The immunology of asymptomatic SARS-CoV-2 infection: what are the key questions?

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Abstract | An important challenge during the COVID-19 pandemic has been to understand asymptomatic disease and the extent to which this may be a source of transmission. As asymptomatic disease is by definition hard to screen for, there is a lack of clarity about this aspect of the COVID-19 spectrum. Studies have considered whether the prevalence of asymptomatic disease is determined by differences in age, demographics, viral load, duration of shedding, and magnitude or durability of immunity. It is clear that adaptive immunity is strongly activated during asymptomatic infection, but some features of the T cell and antibody response may differ from those in symptomatic disease. Areas that need greater clarity include the extent to which asymptomatic disease leads to persistent symptoms (long COVID), and the quality, quantity and durability of immune priming required to confer subsequent protection.

Early in the COVID-19 pandemic it became clear that infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) would pose greater challenges to infection control than infection with SARS-CoV in 2002–2003 or Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, as SARS-CoV-2 is spread readily between people, even by those who are presymptomatic or asymptomatic[1–3]. Thus, surveillance and case-finding would require far greater resources and vigilance than previously thought; for example, temperature monitoring is not sufficient to detect all individuals infected with SARS-CoV-2. In retrospect, it became apparent that a significant proportion of infections with MERS-CoV are asymptomatic[4], but this virus is significantly less infectious than SARS-CoV-2. An account of a COVID-19 outbreak in a US care home in April 2020 described how one positive case led to 63% of residents becoming infected, although half of these had asymptomatic infections that would not have been identified except by regular PCR testing[5]. Asymptomatic transmission was thus described as the "Achilles' heel" of SARS-CoV-2 infection control strategies[1].

Asymptomatic COVID-19 case-finding depends on regular community screening by PCR-based approaches and/or serology, either in a cohort study setting[6,7] or to trace potential transmission after possible exposure, as in the care home example. Although studies such as this established the likelihood of asymptomatic transmission, a mass screening study from Luxembourg at the end of 2020 formally demonstrated that asymptomatic carriers infect a similar number of people to symptomatic individuals[8].

Asymptomatic infection is a tricky and uncharted territory for infectious disease immunologists and clinicians. Our natural territory is understanding the mechanism underlying severe infection and protection against it. As with so many other aspects of medical research in the time of COVID-19, the pandemic has fast-tracked us into deploying our skillsets to address completely new questions. For this Progress article, we aim to build a picture of the emergent field of the immunology of asymptomatic infection. We start by considering the now well-established data showing that asymptomatic presentation is relatively common and is a major route of transmission — a key point in the past year's steep learning curve in attempts to explain the rapid spread of the virus, which necessitated a complex reappraisal of pre-emptive mitigation strategies. We then discuss the preliminary evidence that asymptomatic infection is similar to mild or severe COVID-19 in its ability to trigger long-term, persistent symptoms termed 'long COVID'. More data are urgently needed, but whether or not asymptomatic infection poses a risk similar to that posed by symptomatic infection with regard to long-term complications is clearly a decisive question. If there is no health cost to asymptomatic infection, there is no need to fear it or count it, and it could indeed serve it as a natural top-up to SARS-CoV-2 immunity. If, on the other hand, it is an invisible precursor to long-term disease, it should be a cause of great concern.

We then discuss current insights into innate and adaptive immunity in asymptomatic infection, an area which is very much a work in progress, yet with massive ramifications. The first point we discuss is the role of immune recognition of the virus during asymptomatic infection — it is certainly clear that innate and adaptive immune activation are triggered during even the mildest infection. What has been less clear and currently remains unresolved is the nature of the immune determinants that dictate why, after a similar viral inoculum, some individuals will develop no detectable symptoms, others will develop mild symptoms and yet others will develop severe or fatal symptoms. Ultimately, our aim has been to pull together and appraise the current state of knowledge, allowing some assessment as to whether asymptomatic transmission might be considered beneficial in that it induces immunity with a low cost in disease burden, thus enhancing herd immunity, or detrimental in that it fuels untracked transmission and facilitates the evolution of vaccine escape variants and carries the risk of long COVID. Despite the impressive progress in research over the past 18 months, datasets are sparse and informed consensus is hard to reach. We conclude that asymptomatic spread indeed carries a risk, not least the potentially long-term burden of long COVID. Asymptomatic COVID-19 is

invisible and therefore easily missed, but this should not allow us to ignore its potentially detrimental contributions through silent percolation and transmission in society.

