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SARS-CoV-2 variant biology: immune escape, transmission and fitness

Alessandro M. Carabelli[1*], Thomas P. Peacock[2*], Lucy G. Thorne[3], William T. Harvey[4], Joseph Hughes[4], The COVID-19 Genomics UK (COG-UK) Consortium[5], Sharon J. Peacock[1], Wendy S. Barclay[2], Thushan I. de Silva[6], Greg J. Towers[3], David L. Robertson[4†]

[1]Department of Medicine, University of Cambridge, Addenbrookes Hospital, Cambridge, UK
[2]Department of Infectious Disease, St Mary's Medical School, Imperial College London, London, UK
[3]Division of Infection and Immunity, University College London, London, UK
[4]MRC-University of Glasgow Centre for Virus Research, University of Glasgow, Glasgow, UK
[5]https://www.cogconsortium.uk. Full list of consortium names and affiliations are in Appendix 1
[6]Department of Infection, Immunity and Cardiovascular Disease, Medical School, University of Sheffield, Sheffield, UK

*Authors contributed equally

†email: david.l.robertson@glasgow.ac.uk

Abstract

In late 2020, after circulating for almost a year in the human population, SARS-CoV-2 exhibited a major step-change in its adaptation to humans. These highly mutated forms of SARS-CoV-2 had enhanced rates of transmission relative to previous variants and were termed 'variants of concern' (VOCs). Designated Alpha, Beta, Delta, Gamma and Omicron, the VOCs emerged independently from one another and in turn each rapidly became dominant, regionally or globally, out-competing previous variants. The success of each VOC relative to the previously dominant variant was enabled by altered intrinsic functional properties of the virus and, to varying degrees, changes to virus antigenicity conferring the ability to evade a primed immune response. The increased virus fitness associated with VOCs is a complex interplay of virus biology in the context of changing human immunity due to both vaccination and prior infection. In this review, we summarise the literature on the relative transmissibility and antigenicity of SARS-CoV-2 variants, the role of mutations at the furin spike cleavage site and of non-spike proteins, the potential importance of recombination to virus success, and SARS-CoV-2 evolution in the context of T-cells, innate and population immunity. SARS-CoV-2 shows a complicated relationship between virus antigenicity, transmission and virulence, which has unpredictable implications for the future trajectory and disease burden of COVID-19.

Introduction

Since its initial emergence in Wuhan in December 2019, SARS-CoV-2 has caused over 641 million cases of COVID-19 and more than 6.6 million deaths as of December 2022[1]. SARS-CoV-2 (along with SARS-CoV, the cause of SARS) is a member of the severe acute respiratory syndrome-related coronavirus (SARSr-CoV) species, the sole member of a genus of viruses, *Sarbecoviruses,* primarily found in horseshoe bats[2]. Like other coronaviruses, SARS-CoV-2 possesses a large RNA genome, comprised of ~30,000 nucleotides, whose replication is mediated by RNA-dependent RNA polymerase (RdRP) and an associated proofreading enzyme exoribonuclease (ExoN). This, combined with the discontinuous nature of coronavirus transcription, has resulted in coronaviruses with high rates of recombination, insertions and deletions, and point mutations (although lower than other RNA viruses due to the proofreading), as previously reviewed[3]. The success of novel genetic variants generated, although prone to stochastic sampling processes, will be very dependent on natural selection; in particular, positive selection associated with mutations that are beneficial to the virus they occur in.

SARS-CoV-2 has proven to be a highly capable human pathogen, but also a generalist in terms of host tropism, establishing infections in a variety of mammalian species including farmed mink[4], a stable reservoir in white-tailed deer[5,6], and incidental infections of many other animal species (ref. 209). Once in humans, the first months of SARS-CoV-2 evolution were characterized by limited adaptation and phenotypic change relative to its later evolution[7]. The first notable change, a single spike substitution D614G, arose early in the pandemic and conferred a ~20% growth advantage relative to preceding variants[8]. A lineage defined by D614G (PANGO[9] B.1) quickly became dominant in Europe, giving an early indication of the potential for SARS-CoV-2 to increase its transmissibility in humans. As we described previously[3,10], from October 2020 onwards, novel, more heavily mutated SARS-CoV-2 variants began to emerge. These variants were distinguished by higher numbers of nonsynonymous mutations principally in the spike protein - particularly the case for Omicron - and distinct phenotypic properties, including altered transmissibility and antigenicity. To date, five SARS-CoV-2 variants have been declared variants of concern (VOCs) by the World Health Organization (and national public health agencies) on the basis that they exhibit substantially altered transmissibility or immune escape, warranting close monitoring. Each VOC showed transmission advantages over preceding variants and became dominant, either regionally in the cases of Alpha (PANGO lineage[9] B.1.1.7), Beta (B.1.351) and Gamma (P.1)

- in Europe, Southern Africa, and South America, respectively - or globally, in the cases of Delta (B.1.617.2/AY sub-lineages) and the many Omicron sublineages (B.1.1.529/BA sub-lineages, such as BA.1, BA.2, BA.5).

In contrast to the expectation that viruses undergo rapid host adaptation following spillover[11,12], selection analysis indicates that SARS-CoV-2 lacked notable levels of observable adaptation early in the pandemic[13]. It subsequently became clear that SARS-CoV-2 is a generalist virus capable of using a variety of mammalian angiotensin-converting enzyme 2 (ACE2) membrane proteins for cell entry[14], enabling infection of a wide range of mammals[13,15]. The sarbecoviruses transmit frequently between different horseshoe bat species[16] and non-bat species with ACE2-binding capability (the inferred ancestral trait in sarbecoviruses[17]), which happens to include humans. The SARS-CoV-2 spike protein contains important properties that are responsible and required for efficient human-to-human transmission, in particular: human ACE2-binding and the polybasic furin cleavage site (FCS) at the S1/S2 junction[18,19]. At present the SARS-CoV-2 S1/S2 FCS is unique among sarbecoviruses although analogous sequences are observed in other betacoronaviruses.

The entry of SARS-CoV-2 into airway cells requires furin-mediated cleavage at the FCS, enabling membrane fusion. The FCS is therefore a key determinant of SARS-CoV-2's high transmission rates, contributing to its efficient spread in humans[18,20]. Further optimization of the wild-type FCS during the course of the pandemic has resulted in enhanced furin cleavage of the Alpha and Delta spikes[21-24]. In concert with other mutations, notably those enhancing ACE2-binding[25,26], mutations optimizing furin cleavage are thought to have contributed to enhanced transmissibility, and thus fitness, of the Alpha and Delta VOCs, with a 65% and 55% higher relative transmissibility compared to the variants they replaced, respectively[27-29]. In contrast to Alpha and Delta, the evolutionary success of the Omicron variant is not linked to optimization of furin cleavage. Rather, Omicron is characterized by an altered entry phenotype[30,31], coupled with significant immune escape[30,32,33], enabling efficient infection of vaccinated or previously infected individuals. Although transmissibility in a naive population is largely determined by intrinsic viral properties, the increasingly complex immune landscape in which SARS-CoV-2 now circulates means that antibody escape (as opposed to transmissibility being enhanced by virus biology alone, a trait that might be difficult to optimize further than that achieved by Omicron) is becoming the prime driver of variant success. Prior to Omicron's emergence (Box 1), each of the dominant variants had evolved from pre-VOC progenitors, rather than evolving from one another. In contrast, successive waves are now being

caused by Omicron sublineages, for example, BA.5, one of its sublineages BQ.1 and BA.2.75, a sublineage of BA.2. Of note, it is possible that an undetected variant, potentially a recombinant (Box 2), could emerge with high transmissibility linked to intrinsic biology and novel antigenic properties.

Whether an entirely novel variant emerges, or future viruses evolve from the Omicron sublineages with novel antigenic changes (increasingly probable as previous VOCs are no longer circulating), it is clear that novel SARS-CoV-2 variants possessing unique combinations of mutations will continue to emerge and those with a fitness advantage will dominate relative to previous variants. To date, successful variants have also exhibited variation in clinically relevant traits including both disease severity, immune evasion and sensitivity to therapeutics (particularly monoclonal antibodies). It is therefore of public health and clinical importance to understand the drivers of SARS-CoV-2 fitness. Variant fitness - a viruses' reproductive success - depends on a variety of factors that determine its ability to infect, replicate within, and transmit between hosts. In this review, we provide an overview of observed mutations that are described as impacting SARS-CoV-2 infectivity and transmissibility, and discuss the viral capacity to escape T cell-mediated, innate or humoral immunity[10]. See our previous review[10] for more details on spike-mediated humoral immunity.

Antigenic escape and SARS-CoV-2 variants

An early concern regarding SARS-CoV-2 evolution was the potential emergence of antigenically distinct variants with the ability to evade vaccine- or infection-acquired immunity, as exemplified by the N439K spike substitution[34]. Prior to their update in late 2022, all widely used COVID-19 vaccines were based on the spike antigen of early variants, most using the reference sequence Wuhan-Hu-1, sampled from a lineage B infection at the Huanan Seafood market; often with mutations that stabilize spike in a pre-fusion conformation[35]. Although limited antigenic change was reported for Alpha[24,36,37], moderate escape from vaccine-derived antibodies and convalescent sera was observed for Beta, Gamma and Delta in laboratory experiments[22,36-38]. Nonetheless, epidemiological studies provided evidence that vaccine effectiveness against Delta and Beta was largely preserved[39-41]. Thus, despite a design based on very early spike sequence, first generation SARS-CoV-2 vaccines conferred remarkable protection against severe disease and allowed much of the world to return to a semblance of normality.

