to a heterologous strain, the previously matured antibody pool is recalled again, but this pool predominantly consists of non-neutralizing or low-affinity binding antibodies, and provides low to no protection against the new strain. On exposure to the heterologous strain, a pool of novel antibodies is also generated in addition to the existing pool of antibodies. The bottom panel shows the binding of the antibodies to each strain. Low-affinity antibodies are tagged with a light-blue filled circle, high-affinity binding antibodies with a dark-blue filled circle and non-neutralizing antibodies with a stop symbol. Structure visualization and image rendering were performed using PyMOL.

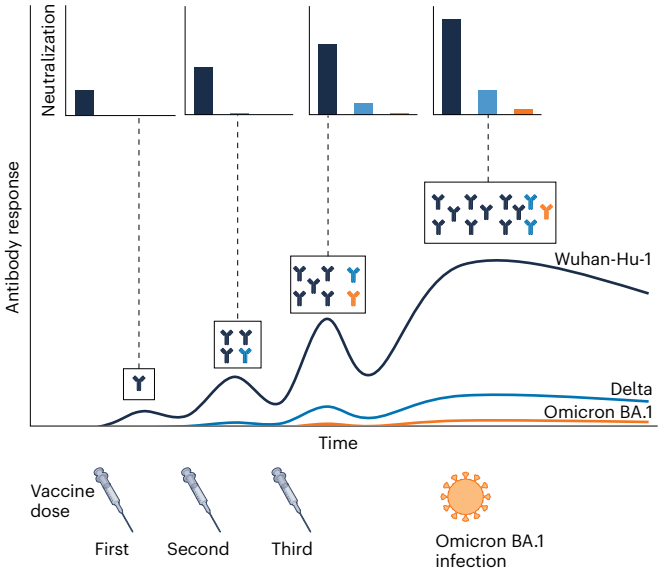

Fig. 3 | The antigenic seniority model, showing the hierarchical nature of imprinting on the antibody responses to SARS-CoV-2 and its variants. Repeated vaccination of a Wuhan-Hu-1 spike-based vaccine primes and imprints Wuhan-Hu-1-specific antibody responses, with virus neutralization potential increasing 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, while 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 the Wuhan-Hu-1 spike. Successive exposures to related antigens back-boost pre-existing antibodies to the ancestral strain. Over time, increasing breadth is acquired in the host antibody repertoire against subsequent viral variants.

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 neutralization 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 neutralizing activity of the Wuhan-Hu-1 booster against both Wuhan-Hu-1 and matched Omicron sublineages[20,51]. A US cohort receiving the BioNTech BA.4/BA.5 bivalent booster as a fourth dose also demonstrated enhanced neutralization 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 neutralizing antibody titres when compared with those receiving the monovalent Wuhan-Hu-1 booster at approximately three to four 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 neutralization in a separate study[54]. In BA.4/BA.5 bivalent vaccinees, neutralization against the latest and most evasive BA.2.75.2, BQ.1.1 and XBB variants was also markedly lower than against Wuhan-Hu-1, despite being comparatively better than groups receiving one or two 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 authorization[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 fifth dose at approximately eight months post-vaccination after the Wuhan/BA.4/BA.5 bivalent booster, reported comparable increases in neutralizing antibody titres against the ancestral D614G strain and Omicron subvariants. Specifically, both vaccines induced similar neutralization of BA.4/BA.5 and BQ.1.1, whereas the XBB.1.5 monovalent vaccine induced numerically higher titres against the