How common is asymptomatic infection?

Cohort screening studies have allowed estimates of the proportion of individuals with asymptomatic cases of SARS-CoV-2 infection, although there has been considerable variability in findings. Part of this variation derives from the rigour with which the term 'asymptomatic' is defined. Does it apply only to someone identified as testing positive for SARS-CoV-2 (by serology or PCR) who had not felt unwell with specific, COVID-19 case-defining symptoms (such as fever, cough or anosmia), or does it apply to someone who felt completely well throughout the period with no symptoms whatsoever? Scientific stringency may argue that an asymptomatic case is one with laboratory-confirmed SARS-CoV-2 infection as determined by PCR and/or serology but with no symptoms whatsoever for the duration of infection[9]. The complexities of a working definition for the term 'asymptomatic' in this disease are exemplified by an early report from Wuhan[10], which showed that about one-third of individuals with asymptomatic infections had lung changes that were visible on computed tomography scans, a finding that was subsequently confirmed in several other studies[11]. That is, end organ damage can ensue in individuals who were otherwise unaware of their infection. Meta-analyses and studies of large cohorts have shown that around 20–40% of individuals infected with SARS-CoV-2 have asymptomatic disease[11–13]. For example, a serological screen of residents in the Italian municipality of Vo' in early 2020 found that 42% of individuals who had been infected had been completely asymptomatic[14]. However, with looser criteria of 'no COVID-19-related symptoms', some studies, such as a survey of 9,500 residents of Wuhan, place the proportion of asymptomatic cases above 80%[15,16]. An important aspect of these data is whether there are differences between age groups, especially the often-quoted assumption that children and adolescents are more likely to be asymptomatic following infection. Early data suggested that asymptomatic disease was indeed inversely correlated with age[17]. However, the Real-time Assessment of Community Transmission 2 (REACT-2) UK national study considered asymptomatic disease in a large cohort of 106,000 adults sampled by specific antibody tests during the first wave of the pandemic in the UK (March to June 2020); 5,544 tested positive, of whom one-third had reported no symptoms, although this rose to more than half of those older than 65 years[6]. That is, asymptomatic disease was more common in older adults in this sample. This finding obviously does not contradict the observation that severe and fatal disease is also heavily increased in this age group. This set of data cannot be specifically compared with reports of common asymptomatic presentation in children because the REACT-2 study included only adults. There is indeed evidence for milder infections with a higher chance of asymptomatic presentation in children and adolescents[18–20]. A systematic review and meta-analysis of 350 studies concluded that asymptomatic presentation in elderly people occurred in 19.7% of cases, compared with 46.7% in children[21]. The extent to which this makes young individuals potential hubs for community spread (for example, out of schools) remains a subject of debate. It is also important to note that any discussion of differential presentation of symptoms across the life course has become more complex as the world has endured sequential waves of infection driven by different variants of concern, with a trend to a lower mean age of individuals affected relative to the first wave, which is considered to be, at least in part, a feature of the differential pathophysiology of variants in the subsequent waves[22].