The Omicron 'complex' - comprising the distinct sub-lineages BA.1-5 (**Box 1**) – is capable of infecting the vaccinated and previously infected, bringing about the challenge of vaccine-sequence selection and universal vaccines to the fore of SARS-CoV-2 control strategy discussions. With >15 spike receptor binding domain (RBD) mutations and a number of antigenic deletions and substitutions in the N-terminal domain (NTD)[42,43], BA.1, BA.2, BA.4 and BA.5 are very poorly neutralized with first generation vaccines and by pre-Omicron infection-derived antibodies (**Fig. 1**). In addition, escape from the vast majority of current therapeutic monoclonal antibodies has been demonstrated; at present, only Bebtelovimab – a monoclonal antibody targeting the RBD of spike – has been reported to retain its efficacy against all SARS-CoV-2 variants[30,32,33,37,44-49]. This large antigenic 'shift' has led some to propose that Omicron lineages should be considered a separate strain or serotype compared to pre-Omicron lineages[50,51]. Importantly, the magnitude of this antigenic change is reflected in real-world vaccine effectiveness data against infections and symptomatic disease[40,52-56]. Booster doses are required to maintain any vaccine effectiveness against Omicron, which reduces as antibody titres wane[40,54]. Indeed, vaccine effectiveness against severe disease for Omicron remained high four months after a booster dose and then decreased quickly, although the reduction was less rapid than that seen after primary vaccination[57]. Due to the short duration of protective immunity against Omicron infection with current vaccines, many vaccine manufacturers and academics are focusing on second generation vaccines, such as monovalent or bivalent Omicron-specific boosters[58] (which are being deployed at present), nasal vaccine delivery to stimulate greater mucosal immunity[59], or universal vaccine approaches[60]. In common with seasonal human coronaviruses, the extent to which long-term acquired immunity can prevent reinfection by SARS-CoV-2 is limited due to a combination of antibody waning and virus antigenic drift - the incremental acquisition of mutations that permit immune evasion[61,62]. In addition to step-changes in antigenicity, virus evolution during persistent infections has enabled SARS-CoV-2 to accumulate multiple mutations in the context of a single or a few long-term infections, contributing to antigenic shift events when these variants go on to infect others (**Box 1**).

The furin cleavage site in SARS-CoV-2 variant emergence

Compared to known sarbecoviruses, a unique feature of SARS-CoV-2 is the presence of a FCS within its spike protein that can be cleaved by furin. FCSs are nonetheless present in many other betacoronaviruses of the *Embeco-* and *Merbecovirus* subgenera, such as HCoV-OC43, HCoV-HKU1 and MERS-CoV (**Fig. 2A**). The SARS-CoV-2 FCS has been shown to be vital

for optimal virus replication in human airway cells[84], transmissibility[18,20] and pathogenicity[85]. Furin, a host protease, is most abundant in the Golgi apparatus, allowing cleavage during trafficking of the virus to the cell surface[86]. It is now clear, however, that the FCS of early SARS-CoV-2 variants was suboptimal and not efficiently cleaved by furin[18,23,85]. Interestingly, one of the roles described for the early substitution mutation, spike D614G, was modestly enhanced spike cleavage (reviewed in detail previously[3]). A number of subsequent SARS-CoV-2 variants harbor mutations adjacent to the FCS, which increase the number of basic amino acid residues – the known recognition site for furin – for example, Alpha, Mu and Omicron contain the FCS mutation P681H[43,69,87], which is predicted to increase cleavage activity. Moreover, a different mutation at the same position, P681R, enhances the replication and pathogenicity of the Delta VOC (**Fig. 2B**)[22,29,89]. Of note, Omicron contains P681H, as well as a further mutation N679K[43], which together result in an optimized FCS[18,22-24,31,88]. Importantly, however, furin site optimization alone does not enhance the transmissibility or replication of SARS-CoV-2 and might be detrimental to efficient virus transmission[26], indicating that additional mutations seen in these variants are required for optimized replication and transmissibility.

The exact mechanism by which the observed FCS mutations enhance furin cleavage remains a topic of debate. Although there is fairly strong evidence that P681R directly enhances furin engagement and cleavage of the S1/S2 site[18,23,89], the functional consequence of P681H[92] is less clear. Mechanistically, it is likely relevant that position T678 of the spike protein, located within close proximity to the P681 residue, can be post-translationally modified with an O-linked glycosylation site[93-95]. Downstream prolines are known to promote O-linked glycosylation; as such, an alternative explanation could be that the removal of proline 681, rather than the addition of the histidine per se, leads to loss of the potentially furin-obstructing glycosylation site and associated enhancement of cleavage.

Recent insights into Omicron biology have challenged the hypothesis that enhancing spike cleavage is essential for increased viral transmission. All Omicron lineages contain P681H and N679K[42,43], which alone, or together, enhance cleavage of the S1/S2 site in wild-type spike[31,88]. However, in the context of full Omicron spike, evidence of such improved cleavage is less clear, with some studies finding that the S1/S2 junction appears more poorly cleaved than previous VOCs[49,90], whereas others show comparable cleavage efficiency to Delta[31,91,96]. Regardless of the cleavage phenotype, many groups have described how Omicron is able to efficiently utilize an alternative cell entry pathway. Indeed, whereas previous VOCs

such as Delta are highly reliant on fusion priming by Transmembrane Protease Serine 2 (TMPRSS-2) at the cell surface, Omicron is also able to be efficiently primed by endosomal proteases, such as cathepsins in a manner similar to SARS-CoV[30,31,49,90,91,97,98]. This alternative mechanism is hypothesized to be partly responsible for the reduced severity of Omicron, at least in rodent models[31,90,97,99,100], due to lower fusogenicity and potentially altered tissue tropism, with a bias towards infection of the upper respiratory tract over the lower respiratory tract. Several studies have suggested molecular mechanisms for this reduced fusogenicity and altered entry route, including mutations in the spike receptor binding domain (RBD) of Omicron[30,101], H655Y[102] or mutations in the S2 domain[30,31,91], specifically N969K[31], although it is contentious whether this trait is conserved across all Omicron lineages[31,103]. Nontheless, Omicron lineages continue to also show high transmissibility, at least equivalent to Delta[104,105], implying a potential decoupling between efficiency of furin cleavage and fusogenicity and their contributions to virus transmissibility.

Beyond the S1/S2 site, betacoronaviruses also require cleavage of a second protease cleavage site, known as the S2' site. Following cleavage of the S1/S2 site and cognate receptor binding, the S2' site becomes exposed in the S2 domain of spike[106]. Cleavage of S2' directly liberates the fusion peptide leading to viral-host membrane fusion[107-109]. For SARS-CoV-2 entry into airway cells, this site is preferentially cleaved by host serine proteases, such as TMPRSS2, but it can alternatively be cleaved by endolyosomal cathepsins [18,110]. A number of recent papers have suggested that variation in the NTD domain of SARS-CoV-2, particularly via remodelling of external loops through the acquisition of deletions or insertions, can allosterically influence both S1/S2 and S2' cleavage, and therefore fusion[111-113].

Overall, the relationship between protease usage, S1/S2 cleavage efficiency, tropism, pathogenicity and transmissibility of SARS-CoV-2 is highly complex and, at times, results between studies have been inconsistent. Further work is, thus, required to bridge these gaps in knowledge and to fully understand this system.

Other structural and non-structural proteins and infectivity

Several recent studies have investigated the consequence of mutations in structural proteins other than Spike, including Membrane (M), Envelope (E) and Nucleocapsid (N) proteins. The B.1.1 lineage is defined by a pair of substitutions in the N protein – R203K and G204R. Variants derived from B.1.1 (such as Alpha, Gamma and Omicron) inherited the same mutations, whereas Delta and Beta independently evolved R203M and T205I, respectively,

with convergent functional properties. Specifically, these mutations have been shown to increase viral infectivity[114-116], although the exact mechanism of action remains disputed. On the one hand, data from a virus-like particle-based reporter-based assay[114] suggested that these mutations directly increase virus particle formation, whereas another report suggested a role for phosphorylation of N protein allowing escape restriction by GSK-3 kinase[116]. An alternative explanation is that the R203K and G204R mutations introduce a novel transcriptional regulation site (TRS) into the middle of the N gene, allowing the expression of a truncated form of N protein (termed N* or N.iORF3), which might enhance virus infectivity through enhanced interferon antagonism[117,118]. Interestingly there are several further examples of SARS-CoV-2 lineages evolving novel TRS sequences that could result in expression of truncated protein products, either in- or -out-of-frame, most prominently in non-structural protein (NSP) 16[117].

Alongside N protein, mutations in M and E proteins have also been implicated in modulating SARS-CoV-2 infectivity. Substitutions in the M and E proteins of BA.1 (Omicron) have been shown to reduce cell entry of virus-like particles, although these mutations are compensated for by further substitutions in S and N proteins[119]. Coronavirus E proteins have several functions, one of which is to act as a cation channel, potentially within the ER and Golgi compartments to regulate multiple stages of the viral life cycle[120]. The T9I mutation found in Omicron E protein has been shown to attenuate this ion channel activity *in vitro*[121], although its functional consequences are unclear.