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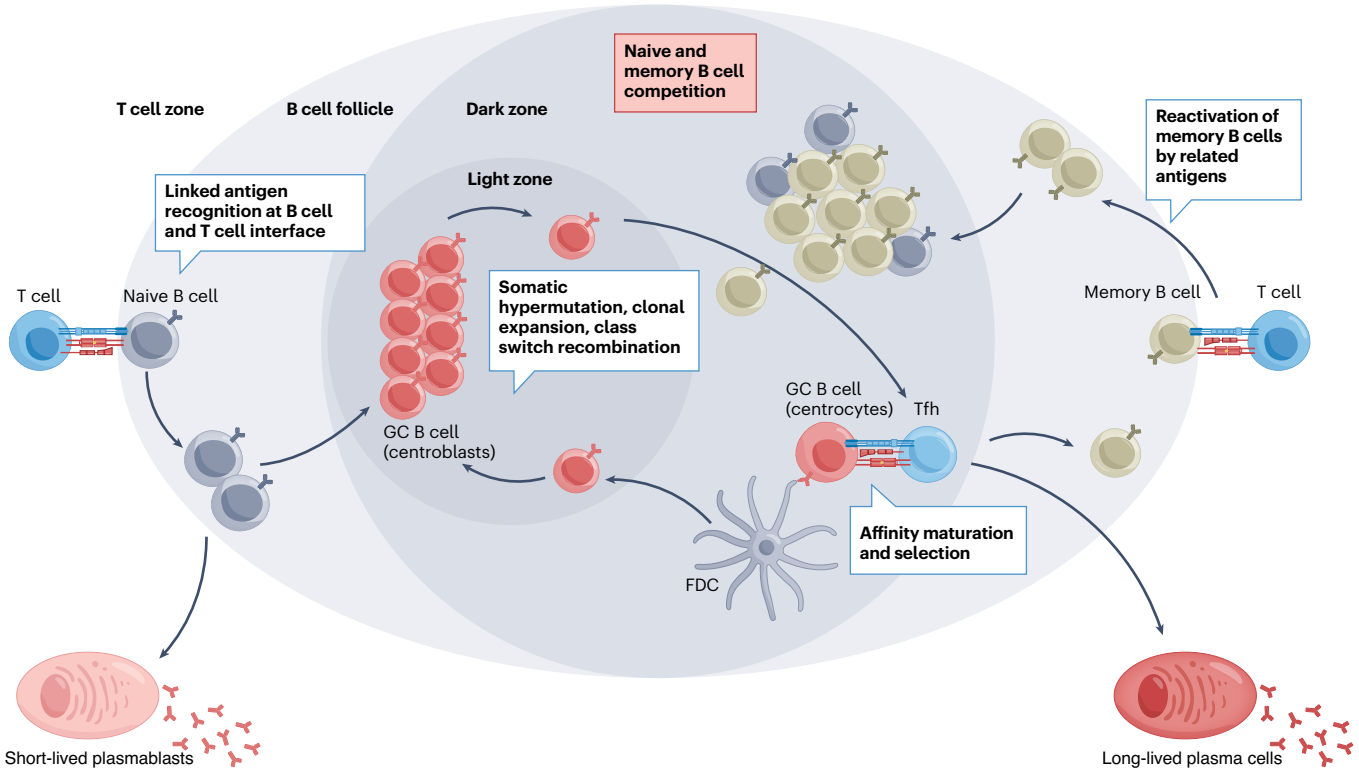

Fig. 4 | Germinal centre dynamics of imprinting—distraction, exhaustion and competitive inhibition. B cell responses to viral antigens are tailored to generate plasmablasts and plasma cells, which produce antibodies that neutralize, opsonize and activate complement against specific antigens on the invading pathogen. Long-lived memory cells are capable of rapid effector differentiation following secondary exposure to the same antigen[167]. A typical T cell-dependent B cell response in peripheral lymphoid organs includes linked antigen recognition, an extrafollicular response leading to formation of the primary focus and plasmablast production, a germinal centre (GC) response leading to formation of the secondary follicle, and effector plasma cell differentiation. The GC response involves complex cellular interactions between resident stromal and follicular dendritic cells (FDCs) and naive B and T cells arriving from the 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 GCs 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, the inflammatory cytokine transforming growth factor-β stimulates a chronic inflammatory state with continuous plasmablast release and persistence in the 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 naive 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 the generation of high viral antigen loads for naive 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 GC reaction, naive 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 GCs. Intrinsically, memory B cells exhibit lower activation thresholds than naive B cells, characterized by higher-affinity antigen recognition via optimized B cell receptors (BCRs) and elevated expression of major histocompatibility complex class II (MHC class II) and co-stimulatory molecules. As the number of B cells within any one GC is limited, rapid antigen acquisition and presentation by memory B cells occur at the expense of naive B cell activation through competitive inhibition. Variable gene expression profiles between naive and memory B cells also contribute to elevated effector differentiation activity of the latter, further facilitating a potent recall response upon antigen re-encounter[167].