Transmission by asymptomatic individuals

The degree to which individuals with asymptomatic infection can transmit SARS-CoV-2 is keenly debated, as this has profound consequences for public health strategy: if asymptomatic spread is a significant route of transmission, then substantial, ongoing, population-level surveillance is a public health need[21,23–25], whereas this level of screening could be a considerable waste of resources if asymptomatic spread was uncommon[26]. A general finding is that individuals with symptomatic and asymptomatic infections have a comparable viral load, supporting the idea of a similar potential for transmission, although virus clearance may be faster in asymptomatic infections, which suggests a shorter period of transmission[27]. For example, a prospective study at a containment centre in Vietnam showed similar viral loads at enrolment between participants with asymptomatic and symptomatic infections, but the virus seemed to be more rapidly cleared in individuals who were asymptomatic[28]. In an early study of 300 individuals with asymptomatic infections in Wuhan, it had been posited that such individuals may not be infectious and carried no culturable virus[15,16]. This may have reflected the time of sampling, especially if the window for detecting infectious virus is shorter in asymptomatic infections. However, another study from early 2020, which reported the contacts of 63 PCR-positive individuals with asymptomatic infections in Chongqing, China, found that 9 of the 63 individuals (14.3%) transmitted infection to their contacts[29]. A meta-analysis subsequently suggested a low but significant secondary attack rate from individuals with asymptomatic infections, with an overall relative risk of 0.35 (REF.[12]). An analytical model of SARS-CoV-2 transmission argues that more than half of all transmission is from asymptomatic carriers, including both individuals who are presymptomatic and individuals with true asymptomatic infections who never develop symptoms[23]. During a period when those with known PCR-positive symptomatic infections have tended to self-isolate, 'superspreader' clusters after large events (such as the Sturgis Motorcycle Rally (South Dakota, USA) attended by half a million bikers in August 2020)[30] were attributed to spread from individuals with presymptomatic and/or asymptomatic infections. A recent study estimated the infectiousness of individuals who were presymptomatic, asymptomatic or mildly symptomatic[31]. The study was conducted in Germany during high prevalence of the Alpha (B.1.1.7) variant of concern. The conclusion was that, as for the wild-type Wuhan Hu-1 strain, there is a similar potential for transmission from individuals with presymptomatic, asymptomatic or mildly symptomatic infections as for transmission from individuals with symptomatic presentation[31].

In summary, it is not surprising that any granular analysis of asymptomatic infections — which are, by definition, invisible — is fraught with caveats of study design, with consensus hard to reach. It is assumed that many of the unknowns will be resolved in human challenge studies that are currently in progress[32]. Viral load is not substantially different between symptomatic and asymptomatic infections, although the period of shedding may be longer in the former group. However, individuals with asymptomatic infections have consistently been shown to shed virus, and this group likely accounts for substantial transmission by virtue of being out and circulating in

society, rather than unwell and isolating. The big question, discussed in more detail later, is how to better decode the immune determinants of faster virus clearance, which appears to be correlated with asymptomatic presentation.

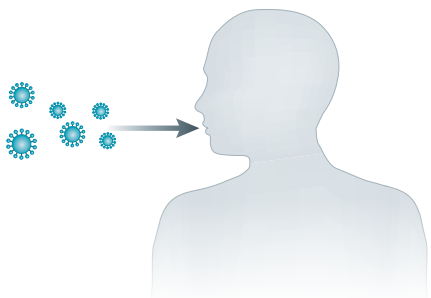
Risk of long COVID

In addition to the potential for individuals with asymptomatic infection to transmit the virus, a key question in evaluating whether asymptomatic infection might be detrimental in terms of public health is whether an initial asymptomatic episode carries with it the risk of progressing to long COVID. Long COVID is defined as a protracted period of persistent, fluctuating symptoms including fatigue, shortness of breath, headache, cognitive impairment, cough, chest pain and muscle pain[33]. Long COVID is estimated to ensue from about 20% of symptomatic infections[34,35]. The UK Office for National Statistics reporting includes all those reporting symptoms beyond 4 weeks from acute infection, although many of these individuals will go on to have symptoms lasting well over a year[36]. If asymptomatic infection also poses a risk for developing long COVID, the risk associated with asymptomatic or mild infection with SARS-CoV-2 may have been underestimated. One study estimated that one-third of cases of long COVID ensue from asymptomatic acute infection events[37]. The debate regarding this risk has underpinned strategies for moving out of lockdowns and learning to 'live with the virus'. Particularly in countries with high levels of vaccination, there is a view that the link between infection and severe outcome has been weakened, leaving COVID-19 as just another ailment for us to tolerate alongside influenza and common colds. The UK is currently experiencing a wave of a considerable number of new infections per day with the Delta variant but, compared with previous waves of infection, a low translation of these numbers into acute hospital admissions and fatalities[38]. A key question is the extent to which these milder or asymptomatic infections could contribute to a future long-term disease burden of long COVID. If one took the view that a predicted tolerance for 25,000 new infections per day could translate into a long-term legacy of about 5,000 individuals per day who will develop long COVID, this could be building a staggering public health burden for the future. A large US study from FAIR Health looked at 2.2 million cases of COVID-19 and long-haul disease, estimating that 19% of cases of long COVID have resulted from asymptomatic infections[39]. Currently, asymptomatic infection remains difficult to diagnose and track, as does the long-term consequence in the form of long COVID symptoms. Improved biomarkers and diagnostic tests are needed on both counts. The prospect of tolerating a 'new normal' of an infection with a low risk of fatal outcome but a high risk of leading to long-term disease is a difficult one. It may be useful to consider the analogy of acute and chronic outcomes after infection by chikungunya virus: the acute infection is serious and challenging, with fever, malaise, rash and potential for neurological complications. However, the greater devastation, health-care burden and thus policy challenge arguably come from the sizeable minority who progress to a persistent, disabling arthralgia[40].