Although ORF1ab makes up two thirds of the SARS-CoV-2 genome, it remains the region where the impact of variant mutations is least understood. One exception is a deletion at positions 106-108 within NSP6, a mutation that is conserved among all the VOCs except Delta. NSP6 is a multi-pass transmembrane protein associated with the formation of the coronavirus replication organelle - the endoplasmic reticulum (ER)-derived membranous structures that are produced during infection, providing a compartment for viral RNA replication shielded from innate immunity[122]. A recent study showed that NSP6 forms homodimers and mediates the formation of ‘zippered ER’ - narrow and exclusive membranous channels that connect ‘double membrane vesicles’ (DMV), which are the primary site of viral genome replication[122]. It was found that the 106-108 deletion in NSP6 specifically enhances the formation of this zippered ER, indicating a potential host-specific adaptation. The exact mechanism of this enhancement remains to be elucidated, although the authors postulate that the deletion removes a putative O-linked glycosylation site. At present it remains unclear why

Delta and several other variants were never observed to have gained this adaptation despite the ease at which this deletion can occur.

Beyond the structural and ORF1ab proteins, there is some very limited evidence showing adaptive changes in accessory proteins, which is covered later in this review. Unfortunately, experimental characterization of non-spike mutations and any associated adaptations to humans in SARS-CoV-2 variants remains far behind those of spike. This is due to a number of factors, including the ubiquitousness of pseudovirus technology used to study Spike phenotypes compared to the technical complexity of reverse genetics (usually required for virological studies of non-spike mutations) and the scarcity of *in vitro* systems for investigating non-Spike proteins. It is clear from current work that non-Spike adaptations contribute largely to virus fitness and pathogenicity, and the continued development of systems to study these regions is vital to ongoing research.

T cell responses and antigenic escape

T cells are a major part of the adaptive immune response to SARS-CoV-2 infection, with a profound $CD4^+$ and $CD8^+$ T cell response observed in most infected individuals[123]. Several studies suggest an important role for T cell immunity in protection from severe COVID-19, although this is likely to be more nuanced and complex than the well-characterised correlate of protection from infection of neutralising antibody responses[124,125]. Although $CD4^+$ helper T cell responses are likely to be broadly important for antibody generation, the importance of T cells in reducing disease severity might be relatively more important in scenarios where neutralizing antibody responses are diminished or not yet detectable. Early induction of SARS-CoV-2-specific T cells is seen more frequently in mild compared to severe infections[126], and $CD8^+$ T cells may be particularly important in reducing severe outcomes in patients with B cell deficiency[127]. Furthermore, a fully functional $CD8^+$ T cell response is mobilized one week after the first dose of mRNA BNT162b2 vaccine (Pfizer-BioNTech), at a time when neutralising antibodies are not fully induced, raising the possibility that early vaccine-induced protection may be mostly reliant on T cells[128].

Given the integral role of T cells in SARS-CoV-2 immunity, the potential exists for selective pressure to lead to T cell escape, although the extent to which SARS-CoV-2 mutations affect T cells is currently poorly understood. Functional T cell responses are directed against multiple virus proteins, with the magnitude of response correlating with viral protein expression levels. Responses to spike, N protein and M protein dominate, with appreciable

responses also seen against ORF3a and non-structural proteins NSP3 and NSP12[129]. As the T cell response targets epitopes across the SARS-CoV-2 genome, the footprints of T cell escape are more broadly distributed than antibody-driven changes that are concentrated within dominant epitopes of the spike protein alone (**Fig. 3**). Few studies have documented intra-host evolution within T cell epitopes, which would serve as direct evidence of T cell escape. Mutations within $CD8^+$ epitopes in N protein (M322I, L331F), M protein (L90F), and spike (L270F) were noted within minority variants in one study during the course of acute infections, resulting in loss of epitope-specific responses[130]. Prolonged SARS-CoV-2 infections in immunocompromised hosts may offer greater opportunities for T cell escape, akin to the extensively described examples in HIV-1 infection[131]. Emergence of the NSP3 T504P mutation resulting in loss of a $CD8^+$ epitope response has been reported in multiple individuals with impaired humoral immune deficiency, but preserved T cell responses in the context of chronic SARS-CoV-2 infection[78,132]. These findings are limited to a few cases, demonstrating the need for more prospective cohort studies systematically evaluating the risk of T cell escape in certain patient populations.

Several mutations within immunodominant ORF3a and N protein $CD8^+$ T cell epitopes that result in complete loss of recognition have arisen independently in multiple SARS-CoV-2 lineages[133]. Among these is N protein P13L, present in Omicron within a B*27:05 restricted $CD8^+$ epitope. Given the hypothesis that VOCs emerge in chronic infections, it is tempting to speculate that the presence of P13L in the Omicron VOC reflects selection due to T cell pressure during a chronic infection, in addition to the constellation of spike mutations that are likely driven by antibody pressure. L452R found in Delta, Epsilon, Kappa and the BA.4/BA.5 variant spike proteins results in loss of an A*24:02-restricted $CD8^+$ response[134]. The Spike P272L substitution has arisen in multiple lineages worldwide and leads to loss of a dominant HLA A*02:01 restricted $CD8^+$ epitope[135]. The role that T cells have in driving this change, in addition to antibody evasion and enhanced ACE2 binding affinity, is uncertain. Other spike mutations in VOCs that have been associated with loss of specific $CD4^+$ responses include L18F, D80A and D215G in Beta, and D1118H in Alpha[136,137]. The degree to which these observations also represent incidental effects on T cell responses with mutations driven by other pressures is currently unknown.

Despite the loss of these specific responses, several studies show that the overall T cell response induced by infections and first-generation vaccines is preserved against most VOCs[124,136-138]. Even the extensive mutations in Omicron spike only result in a modest <30%

reduction in total $CD4^+$ and $CD8^+$ responses, with considerable inter-individual variability[137,138]. Most high frequency spike $CD4^+$ epitope responses are focused on discrete regions of the N-Terminal Domain (NTD), C-Terminus and fusion protein regions, with very few in the RBD[129]. There are no clear hotspots of spike $CD8^+$ epitopes[129]. Mutations concentrated in the spike RBD and NTD found in many VOCs that are thought to be driven by antibody evasion and increased ACE2 binding affinity might therefore have limited impact on the overall T cell response. Thus, the majority of T cell epitopes are conserved in different VOCs and this is likely to contribute to the preserved vaccine effectiveness against hospitalization and death with Omicron seen after both 2nd and 3rd doses when compared to unvaccinated individuals[139].

The other key reason for the modest impact of variants on T cell immunity is the breadth of response generated, with each individual mounting responses to 30-40 epitopes following infection[129]. At a population level, there is also far greater heterogeneity to the T cell response than antibody immunity due to a number of polymorphisms present in human HLA genes. At an individual level, however, significant reductions in spike-specific $CD8^+$ responses to Omicron have been reported in ~15% of convalescent and vaccinated donors[135], with another study noting >50% loss of $CD4^+$ and $CD8^+$ T cell responses in ~20% of individuals tested[140]. Although the generalizability of these results are limited by small sample sizes and the HLA distribution of the populations studied, it nonetheless highlights the potential impact of VOCs on T cell responses in certain individuals who only have spike-specific immunity generated through vaccination.

It seems likely that antibody evasion and enhanced transmissibility will continue to be greater drivers of emerging VOCs than significant T cell escape. Whether we will see slow and sequential loss of $CD8^+$ epitopes over time, similar to long-term adaptation in H3N2 influenza, is difficult to predict[116]. T cell escape can occur through several mechanisms[117]. Amino acid changes within epitopes or flanking regions can disrupt antigen processing, and changes to anchor residues can interfere with major histocompatibility complex (MHC) binding to epitopes[141]. Both these mechanisms can result in irreversible loss of T cell responsiveness to a particular epitope. Changes that impair T cell receptor binding to the peptide-MHC complex can, in contrast, result in partial or complete escape. This latter scenario might also be overcome by *de novo* T cell responses using alternative T cell receptor repertoires, as previously described in HIV-1 infection[142].

As well as potential T cell escape, SARS-CoV-2, like many other viruses, directly downregulates MHC-I expression on infected cells in order to evade T cell recognition, best described for the accessory protein ORF8[143,144]. ORF7a (as well as ORF3a and ORF6[145,146]) has also been reported to downregulate MHC-I[146,147] , although it is unclear if this is a specific effect or merely the result of non-specific golgi fragmentation[148]. Recent work has shown that common substitutions seen in VOCs do not alter the ability of ORF8 to suppress MHC-I expression, with the exception of a premature stop codon at amino acid 27 in Alpha, resulting in the expression of a truncated, non-functional form of ORF8[70,144]. Despite the truncationof ORF8, in the context of infection, Alpha still downregulates MHC-I expression, implying that this variant, or possibly SARS-CoV-2 more generally, has evolved redundant mechanisms to inhibit this pathway[144].