ancestral D614G strain, XBB.1.5 and XBB.1.16. Monovalent vaccinees also cross-neutralized 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 against 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] and 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 are needed to unpick the relationships between different antigenic epitopes on host immune memory against influenza.

For SARS-CoV-2, the nature and extent of correlations between pre-existing antibodies and antigenically related viruses and protection against infection and disease are controversial. For susceptibility to infection, despite the 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. Although 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 hospitalized COVID-19 patients commonly correlated pre-existing antibodies to CCCoVs with increased risk of severe disease[37,73–75]. The mode of action was hypothesized 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 neutralizing antibody interactions with the RBD by Dejnirattisai and colleagues revealed an inverse correlation between pre-existing CCCoV antibody titres and de novo generation of SARS-CoV-2 antibodies[77]. In contrast, Kaplonek and colleagues 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 have 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% to 31% at two to three months since the last monovalent dose, as reported in a nationwide study conducted by the US Centers for Disease Control and Prevention (CDC)[81]. Although the 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 and colleagues 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 corroborates 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 immune responses than recalled memory responses to prior seasonal strains[68]. Similarly, individuals born prior to the 1890s H3N8 influenza pandemic, probably infected by a putative H1-like strain during initial exposure, exhibited high H3-specific antibody titres in the 1950s[66]. Serological studies after the 2009 H1N1 pandemic isolated broadly neutralizing antibodies following infection[83] and vaccination[84], and 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] (Fig. 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 a 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, neutralizing 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 will be critical for accurately predicting the impact of immune imprinting on SARS-CoV-2. For example, 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 cells. In contrast to abrupt antigenic specificity changes upon exposure to immunologically distinct viruses, driven by naive B cell activation, clonal evolution of the memory B cell repertoire can also progressively modify imprinting over time (Fig. 4). Memory B cells emerging early during the germinal centre (GC) response typically have low levels of somatic hypermutation, and are retained in circulation for prolonged periods and recalled into the GC for further rounds of affinity maturation within clonal lineages during secondary exposure. Following additional GC 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 naive B cells, activated by SARS-CoV-2, and memory B cells, primed by prior CCCoV infections, into antibody-secreting plasmablasts[76]. The GC response continues for at least six months, allowing extensive memory B cell evolution to optimize their binding affinity, neutralizing potency and breadth to viral variants[86,88]. Monitoring of convalescent individuals detected memory B cell clonal evolution that lasted more than 12 months post-infection, as well as the emergence of broadly neutralizing 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 re-infection rates, suggesting

that a previous 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 an alarming progression of memory B cell responses away from the SARS-CoV-2 S protein towards internal viral nucleoproteins and open reading frame 8 in elderly patients, which are non-neutralizing 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 to approximately five months, limited mutation levels in memory cells and suboptimal protection against variants. Although the RBD-binding antibody response narrows in vaccinees and broadens in convalescent sera over time, vaccine-induced responses still supersede convalescent responses at three to four 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 the immune imprinting 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 have emphasized the differences between exposure from infection and from 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 neutralizing antibody responses in vaccinees compared to convalescent sera. Vaccine-induced binding antibodies also demonstrated greater resistance to single mutations arising from antigenic drift, retaining protection against triple mutants with disrupted major neutralizing epitopes[105]. Consistent findings were obtained for the polyclonal serum antibodies induced by BNT162b2[106]. Human lymph node analysis by Röltgen and colleagues showed robust formation of GCs 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 GCs observed during severe SARS-CoV-2 infection[108]. As abundant GC induction and functional organization 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 great interest. Numerous independent studies also highlighted a beneficial role of breakthrough infections in increasing the breadth of neutralizing 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 sublineages 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-Hu-1/BA.5 bivalent mRNA vaccine. However, even this group demonstrated inferior Omicron-specific neutralizing 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.