Immunity after asymptomatic priming

From the currently available data, it is unclear what determines asymptomatic versus symptomatic presentation following SARS-CoV-2 infection. Possible contributing factors include lower age, lower challenge dose, prior immunity (for example, cross-reactive immunity from human common cold coronaviruses or innate immune subsets) or differences in the specific immune response that is mounted. Here, we consider in detail the available data for the specific immune response that is induced in the context of asymptomatic infection. This is a point of considerable significance if, as proposed, 20–40% of global infections are asymptomatic. Depending on the level and type of the immune responses induced by asymptomatic infection, ongoing asymptomatic transmission in a population could contribute to generating herd immunity. Any models of herd immunity therefore need to take into account natural infections (both symptomatic and asymptomatic) and vaccine-induced immunity, and assume that both contribute to lowered virus transmission.

Many studies have now compared adaptive immunity to SARS-CoV-2 across the spectrum of asymptomatic infection to mild, severe and fatal infection (FIG. 1). This comparison goes to the heart of the nature and implications of asymptomatic COVID-19: is this simply a very mild form of infection that never quite takes hold or is this an example of a form of potent immune control that suppresses symptoms, yet may nevertheless contribute to the acquisition of normal immune memory, thus driving collective herd immunity? Some studies find that severer, symptomatic infections (although, not lethal infection) tend to be associated with a higher or more enduring viral load, greater immune priming and higher levels of antibodies, neutralizing antibodies and $CD4^+$ T cell and $CD8^+$ T cell responses[41], while others find mild disease poorly correlates with the levels of neutralizing antibodies but is significantly associated with the magnitude of $CD4^+$ T cell and $CD8^+$ T cell responses[42]. A retrospective analysis of basic immune parameters comparing 25 individuals with asymptomatic infection with 27 individuals with symptomatic infection found similar



No infection	Asymptomatic infection	Mild symptomatic infection	Severe	Lethal
• Innate resistance? • Pre-existing polymerase-specific T cells?	• Variable T cell response including IFNγ, IL-2, TNF, IL-6 and IL-10 • High neutralizing Ab levels	• Some evidence for greater T cell response than in asymptomatic individuals • High neutralizing Ab levels	• Delayed seroconversion, then high Ab titre • Lymphopenia, especially $CD8^+$ T cell, B cell, NK cell and γδ cell cytopenia • Raised IL-10 and IP10 levels	

Fig. 1 | **Immune responses across the spectrum of COVID-19 from asymptomatic to severe disease.** Ab, antibody; IP10, IFNγ-induced protein 10; NK, natural killer.

levels of SARS-CoV-2-specific IgG and serum cytokines, although the total numbers of $CD3^+$ T cells, $CD4^+$ T cells, natural killer (NK) cells and $CD19^+$ B cells were significantly higher in the asymptomatic group[43].

By contrast, relatively few studies have investigated potential differences in innate immune responses in individuals with symptomatic or asymptomatic infection. An initial question was whether some people become infected but are able to rapidly control the virus through innate immune pathways and therefore do not become ill. A rationale for considering efficient virus control or clearance by the innate immune system in the absence of symptomatic disease comes from considering coronavirus infections in bats, where the evidence is for a distinctive programme of type I interferon activation, which appears to protect from disease[44].

Innate immunity. There has been speculation that some people may have been exposed to SARS-CoV-2 on multiple occasions but without this leaving any footprint of adaptive priming because of the efficiency of innate immune mechanisms. For example, longitudinal studies of a cohort of health-care workers showed that some heavily exposed but consistently PCR- and antibody-negative individuals have a raised frequency of memory T cells that are specific for epitopes from the SARS-CoV-2 replication transcription complex, as well as low-level activation of IFI2, an early marker of innate viral recognition[45,46]. Such 'neutralizing' effector pathways will remain hard to validate until more insight is gained from human challenge studies. An analysis of potential protective immunity induced by asymptomatic infection that is purely based on measuring systemic antibody and T cell responses in peripheral blood may be too narrow in scope: it will be important to consider differences in terms of local control, for example through mucosal antibodies or via lung-specific tissue-resident T cells[47].