Innate immunity and SARS-CoV-2 variants

Innate immunity is an integral part of host defense against pathogens, with a key role in early viral control and tuning of adaptive immune responses[149]. Innate immune responses are particularly critical against novel viruses, such as zoonotic pathogens, for which there is usually no pre-existing adaptive immunity. In fact, it is surprising when novel zoonotic viruses are effective at transmitting between humans through effective antagonism of innate host defences, despite recent successful replication and transmission in a distantly related host species, with its typically divergent innate immune system. A consequence of the robustness of the human innate immune system is that the proportion of zoonotic viruses that go on to cause pandemics is extremely low and properties that evolved in the SARS-CoV-2 reservoir species, specifically generalist host tropism (which happened to include humans) coupled with acquisition of its FCS in spike, possibly in an intermediate host species, were critical features for starting the COVID-19 pandemic. It is noteworthy that the related SARS virus, SARS-CoV, did not establish in the human population, despite also being highly transmissible; its eradication being attributed to less asymptomatic spread compared to SARS-CoV-2 making it easier identify infections. Importantly, as SARS-CoV-2 VOCs have emerged, it has become clear that they are acquiring adaptations to more efficiently infect humans, and this in part is through enhanced innate immune evasion. Indeed, Alpha[150,151], as well as more recent VOCs[96,152,210] have evolved reduced sensitivity to interferons, consistent with this being a key selective pressure for virus transmission in humans. Surprisingly, rather than adapting to specifically antagonize human proteins, Alpha, and Omicron sublineages BA.4 and BA.5 have in part achieved this by upregulating expression of innate immunity suppressing viral proteins, particularly the ORF6

protein[96]. ORF6 inhibits nuclear transport of transcription factors including STAT1 and IRF3[153], which control the expression of antiviral proteins and soluble pro-inflammatory mediators. Alpha has also enhanced expression of ORF9b (which inhibits signalling downstream of RNA sensing[154]) and N protein (which sequesters viral RNA to prevent activation of sensing mechanisms[117]), as well as *de novo* expression of N*/N.iORF3 - the N-terminally truncated form of N protein, which displays some interferon antagonism but is expressed at low levels[117]. Increased levels of these proteins are likely the result of mutations in regulatory regions that modulate subgenomic RNA (sgRNA) synthesis and protein expression. This highlights the importance of changes outside spike in determining VOC properties, particularly in regulatory regions. Critically, mutations in the N protein Kozak sequence that are expected to influence expression of Alpha N protein and the overlapping ORF9b protein also appear in the dominant VOCs Delta and Omicron, but their full impact on innate immune antagonism in these VOCs remains to be determined. The relationship between SARS-CoV-2 and innate immunity is highly complex. For example, the viral antagonist ORF9b appears to be negatively regulated through phosphorylation by host kinases, suggesting its innate immune inhibition could be switched off at some point during infection, perhaps once host responses are triggered in infected cells[150]. Such an inflammatory switch mechanism, which essentially regulates host responses to infection, could drive symptomology and subsequent viral spread by altering cellular activation.

At least fifteen SARS-CoV-2 proteins have been suggested to contribute to antagonism of innate responses to date (reviewed in detail elsewhere[155,156]). These proteins have typically been identified via reporter screens in which the SARS-CoV-2 protein of interest is expressed during a simulated in vitro innate immune response, and its capacity to antagonize the response is evaluated. Such experiments do not typically provide mechanistic insight but are effective in discovering novel protein functions. Several of the VOCs exhibit amino acid changes in many of the proteins implicated as innate immune system antagonists, including NSP1, NSP3, NSP6, ORF3a, ORF6, ORF7b, ORF8, and N protein. Whether these coding mutations reflect adaptations to better antagonize human innate immunity is yet to be established in most cases. Moreover, the contribution of these proteins to the VOC phenotype is poorly understood, as this requires the painstaking generation of isogenic mutants using reverse genetics and assessment of their impact on replication, interferon production and sensitivity.

As well as reducing the induction of interferon, the Alpha and Omicron VOCs are more resistant to inhibition by its antiviral effects[144,150-152,158]. This has been best described as being

related to spike adaptations which reduce sensitivity to interferon-induced transmembrane (IFITM) restriction factors[91,151,159]. The exact effect of IFITM proteins during SARS-CoV-2 replication is contentious, with some studies showing that IFITM proteins inhibit cellular entry of SARS-CoV-2, in a similar manner to influenza[18,91,151,159], whereas others suggest that in some contexts they enhance infection, in a manner similar to that described for OC43 and HKU1[91,151,160-162]. The exact role of IFITM proteins is likely to be context specific, such as the particular entry pathway utilized by a particular virus in a given cell type or cell line, the use of live virus versus pseudovirus, and the level of IFITM protein expressed. IFITM proteins are small transmembrane interferon-stimulated genes (ISGs) that are associated with different cell membranes; human IFITM1 is generally associated with cell surface membranes, whereas IFITM2 and IFITM3 are more localized to late and early endosomes, respectively[91,163]. The exact mechanism by which IFITMs impact viral entry is not fully resolved but inhibition of viral glycoprotein fusion with host membranes is thought to be involved[164,165]. Several VOCs have been shown to have different degrees of sensitivity to IFITM inhibition or enhancement, quite often associated with specific entry pathways or furin cleavage phenotypes. For example, Omicron, which is more efficient at endosomal entry compared to early variants and other VOCs, appears to show greater inhibition (or in some cases, enhancement) by endosomal IFITMs, although this does appear to be highly dependent on the cell system used[31,91,162]. This is possibly due to Omicron either having to compromise innate evasion due to adaptive immunity avoidance, particularly in the context of vaccine-induced spike antibodies, or indeed, adaptation to use IFITMs as cofactors for entry.

It is not yet clear how enhanced antagonism of innate immunity might influence virus transmission. We hypothesize that the capacity of SARS-CoV-2 to transmit efficiently is strongly linked to its ability to evade and antagonize innate immune responses in the first cells that encounter virus in the airway. Indeed, the efficiency of infection events is expected to influence viral spread through the airway and thus the likelihood of seeding a productive infection at all. Certainly type I interferon responses have been shown to be important in determining infection efficiency and outcome for other viruses, and have been well characterized for lentiviral infection of macaques[166].

Finally, the fact that at present each VOC has evolved independently from an ancestral virus circulating early during the pandemic means that each VOC has taken a different mutational pathway to acquire distinct adaptations to humans. As a consequence, VOCs, or

indeed other variants of SARS-CoV-2, could potentially recombine to unite independently encoded adaptations and thus phenotypic advantages from different variant genomes (**Box 2**).

Antigenic distance in determining transmission and fitness

Ever-changing population immunity creates a dynamic fitness landscape for virus variants because their fitness is as much dependent on acquired immunity as their set of unique mutations. Although complexities such as the breadth and duration of immunity are important considerations[173,174], the cumulative number of humans exposed to SARS-CoV-2, via infection and/or vaccination, results in a much less susceptible population to most circulating (and past) variants with an ever decreasing number of immunologically-naïve, fully susceptible hosts (**Fig. 4**).

Early in the COVID-19 pandemic, when the fraction of naïve hosts is greatest, there is little evolutionary benefit of antigenic novelty relative to wild-type variants. Instead, selection favored variants capable of maximizing reproductive success through adaptation of intrinsic biological features such as the conformational change in spike caused by D614G, the defining mutation of the Pango B.1 lineage, or the enhanced furin cleavage phenotype coupled with increased ACE2-binding exhibited by Alpha[26,28]. As immunity in the host population increased, a variant's antigenic novelty played an increasingly important role in its reproductive success, relative to intrinsic biological changes[175]. Subsequently, the Delta VOC became dominant globally, displacing previous variants in countries with partially immune populations with moderate-to-high vaccination coverage[22,176]. Virus neutralization data indicated moderate immune escape from neutralizing antibodies by the Delta VOC[22,36-38], and vaccine effectiveness data indicated that antigenic novelty was not the primary driver of increased transmissibility[39-41,177,178], indicatinging the high fitness of Delta was more the result of intrinsic viral properties such as optimization of spike furin-cleavage[21,22,89].

Compared to previous variants, Omicron showed an unprecedented degree of antigenic novelty[30,32,33], arguably comparable to an influenza-like antigenic shift event[50]. The 'shift' here, the accumulation of mutations contributing to antigenic distance, probably occurs at least partly in the context of chronic infection(s) (Box 1). Comparison of transmission dynamics within vaccinated and unvaccinated households has indicated that immune escape was a critical component of the increased transmissibility of Omicron (BA.1) relative to Delta during their period of co-circulation[105]. The Omicron sublineage BA.2 has proved even more capable of infecting both unvaccinated and vaccinated individuals, potentially driven by similar immune

evasiveness properties to BA.1 but with higher intrinsic transmissibility[179]. Most recently BA.4, BA.5, BA.2.75 and their sublineages have shown not only even greater evasion of immunity compared to pre-Omicron variants, but also escape from immunity generated from prior infection with Omicron, particularly BA.1[45-48,83,180,181]. This has been largely attributed to mutations at antigenically potent RBD positions, particularly L452R and F486V in the example of BA.4/BA.5[182]. Although there may remain the potential for SARS-CoV-2 to further optimise for transmission within humans, it now seems likely that the antigenic novelty and immune evasiveness of emerging variants are the primary determinant of variant fitness and evolutionary success going forward. Consequently, understanding the complexities of cross-protection between variants is a major research priority.