Immunization in the context of imprinting

Understanding the constraints of immune imprinting is likely to better inform future influenza and SARS-CoV-2 immunization 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 naive 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 immunization 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 following subsequent infections and vaccinations (Fig. 4)[110]. For example, most individuals are first infected by seasonal influenza virus between the ages of two and three years[111], but intranasal LAIV immunization, which effectively induces localized mucosal antibody responses and virus-specific $CD4^+$ memory T cells in the respiratory tract, is approved for children above the age of two years due to wheezing reactions in younger children[112]. Safely lowering the age limit of LAIV use to one year would allow immunization of the predominantly naive population. Various SARS-CoV-2 live attenuated mucosal vaccine candidates are also under clinical investigation that have the potential of expanding indication to younger children[113]. Furthermore, simultaneous administration of naive infants with group 1 and group 2 influenza strains contained in the current seasonal vaccines is hypothesized 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 immunization[110]. If proven safe and effective, multivalent strain selection would need to be carefully considered as potential priming immunizations to induce maximum breadth and avoid biased imprinting responses from seasonally circulating strains.

Sequential vaccination of adults with antigenically distinct strains. Unlike naive 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 immunizations with antigenically distant HAs that harbour conserved, broadly neutralizing epitopes in the stem region have been proposed and trialled with some success[114]. However, as substantial heterogeneity exists across the population in terms of influenza exposure history and immune imprinting patterns, the optimal vaccine regimens can vary between different birth cohorts. Personalized influenza vaccines tailoring sequential immunization regimens to specific cohorts have been explored, but faced substantial challenges[8]. On the other hand, the COVID-19 pandemic, with its recent population-wide exposure history to SARS-CoV-2 variants, provides a unique opportunity to explore sequential immunization regimens with strategically designed and selected antigens of the

virus as an intervention to imprinting. Implementation of the bivalent S booster vaccines has evidenced the potential to increase the protective breadth in an imprinted population[21]. A 'prime and boost' strategy has also recently been explored; this 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, which will allow the impact on imprinting to 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 example, 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 naive and exposed populations include subunit antigen structures that focus immune responses to highly conserved sites, chimaeric and in silico optimized 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 redirect host responses towards broadly protective epitopes. A diverse range of modified headless HA vaccine candidates have demonstrated superior stem-reactive, broadly neutralizing antibody responses to full-length HAs in mice[115]. In particular, animals immunized with headless H1N1 HAs were protected against lethal challenge by heterosubtypic avian H5N1 viruses[115]. Recently, more advanced attempts harnessed a 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 the 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].

Chimaeric immunogens. Another approach to refocus 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 chimaeric immunogens. Chimaeric HAs (cHAs) have been generated from the fusion of non-human globular head structures with conserved stem domains. Sequential prime–boost immunization with these cHAs was hypothesized 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 an exotic avian influenza head fused to H1 HA stems showed promising safety and broad protection against group 1 IAVs[122]. The latest fine-tuning of this approach focused on minimizing 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, chimaeric 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 chimaeric 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 and colleagues 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 the wild-type strain[128]. Through comprehensive phylogenetic analysis and sequence comparison, Ross and colleagues generated computationally optimized 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 optimization of key viral antigens through in vitro selection. Best-in-class, digitally designed, immune-optimized 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 neutralizing potency at much lower doses, as well as 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 neutralizing activity against several VOCs and SARS-CoV-1[138]. In addition to natural nanoparticles, Baker and colleagues generated a pool of de novo synthesized 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 a phase 3 clinical trial[141]. Quadrivalent mosaic I53_50 nanoparticles displaying heterotypic copies of RBD[142] or pre-fusion stabilized 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 and colleagues[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 the 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 and colleagues 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 neutralization of Wuhan-Hu-1, Delta and SARS-CoV-1 in mice[148].