The idea that asymptomatic infection induces an altered innate immune signature compared with symptomatic infection was considered in a study using single-cell RNA sequencing to look at comparative immune phenotypes in asymptomatic, mild or severe COVID-19 (REF.[48]). The defining biosignature of asymptomatic infection was a reduced expression of transcripts encoding components of the type I interferon pathway — this might be taken to indicate that the route to asymptomatic outcome was a rapid and effective type I interferon response, whereas individuals who mount a longer, more pronounced response are those who control the virus less effectively and are destined for symptomatic infection. The asymptomatic biosignature also involved increased numbers of $CD56^{hi}CD16^-$ NK cells and a clonal expansion of $CD4^+$ T cell populations. By contrast, symptomatic, acute infection involved a reduction in the number of $CD56^{hi}$ NK cells and an increased number of $CD56^{low}$ NK cells[49]. As $CD56^{hi}CD16^-$ NK cells are the subset associated with cytokine production rather than cytotoxicity, these findings may suggest a regulatory function for NK cell cytokines in asymptomatic disease.

Whole-exome sequence analysis of individuals with asymptomatic disease compared with individuals with symptomatic disease highlighted a variant of the cellular viral entry protein TMPRSS2, which is over-represented in the former group[50]. The variant sequence appears to impair cleavage of the SARS-CoV-2 spike protein by TMPRSS2, which may reduce viral entry and thus result in a mild phenotype.

From the above, the innate immune determinants of asymptomatic presentation remain hard to resolve in terms of relative contributions of differential protection by intrinsic antiviral pathways and prior immune subsets, whether NK cells or T cells.

Antibody responses. The activation of adaptive immune responses in individuals with asymptomatic infection is broadly similar to symptomatic disease, as evidenced by the fact that many studies have defined asymptomatic caseload through seroprevalence studies of SARS-CoV-2-specific antibodies. Although the definition of asymptomatic caseload through serology excludes potential asymptomatic infections that may have not resulted in adaptive immune responses, such examples are picked up by community PCR screening programmes. There is evidence for significantly higher levels of SARS-CoV-2 spike protein-specific antibodies in samples from individuals with symptomatic SARS-CoV-2 infection compared with samples from individuals who had asymptomatic infections[7], as well as a more sustained antibody titre that was still detectable at 7 months after infection[51]; from a lower starting titre, individuals with asymptomatic infections were more likely to have reverted to being seronegative. We conducted a long-term study of a cohort of health-care workers through a programme of regular PCR testing, which allowed us to map comparative immunity in individuals with symptomatic and asymptomatic presentation. In that study, we found that titres of neutralizing antibodies were sustained at a similarly high, protective level at 4 months after infection, irrespective of symptomatic or asymptomatic disease[52]. Similarly, a longitudinal serological analysis of inhabitants of Vo' found no difference in antibody responses in individuals with either symptomatic or asymptomatic infection[53]. A 10-month longitudinal analysis of 963 individuals with either symptomatic or asymptomatic disease investigated the durability of neutralizing antibody responses in more detail[54]. In that study, the neutralization capacity of serum from individuals with asymptomatic presentation was indistinguishable from that from individuals with mild disease. Furthermore, the level of serum antibodies (assessed in binding assays) was more sustained in individuals with asymptomatic or mild disease than in individuals who had been hospitalized. Potent, neutralizing anti-spike monoclonal antibodies can be generated from individuals who had asymptomatic disease as well as those who had symptomatic disease[55]. However, a study from Wuhan that compared 405 asymptomatic infections with 459 individuals with COVID-19 symptoms found a more rapid waning of spike-binding and spike-neutralizing antibodies over a 6-month period in those with asymptomatic presentation[55]. Furthermore, sequencing of the B cell receptor repertoire showed a range of differential features across the spectrum from asymptomatic to moderate or severe disease, where symptomatic presentation was associated with longer heavy-chain complementarity-determining region 3 (REF.[56]). Taken together, data from serological studies show that all individuals infected with SARS-CoV-2 can mount a good but heterogenous antibody response, irrespective of whether they are symptomatic or asymptomatic. However, we still lack consensus as to whether symptomatic disease is associated with a more sustained level of neutralizing antibodies[57].