Relative severity of SARS-CoV-2 variants

There is an imperative to understand how the virulence of SARS-CoV-2 variants might evolve in response to changing selection pressures. Pathogen virulence, alongside immunity, individual susceptibility, disease predisposition and other host factors, is a major contributor to disease severity and is defined in the evolutionary literature as the increased morbidity and mortality of individuals due to infection. Modelling data generally show a trade-off between a higher transmission rate and a cost of reducing infectious period due to higher death rates[187,188]. However, the predictability of virulence evolution is complicated by several mechanisms including within-host competition, changing transmission routes and tropism, and interactions with the immune system[188]. For instance, repeated epidemic waves, characteristic of an antigenically evolving pathogen, can select for higher pathogen virulence[189]. Assessing the relative virulence of SARS-CoV-2 variants through disease severity in humans is challenging due to changing immune status and developments in medical interventions throughout the pandemic, although it is possible to compare disease severity of variants that infected the same population within a given period of time[190]. This approach suggests inconsistency in the direction of change in disease severity between successively dominant SARS-CoV-2 variants: the successful variants exhibited increased disease severity as Alpha replaced B.1.177, and as Delta replaced Alpha, correlating with relative changes in transmissibility[190]. In contrast, Omicron exhibited reduced disease severity in the period in which it co-existed with Delta, a decrease which appears to reflect a complex combination of factors including both higher infection rates of those with some degree of prior infection, as well as an intrinsically lower virulence[190-194]. Proposed explanations for the lower disease severity of Omicron infections include reduced fusogenicity of spike, leading to less tissue damage, as well as altered tropism

restricted more to the upper respiratory tract (due to altered TMPRSS-2 usage)[31,90,97,98]. These previous studies have compared the virulence of variants existing in the same population, although they cannot determine the 'intrinsic' virulence of non-overlapping variants that circulated in populations with different immune-statuses, such as Alpha and Omicron. Given that the immune status of an individual influences the severity of symptoms in addition to the likelihood of infection, the antigenic 'distance' between past exposure and a diverged variant has the potential to facilitate onset of disease in an otherwise protected individual, representing the potential for divergence between inherent potential to cause harm and actual virulence in infected people.

A complementary approach to measure severity of SARS-CoV-2 variants involves the use of animal models (reviewed in more depth previously[195]). Common animal models have included naïve rodents, such as transgenic mice expressing human ACE2 under the K18 promoter (expressed abundantly in epithelial cells[196]) or hamsters, whose natively-expressed ACE2 proteins are efficiently used by all current SARS-CoV-2 VOCs[31,197]. Pathogenicity in these rodent models is most commonly measured as a function of percentage weight loss, sometimes alongside survival curves and measures of pulmonary function[198]. These rodent models have largely recapitulated equivalent severity data from epidemiological studies in humans, such as Delta being more pathogenic than earlier variants and Omicron being less pathogenic than Delta[31,89,90,199,200]. However, the models do have several limitations, as exemplified by recent epidemiological evidence from Hong Kong suggesting that Omicron/BA.2 displays a similar disease severity to first wave variants[201], whereas rodent models generally show that Omicron is less severe than previous variants[31,90,199,200,202]. This inconsistency could potentially be explained by ongoing adaptation of SARS-CoV-2 to human hosts, resulting in concomitant adaptation away from rodents and other animal models[31,99,203,204].

Disease severity following SARS-CoV-2 infection is correlated with several risk factors including age, sex, and clinical comorbidities such as obesity and immunodeficiency, and several inflammatory markers[205]. As reviewed elsewhere, several recent genetic studies have focused on characteristics that might explain why some individuals are more susceptible to SARS-CoV-2 infections and others develop more severe symptoms[206]. However, there is an urgent need to combine these findings with data from specific variants and interventions (that is, vaccines, drugs and monoclonal antibodies) that might directly affect the observed phenotypes[205]. Furthermore, although the most common and easily measurable outcomes from

acute infections is hospitalisation or deaths, outcomes that are more difficult to measure also vary widely between variants, such as primary symptomatology[207], or post-acute COVID syndrome, although these likely differ greatly by prior immune status as well.

Conclusion

SARS-CoV-2 has been circulating forthree years within the human population, infecting hundreds of millions of individuals. It remains, however, a relatively new human virus that continues to evolve and acquire adaptations to its new host species. Unprecedented SARS-CoV-2 genome sequence datasets generated globally have found evidence of beneficial mutations arising in real time and have guided laboratory experiments to better understand intrinsic properties of the interactions between virus and host. Although we have this extraordinary understanding of SARS-CoV-2 biology, virus fitness is highly dynamic and the ability of SARS-CoV-2 to infect, replicate within, and transmit among the human population has depended explicitly on the specific immune context at different periods of the pandemic. At present Omicron is dominant worldwide with infections being driven by emergent BA.2 and BA.5 sublineages. Despite the fact that our understanding of SARS-CoV-2 is improving, virus evolution is inherently unpredictable, and a likely future scenario is the emergence of a new VOC that is antigenically and, potentially, phenotypically distinct from the early forms of Omicron. At the same time, population immunity against SARS-CoV-2 continues to accumulate and may well compensate in the case of a future variant emerging with higher severity, leading to milder acute disease.

All of the VOC progenitors have evolved from an ancestral pre-VOC virus present during the first wave of the pandemic, taking different, but often convergent pathways to more efficiently infect and transmit among humans, and to resist antibodies, T-cells driven and innate immunity. The required adaptations, as we discuss throughout this review, are a mixture of changes to host via intrinsic virus properties and escape of innate or adaptive immunity (Box 3). The prevailing hypothesis is that variants originate from chronic infections in immunocompromised individuals, in which the virus is able to establish a persistent infection due to impaired immune function[69,78,82]. This hypothesis explains the step-change of seemingly rapid evolution seen before the emergence of new variants[69,76,79]. Although it should be noted that future variants will probably be directly derived from prior or contemporary VOCs, most recently exemplified by the spate of ‘second generation’ Omicron variants derived from BA.2, such as BA.2.75, BJ.1 and BA.2.10.4[208]. While intra-lineage recombination serves as an

opportunity for the virus to gain additive adaptations and phenotypic advantages from distantly related circulating variants, recombinants have only exerted a minor impact on the course of the pandemic at present (**Box 2**). Furthermore, although there is currently very limited evidence for the establishment, long-term circulation and evolution in animal reservoir species, intensive and active surveillance of susceptible species is needed as reverse zoonosis is being documented[4,5,75]. There are many countries with low sequencing capacity, or places with previously good surveillance that are decreasing or phasing out sequencing altogether. This is troublesome as a lack of genomic surveillance will mean future variants will be detected much later or could be circulating at low levels and unobserved before detection. There is, thus, a need for widespread and equitable surveillance coverage to rapidly detect potential new VOCs among these individuals and communities before they spread more widely.

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Author contributions

A.M.C., T.P.P., L.T., W.T.H., G.J.T., COG-UK Consortium and T.I.dS researched data for the article. D.L.R., A.M.C., T.P.P., J.H., G.J.T., and T.I.dS contributed substantially to discussion of the content. D.L.R., A.M.C., T.P.P., L.T., W.T.H., J.H., G.J.T., and T.I.dS wrote the article. D.L.R., A.M.C., T.P.P., L.T., W.T.H., J.H., S.J.P., W.S.B., G.J.T., and T.I.dS reviewed and/or edited the manuscript before submission.

Competing interests

The authors declare no competing interest.

COVID-19 Genomics UK (COG-UK) consortium
Alessandro M. Carabelli[1], William T. Harvey[4], Joseph Hughes[4], Sharon J. Peacock[1], Thushan I de Silva[6] and David L. Robertson[4]

[1]Department of Medicine, University of Cambridge, Addenbrookes Hospital, Cambridge, UK
[4]MRC-University of Glasgow Centre for Virus Research, University of Glasgow, Glasgow, UK
[6]Florey Institute for Host-Pathogen Interactions and Department of Infection, Immunity and Cardiovascular Disease, Medical School, The University of Sheffield, Sheffield, UK. A full list of members and their affiliations appears in the Supplementary information.
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Related links