Reshaping host immune memory

Although the development of novel, universal vaccine immunogens capable of inducing broadly protective and long-lasting immunity remains the focus of vaccine research, substantial interest has arisen in strategies that directly modulate the host immune system. Building on recent characterization of a prolonged GC 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 (Fig. 4). Although not pragmatic at the population level, such studies provide insight and an important proof of concept. Approaches include (1) selective expansion of immuno-subdominant B cells through guided evolution, (2) selective reduction of immunodominant B cells through targeted elimination and (3) enhancement of B cell function through dendritic cell (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 $CD80^{-}PD\text{-}L2^{-}$ double-negative memory B cell subset preferentially re-enters the GC for additional cycles of somatic hypermutation and affinity maturation upon re-exposure to viral variants, whereas the $CD80^{+}PD\text{-}L2^{+}$ 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 adaptation 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 optimized vaccine-induced immunity against SARS-CoV-2[150]. Definitive characterization 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 neutralizing epitopes can further enhance the robustness of humoral immunity. Understanding the mechanistic basis of progressive displacement of CCCoV-specific or non-neutralizing 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 immunodominant 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 GC 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 GC B cell death in an affinity- and avidity-dependent manner[152,153]. In a recent study, Silva and colleagues immunized 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 GC B cells. Following selective elimination of HNP-specific B cells from the GC, 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 of immunodominant GC 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 hypothesized that transferred antibodies enter peripheral lymphoid organs and compete with GC B cells for antigens presented by follicular DCs 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 GC B cell recognition, thereby favouring 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. The 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 immunodominant B cells, through soluble antigen administration or passive antibody transfer, alleviates the competitive pressure from GC selection and expands desirable B cell subpopulations to provide novel, broadly protective responses in the presence of pre-existing immunity.

Enhancing B cell function through DC activation. Virus-specific B cell responses can also be indirectly enhanced by upregulating antigen uptake, processing and presentation by DCs. Recent advancements in modern vaccinology platforms have enabled several routes for targeted antigen delivery to lymph nodes. Immunization via footpads draining to single lymph nodes increased the local antigen concentration and induced broadly neutralizing antibodies to the HA stem in mice[156]. Translation of the 'albumin hitchhiking' approach used for lymph node visualization in medical oncology has enabled the design of recombinant amphiphile conjugate immunogens, which are composed of an antigen cargo linked to a lipophilic albumin binding tail via an interconnecting, solubility-promoting polar polymer chain. Through enhanced lymph node targeting, structurally optimized 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 DCs to induce a sustained adaptive immune response. Amphiphile vaccine antigens bound to endogenous albumin are prevented from dissemination into the circulation and are effectively retained in lymphoid tissues[157]. Slow-release vaccines can also

be employed for sustained antigen release[156]. Immunogens engineered to target DC-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 DCs, 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 three years, the epidemiology of SARS-CoV-2 has shifted from a pandemic phase, with rapid spread in a naive 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 the influenza and SARS-CoV-2 literature. The nonlinear 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 a comprehensive, longitudinal immune history for individuals, creating another hurdle for drawing conclusive correlations regarding 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 characterization 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 the 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, although immune imprinting may have occurred to a certain degree among some of the human coronaviruses, 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 neutralize SARS-CoV-2 variants[49,52]. In addition, repeated boosting with Omicron S antigens has been shown to alleviate imprints from previous exposure to the ancestral strain through affinity maturation towards neutralizing 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 antigen targets that correlate with the best vaccine protection.

Next-generation SARS-CoV-2 immunization 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; these 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, hospitalizations 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 spillover 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.

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Author contributions

C.Q.H. and J.L.H. conceived the main ideas for this Review. C.Q.H. researched data and wrote the Review. 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, and reviewed and/or edited the manuscript before submission.

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.

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