T cell-mediated immunity. The evidence for similarities or differences depending on disease severity is perhaps even more disparate for T cell responses than for antibodies. In one study of T cell responses in individuals across the spectrum from asymptomatic to severe disease, individuals with asymptomatic or mild COVID-19 were shown to have durable, functional T cell-mediated immunity, often in

the absence of an antibody response[58]. A discordance between humoral and T cell-mediated immunity was also observed in our own studies of health-care workers at 4 months after infection, although in this case, neutralizing antibody titres were often high in individuals with asymptomatic disease, whereas the frequency of SARS-CoV-2-specific T cells was largely indistinguishable from pre-pandemic negative control samples[52]. The two studies used essentially the same approach. When T cell responses were screened across SARS-CoV-2 antigen peptide pools, we observed that T cell responses to the SARS-CoV-2 ORF3a and matrix proteins were reduced in asymptomatic infections. In line with these findings, longitudinal studies show a weaker T cell response in individuals with asymptomatic presentation[59]. A potential confounder in the consideration of T cell memory in individuals with asymptomatic disease is that one study reported SARS-CoV-2-specific T cell responses in COVID-19 household contacts who tested negative for SARS-CoV-2 by PCR[29]. This suggests that there may be some form of T cell-mediated priming (or cross-reactive protection) that efficiently combats the virus before it is detectable by PCR. This emphasizes the need for prospective, longitudinal cohort studies to better understand T cell response parameters predisposing to asymptomatic presentation.

Another study looked at T cell responses in 85 individuals with asymptomatic SARS-CoV-2 infection who were identified in a screen of migrant workers who lived in a dormitory with a major COVID-19 outbreak in Singapore[60]. Of note, of 302 people who seroconverted, 281 remained completely asymptomatic. This study reported an unusually high level of asymptomatic disease. Individuals who were infected were rigorously monitored in the dormitories: thermometers and oximeters were distributed and symptoms were reported twice daily to the medical team. Ultimately, the higher incidence of asymptomatic presentation was attributed the cohort consisting of young, fit construction workers. Around 12 weeks after the initial dormitory case, peripheral blood mononuclear cells were taken for T cell analysis. All of those tested showed T cell responses to SARS-CoV-2 peptide pools, most strongly to the matrix protein. Responder frequencies were high, with median responses of around 100 specific responding cells per million; however, from estimates of the time from infection, it appeared that T cell responsiveness might decline more rapidly in individuals with asymptomatic presentation. An analysis of T cell cytokine production showed that levels of IFNγ, IL-2, TNF and IL-6 were higher in the asymptomatic group than the mild/symptomatic group. From this study, it could be argued that, even in the absence of symptomatic disease, SARS-CoV-2 primes a high frequency of $CD4^+$ effector T cells that secrete high levels of proinflammatory cytokines. These findings are similar to observations of early immune events after SARS-CoV-2 exposure, which had shown that individuals who remain asymptomatic are characterized by an early IL-2 response[61].

To summarize, we are at the very early stages of decoding the immune response associated with asymptomatic infection by SARS-CoV-2. Arguably the biggest question, and the one that is least understood so far, is the nature of the immune response that determines whether the initial course of disease after virus encounter results in an asymptomatic or a symptomatic pathway. The fact that initial viral loads appear to be similar in individuals who progress to symptomatic or asymptomatic disease indicate that it is not determined by the strength of the initial innate response to extinguish the viral load. Studies so far indicate that immune priming for adaptive responses occurs in both symptomatic and asymptomatic infections; however, there appears to be greater consensus in antibody studies than in T cell studies for an equivalence of immune priming.

Boosting of vaccine-induced immune responses. Several teams have reported that prior SARS-CoV-2 infection results in a considerable boost to the immune response after the first vaccine dose (across different vaccine platforms), such that antibody responses in these individuals are similar to levels typically seen after two vaccine doses in uninfected individuals[62–64]. This enhancement is also seen for T cell immunity and memory B cell responses[65]. In this context, a key aspect of the nature of immune priming during asymptomatic infection is whether it has a similar effect on the vaccine response as for symptomatic disease. Our findings, assessed by anti-RBD antibody titre, suggest that this is the case[51,64]. As we now enter a phase of the pandemic in which where there will be intense interest in monitoring the long-term durability of the immune response to SARS-CoV-2, it will be important to understand how symptom severity during any acute infection interfaces with vaccine priming[66].