COG-UK Mutational explorer: https://sars2.cvr.gla.ac.uk/cog-uk/

GISAID: https://gisaid.org

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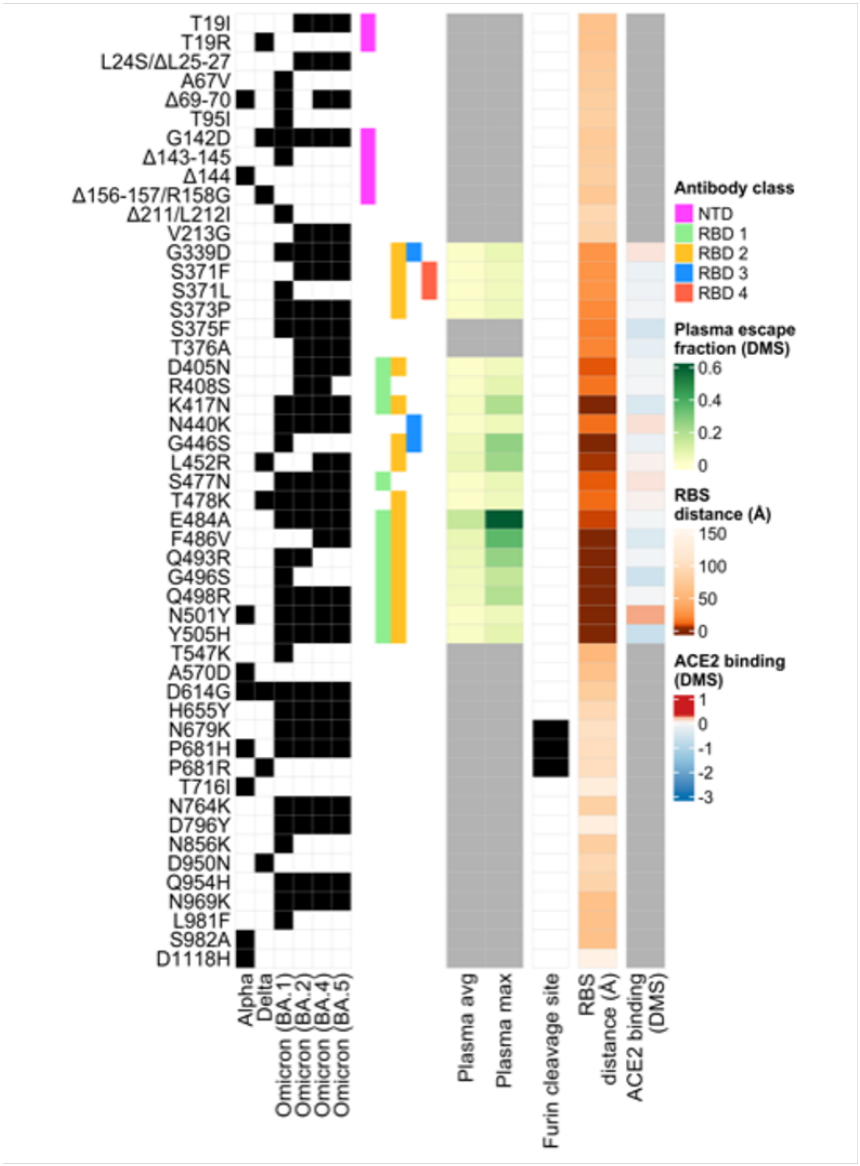

Figure 1. **Properties of amino acid substitutions or deletions in selected SARS-CoV-2 variants of concern (VOCs).** Black boxes denote the presence of each mutation in the VOC. Epitope residues are colored to indicate the amino-terminal domain (NTD) supersite[63] or the receptor-binding domain (RBD) class[64]. For RBD residues, the results of deep mutational scanning (DMS) studies show the escape fraction (that is, a quantitative measure of the extent to which a mutation reduced polyclonal antibody binding) for each mutant averaged across plasma ('plasma avg') and for the most sensitive plasma ('plasma max')[65] illustrating consistency or variation in the effect of a mutation depending on differences in the antibody repertoire of individuals. Mutations in the furin cleavage site are highlighted. Orange shading indicates the distance to ACE2-contacting residues that form the RBS. Note that the RBS is defined as residues with an atom <4Å of an ACE2 atom in the structure of RBD bound to ACE2 (RCSB PDB ID: 6M0J[66]). Finally, ACE2 binding scores representing the binding constant ($\Delta\log_{10} K_D$) relative to the wild-type reference amino acid from DMS experiments are shown in shades of red or blue[25].

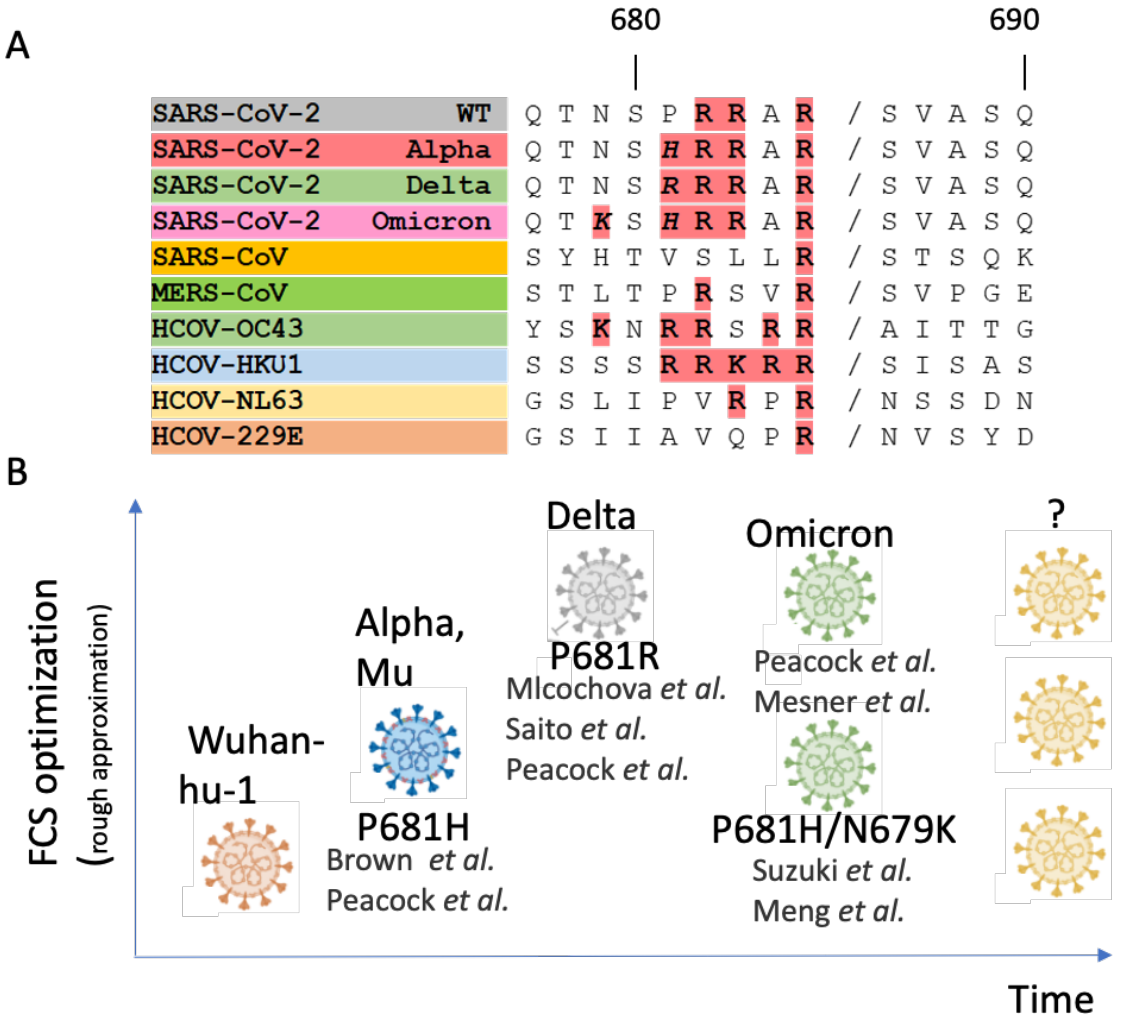

Figure 2. Furin cleavage site and variant success. a) Comparison of S1/S2 cleavage site sequences in wild-type (WT) SARS-CoV-2, Alpha, Delta, and Omicron VOCs, compared to other coronaviruses: SARS-CoV, MERS-CoV, HCoV-OC43, HCoV-HKU1, HCoV-NL63 and HCoV-229E. The slash indicates the putative furin/serine protease cleavage site. Amino acids contributing to monobasic or polybasic cleavages sites are shaded. **b)** Diagrammatic representation of estimated relative optimization of the S1/S2 furin cleavage site (FCS) for the VOCs. The mutations affecting FCS function are indicated. Data on Alpha from refs [18,23,24]; Data on Delta from refs [21,22,89] ; Data on Omicron from refs [31,90.91]. Note that non-concordant results have been observed for Omicron, as indicated. The level of FCS optimization for future variants is uncertain.

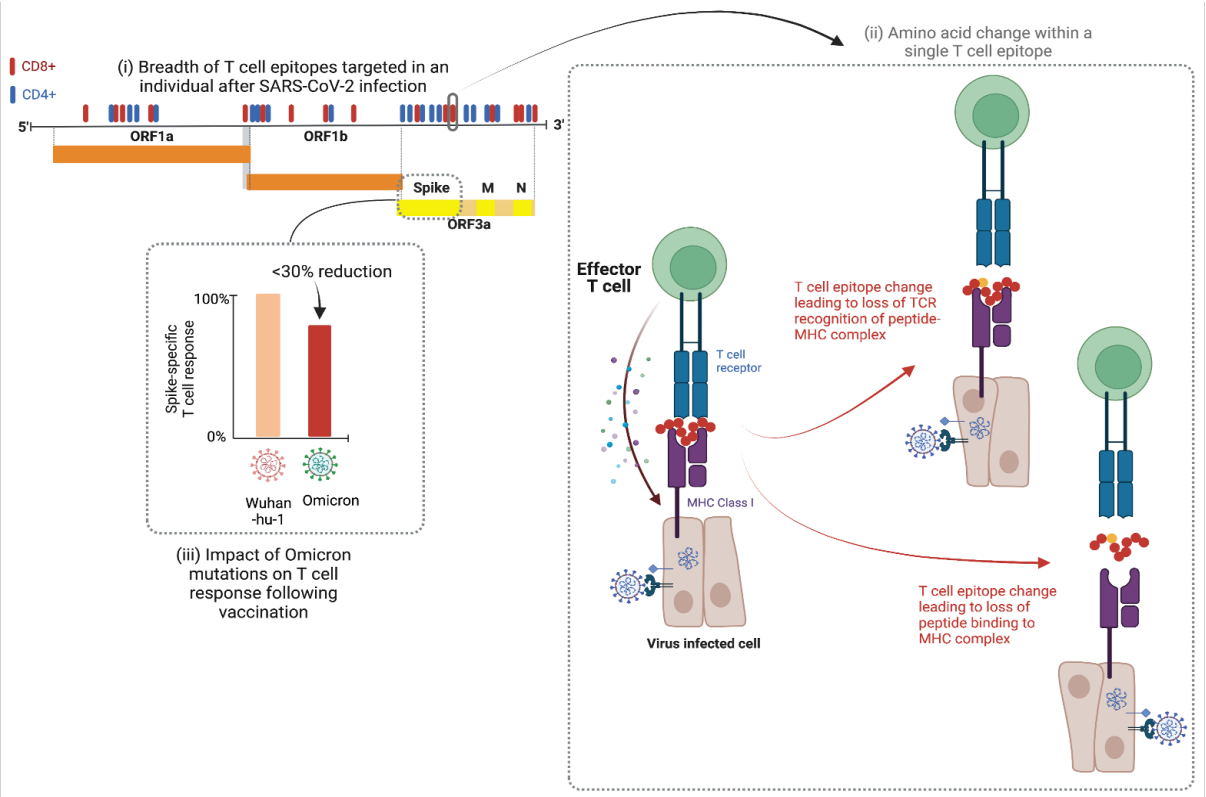

Figure 3. Potential impact of SARS-CoV-2 variants on T cell responses and innate immunity. a) Following SARS-CoV-2 infection, $CD4^+$ and $CD8^+$ T cell responses are generated against 30-40 epitopes across the virus genome[129] (epitopes are shown in red and blue). **b**) An example of how an amino acid change within one epitope might impact epitope-specific cytotoxic T cell responses, thereby inhibiting the elimination of virus-infected cells[133]. T cell evasion of SARS-CoV-2 has been shown to be the consequence of impaired peptide binding to the MHC complex or poor binding of the T cell receptor to the peptide-MHC complex. **c**. Although the T cell response to vaccination is focused on spike alone, even the multiple spike mutations in the Omicron VOC only reduce vaccine-induced spike-specific T cell response by <30%, with considerable inter-individual variability[138,157].

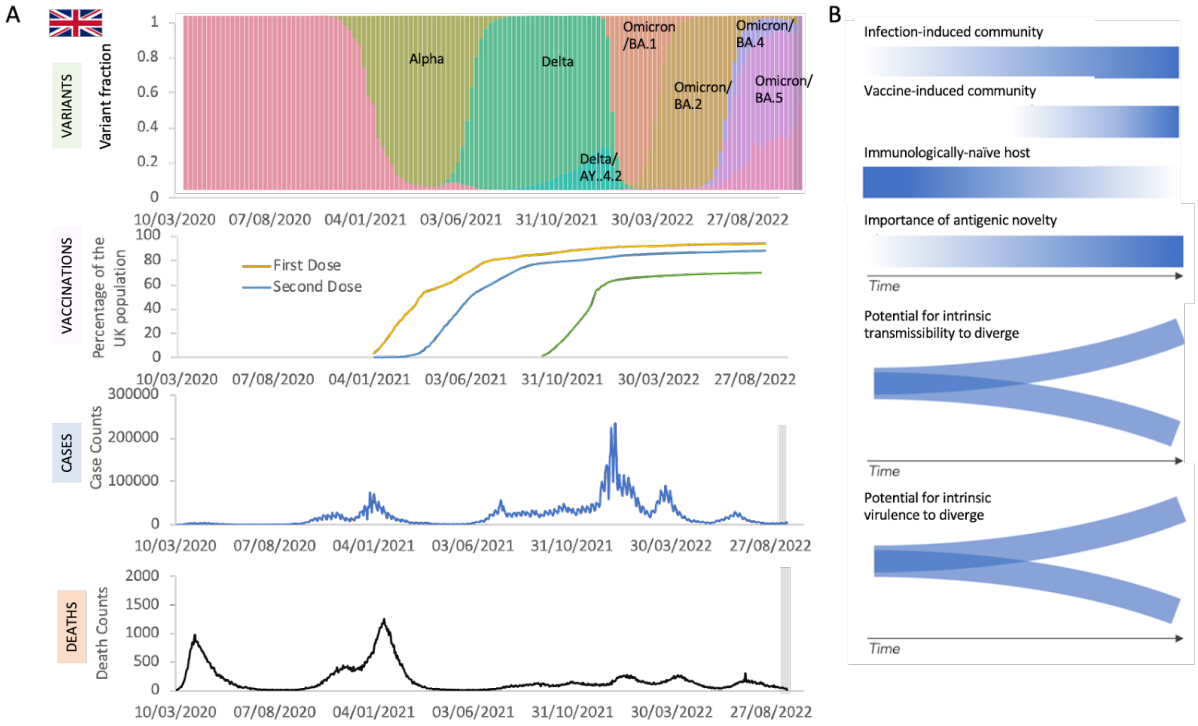

Figure. 4. Dominant SARS-CoV-2 variants, vaccinations, infections and deaths since early 2020 in the UK. a) Waves of dominant variants in the UK (B.1, B.1.1.7/Alpha, B.1.617.2/Delta, AY.4.2/Delta and Omicron sub-lineages: BA.1, BA.2, BA.4 and BA.5), proportion of the UK population with one or two doses and with one booster vaccination, number of COVID-19 cases and reported COVID-19-related deaths. Data from COG-UK Mutational explorer[183] and GOV.UK. **b)** Diagrammatic visualization of the dynamic relationship between variant transmissibility, antigenicity, virulence and fitness. As population immunity derived from infection and vaccination increases, the fraction of completely immunologically-naive hosts declines (gradient blue lines). Consequently, the importance of antigenic novelty in determining variant fitness increases. Antigenic distance to previously circulating variants becomes an increasingly key determinant of variant transmissibility, elevating the potential for intrinsic and real-world transmissibility to diverge. Similarly, antigenic distance influences a variant's potential to infect and cause disease in immune hosts, increasing the potential for a variant's intrinsic virulence to diverge from its real-world clinical impact.

Box 1 | The origin of SARS-CoV-2 variants of concern (VOCs) and the Omicron complex.

SARS-CoV-2 VOCs exhibit a number of distinct properties, one of the most intriguing being a relatively long phylogenetic branch length often with a lack of genetic intermediates prior to their detection[67-69]. Alpha was first detected in the UK during a time of low virus circulation and unprecedented SARS-CoV-2 surveillance[69,70]. Delta was first identified in India in April 2021[71], and then associated with a new epidemic wave in the UK[72], generating several sub-lineages, some of which were even more transmissible than parental Delta, for example AY.4.2[73]. Similarly, the Omicron complex of variants has a long branch length and even more mutations than prior VOCs[42,43].

The lack of intermediate sequences has led to several hypotheses of VOC origins[74]: i) circulation in geographically undersampled regions; ii) cryptic circulation within an animal reservoir following reverse zoonosis of an early variant; iii) evolution within a chronic infection in an immunosuppressed host or multiple human hosts. Although there is evidence for establishment of stable animal reservoirs for SARS-CoV-2, particularly in farmed mink and white-tailed deer[4,5,75], at present the 'chronic infection hypothesis' of variant emergence is the best supported of these scenarios[69,76]. Chronic infections are known to drive mutational profiles that are highly similar to VOCs[77-81]. There is also evidence for limited onwards transmission of chronic infections causing localised outbreaks[78,82]. Long term evolution in the presence of constant sub-neutralising antibody pressure might explain VOC antigenic distance, particularly for Omicron.

Unlike other VOCs, Omicron had evolved remarkable diversity before being first detected, and is currently divided into five major lineages – BA.1-5[42] – with many more sublineages being detected that are accumulating further antigenic changes[83]. BA.1 caused a global wave of infection at the end of 2021, but by early 2022 was replaced by BA.2. In April 2022 further BA sublineages, BA.4 and BA.5, were recognized and, as of September 2022, BA.5 has driven a new Omicron wave internationally[42]. The Omicron lineages show a complex relatedness to each other, which likely included multiple intra-VOC recombination events prior to detection[42,43]. For example, BA.4 and BA.5 contain near identical 5' ends of the genome until the M gene, but show large divergence after this point suggesting a recent recombination event. Similarly, BA.3 could conceivably be the recombinant progeny of ancestral BA.1 and BA.2 viruses[43].