Box 1 | **Current 'Unknowns' about asymptomatic SARS-CoV-2 infection**

- Definition of the innate and adaptive immune pathways on initial exposure to SARS-CoV-2, leading to an asymptomatic rather than a symptomatic outcome
- Improved understanding of the differential disease course following infection by circulating variants of concern — will virus mutation and infection waves from new variants lead to a greater tendency for asymptomatic infection?
- Improved understanding of the differential course of the disease following infection by the different variants across the age range, including greater clarity on asymptomatic presentation in schoolchildren
- As observed in human host–pathogen interactions with the human common cold coronaviruses, will viral adaptations lead to a more common cold-like, endemic disease?
- In a period where infections may increasingly be 'breakthrough' infections in individuals who are vaccinated, are a greater proportion of these are likely to be asymptomatic, and may this in turn fuel undetected transmission and vaccine escape viral variants?
- Is reinfection (with the same strain or a variant strain) more likely to lead to asymptomatic infection?
- Detailed analysis of long COVID risk and features following asymptomatic infection. If initial, asymptomatic presentation can carry a risk of lung computed tomography changes or persistent symptoms, then it cannot be described as asymptomatic.

Concluding remarks

The enormity of the COVID-19 pandemic has generated many questions and exposed uncertainties that we have not previously confronted in our basic research priorities (BOX 1): since January 2020, SARS-CoV-2 has transitioned from a novel virus sequence to being one of the most intensively studied immune targets. In the process, many of our 'textbook' assumptions about viral immune priming, memory and correlates of protection have been stress-tested and reappraised. Among the millions who have been exposed to SARS-CoV-2, many have presumably resisted infection without any record of their exposure in terms of a PCR result, symptom or immune priming. Consider, for example, the many health-care workers treating patients at

the start of the pandemic at a time when personal protective equipment was not routinely used, many of whom remained seronegative. This may suggest that in some cases input virions can be resisted by innate immune effector mechanisms or prior, cross-reactive T cell memory as described earlier herein, which are as yet poorly delineated[44,65]. Here, we have been concerned with the next step of progression along this spectrum — those exposed to the virus who do become infected, seroconvert and yet experience no symptoms. Although the evidence suggests that the time course of shedding of viable virus may be curtailed in this group, individuals with asymptomatic presentation, in general, have viral loads similar to those experiencing combinations of pyrexia, continuous cough or anosmia. Notwithstanding the evidence from the studies we have discussed here, we lack a strong working hypothesis to implicate differences in specific immune pathways in the distinction between asymptomatic and symptomatic infections. It might be suggested that individuals with asymptomatic infections are perhaps those with pre-existing immunity (for example, from cross-reactive T cells) such that disease is completely attenuated. However, this seems to fit poorly with the similarities in viral load and antibody priming between asymptomatic and symptomatic infections. The confidence intervals around estimates of the proportion of asymptomatic presentation are wide, and more clarity is needed on the quality and levels of immunity induced, although this certainly seems to encompass strong adaptive priming of neutralizing antibodies and/or T cells. Does this mean that asymptomatic infection is perhaps a broadly 'good thing' in terms of immune priming with a reduced risk of transmission?

Asymptomatic infection can undoubtedly prime immune memory, but we should not overlook the potential, as yet undefined, risk of long COVID. Arguably, the strongest argument against any complacency regarding a long-term future as a population with low-level asymptomatic carriage is that this would mean ongoing transmission and percolation of significant viral loads, with all that this entails for fuelling future variants of concern. At the time of writing, those parts of the planet with good vaccine access and uptake, moving the situation away from devastating daily fatality metrics, are able to start to reappraise our future relationship with the virus. The case is strongly made for moving on, returning to normal and learning to live with this virus 'in confidence' as a threat no worse than flu. However, there is a powerful counterargument for zero tolerance: living with COVID-19 comes at a high price for future generations in terms of the unfinished business of future waves from new variants and the burden of long COVID.

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