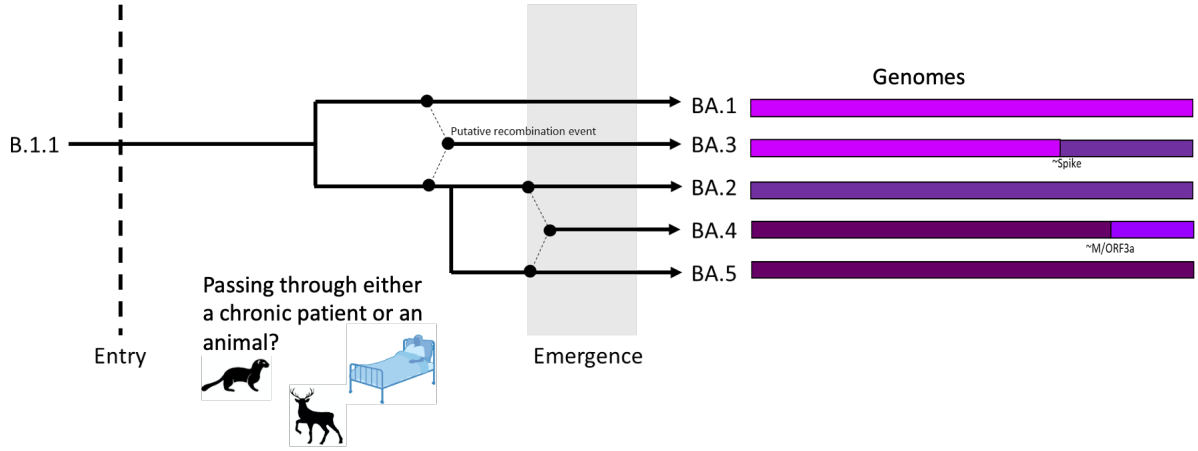

Figure legend for Box 1 figure: Hypothetical phylogenetic origins of the Omicron complex showing one potential pattern of recombination among sublineages BA.1 to BA.5.

Box 2 | SARS-CoV-2 recombinants.

When two RNA viruses happen to co-infect the same cell within an individual, there is a high chance during genome replication that the polymerase will switch from one genome sequence template to the heterologous genome. This results in a recombinant virus with part of its genome from one 'parent' and the remaining genomic sequence from the other. Several recombinant lineages have been identified in different locations and designated by the Pango classification system since 2020[9], identifiable by the 'X-' lineage prefix. Recombinants have been unambiguously identified when genetically distinct variants, such as, two VOCs, have transiently co-circulated leading to co-infections. For example the first assigned recombinant lineage, XA, was a recombinant between Alpha (B.1.1.7) and the previous circulating lineage in the UK, B.1.177[167]. XC was a recombinant between Alpha and Delta found in Japan[168].

From the start of 2022, the number of recombinants identified rapidly increased, which was likely due to high levels of co-circulation between Delta and BA.1, or BA.1 and BA.2, in many countries at a time of phasing out of COVID-19 restrictions. Another explanation is better confidence in identifying recombinants, owing to higher sequence divergence among the genomes of Delta, BA.1 and BA.2. One example recombinant is XD - a Delta x BA.1 recombinant first found in January 2022 in France[169]. XD has two genome breakpoints, with a backbone and part of the spike NTD from Delta and the remainder of spike from BA.1. Functionally, XD has been shown to have an intermediate pathogenicity phenotype between BA.1 and Delta in K18-ACE2 mice, causing moderate weight loss, suggesting part of the differential rodent pathogenicity phenotype of Delta and BA.1 maps outside of the spike protein[169].

A second notable recombinant in the UK is the BA.1 x BA.2 recombinant, XE, first detected in England on January 19, 2022. Before it was outcompeted by BA.5, XE comprised over 2500 genomes, mostly from the UK, and preliminary data suggest a modest increase in growth rate compared to BA.2[170]. XE contains the spike and structural proteins from BA.2 and part of ORF1ab from BA.1.

A recent notable lineage is XAY, a BA.2 x Delta recombinant, first discovered in South Africa in May 2022 alongside a smaller sister recombinant, XBA[171]. These recombinants have a much larger number of breakpoints than any prior known cluster, as well as many unique mutations not found in the parental lineages. One possible explanation for the appearance of these recombinants is a chronic Delta infection with BA.2, iterative recombination (alongside gradual gaining of unique mutations) before multiple emergences back into the general population. XBB is a recently emerged and rapidly growing recombinant between two second generation BA.2 lineage - BJ.1 (aka BA.2.10.1.1) and BA.2.75, and it contains a large number of antigenic RBD mutations and has been shown to be poorly neutralised by previous Omicron breakthrough antisera[211].

As we have described, variant-specific properties can both map within and outside of spike. Recombinants, such as XD, demonstrate it is possible for recombination to generate viruses with phenotypic properties from both parental viruses[169]. At present recombinants haven't had a large impact on the course of the pandemic, usually appearing around the time new variants are replacing previously dominant lineages. It should be noted that such recombination is commonplace among the sarbecoviruses infecting horseshoe bats, as exemplified by the high levels of recombinant genomes detected and the tendency for spike to be swapped, indicative of frequent antigenic shift events[172].

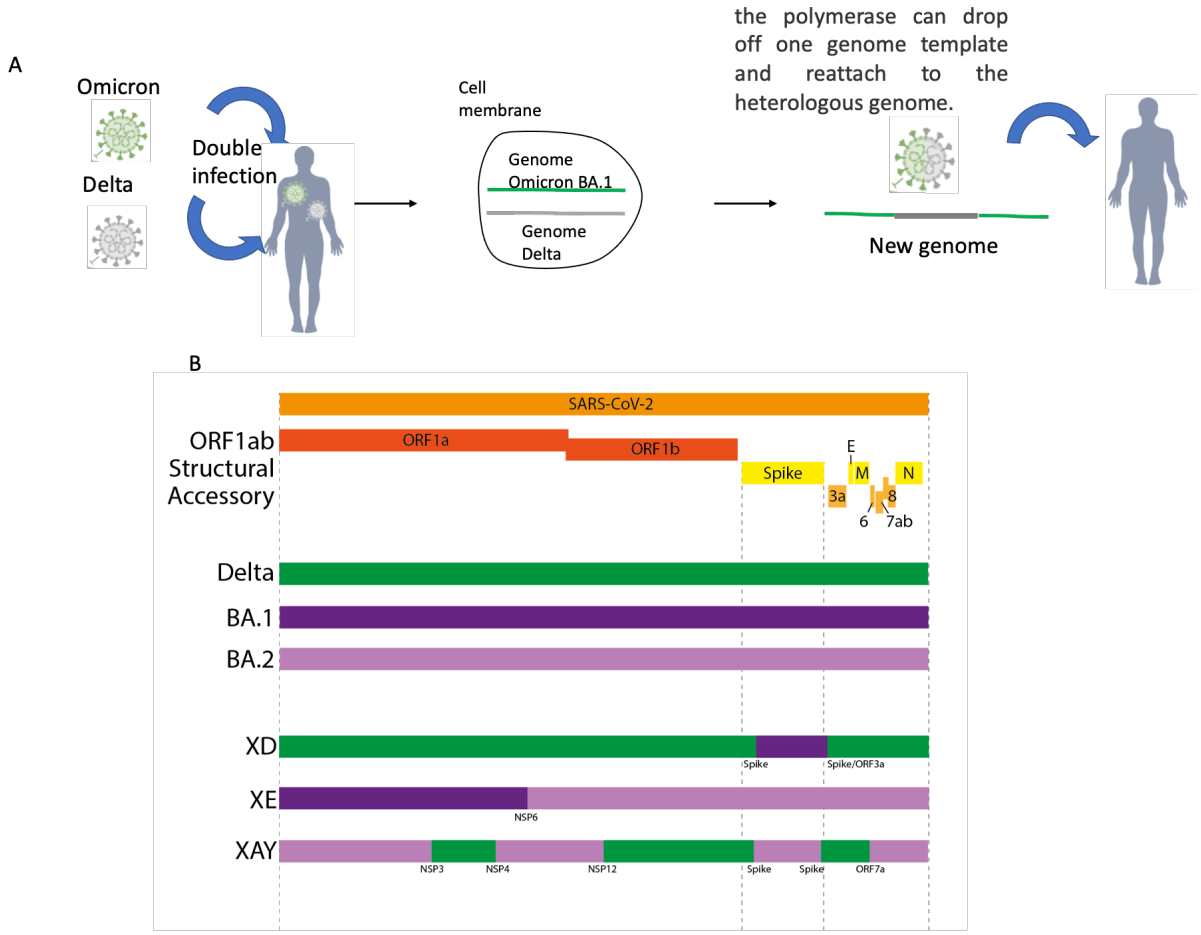

Figure legend for Box 2 figure: a) Diagram of a recombination process occurring in an individual with Omicron and Delta infection. **b)** Genome maps of some notable contemporary recombinants (XD, XE, XAY).

Box 3 | Fitness and antigenicity. Darwinian fitness[184], usually referred to as just fitness, is distinct from replicative fitness: the capacity of a virus to produce infectious progeny, measured experimentally in cultured cells, tissue culture or within individual hosts[185,186]. In contrast, the broader definition of fitness is reproductive success so highly context-dependent often varying over time and between locations. The fitness of a given SARS-CoV-2 variant will depend on the changing immune profile of the host population it's circulating in, and the success of an individual variant is relative to the properties of competing variants within the virus population and stochastic sampling processes. As immunity in the host population increases, the conditions can result in a previously highly transmissible variant, such as in an immune naïve population, now being less fit relative to more evolved variants (Fig. 4B). Although intrinsic transmissibility is thus based on biological properties of the virus only, actual transmissibility depends on these properties in the context of population immunity, including the interplay of host immunity from past exposures, variant antigenicity and stochastic effects. Moreover, although it is possible that variants may differ in their relative antigenicity - the level of immune response they stimulate - variation in the degree of antigenic similarity to other variants is usually of greater impact. As immunity in the host population increases, the antigenic distance

of a variant to previously circulating variants can determine its capacity to infect, replicate within and transmit between hosts. The evolution of antigenic novelty therefore becomes the critical determinant of variant reproductive success and fitness.