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Durability of immunity to SARS-CoV-2 and other respiratory viruses

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**Durability of immunity to SARS-CoV-2 and other respiratory viruses****Authors:** Matthew K. Siggins<sup>1</sup>, Ryan S. Thwaites<sup>1</sup>, Peter J. M. Openshaw<sup>1</sup>,**Affiliations:** <sup>1</sup>National Heart and Lung Institute, Imperial College London, UK.**Keywords:** COVID-19, SARS-CoV-2, Respiratory viruses, Infection, Immunity, Immune responses**Abstract**

Even in non-pandemic times, respiratory viruses account for a vast global burden of disease. They remain a major cause of illness and death and pose a perpetual threat of breaking out into epidemics and pandemics. Many of these respiratory viruses infect repeatedly and appear to induce only narrow transient immunity, but the situation varies from one virus to another. In the absence of effective specific treatments, understanding the role of immunity in protection, disease and resolution is of paramount importance. These problems have been brought into sharp focus by the coronavirus COVID-19 pandemic. Here, we summarise what is now known about adaptive immunity to SARS-CoV-2 and draw comparisons with immunity to other respiratory viruses, focusing on the longevity of protective responses.

### **Importance of understanding immunity**

As the coronavirus disease 19 (COVID-19) pandemic continues worldwide and vaccines against SARS-CoV-2 enter widespread use, it is timely to review our understanding of the immunity generated following infection and how this relates to other endemic and pandemic respiratory viruses. In just over a year, global SARS-CoV-2 infections have exceeded 125 million cases and 2.5 million deaths. Despite an unparalleled rate of progress in scientific understanding, many fundamental questions remain. While there are a great many similarities between the immune response to SARS-CoV-2 and a host of other respiratory viral pathogens, each agent presents its unique challenges. Through comparison with other respiratory viruses, we can now identify the key questions that need to be addressed to further our understanding of immunity to SARS-CoV-2 to manage the COVID-19 pandemic and mitigate future pandemic threats.

### ***Initial antibody response and role in protection***

Protection from infection may be mediated through multiple mechanisms (Fig. 1), but neutralising antibodies can confer sterilising immunity. This type of antibody is the currently optimal correlate of protection for virtually all acute infections and vaccines [1], especially if present in mucosal secretions.

Infections with influenza, rhinovirus, respiratory syncytial virus (RSV), endemic coronaviruses and other common respiratory viruses typically induce neutralising antibodies that are associated with protection from recurrent disease [2-6]. Antibody responses to SARS-CoV-2 infection show substantial heterogeneity and are correlated with severity of infection [7-11], but the vast majority of normal young individuals infected with SARS-CoV-2 generate antibody responses in serum [8, 10, 12] and saliva [13] within 2–4 weeks of symptom onset. Nearly all convalescent sera possess detectable neutralising activity, including those from asymptomatic children and adults [7, 11, 14, 15].

Development of neutralising antibody appears to prevent shedding of infectious virus in hospitalised patients [16] and numerous animal models have demonstrated antibody-mediated protection against COVID-19 [17]. Relatively low neutralising antibody titres protect rhesus macaques from SARS-CoV-2 reinfection, and even sub-sterilizing titres can provide functional immunity and lessen disease severity [18, 19].

Human epidemiological data also support the association between antibody and protection; a study of 12,000 healthcare workers reported antibody responses provided protection from reinfection for the 6 months of study follow up [20]. Additionally, individuals with SARS-CoV-2 positive antibody tests only rarely suffer re-infection in a large COVID-19 outbreaks with high attack rates [21]. Similarly, an investigation of a high attack rate outbreak on a fishing vessel demonstrated an association between protection and the presence of neutralizing antibodies [22]. Furthermore, in a recent study of high-titre convalescent plasma therapy, patients with early COVID-19 showed a 48% relative reduction in severe disease [23], indicating a protective role for antibody early in infection.

As with SARS-CoV-1, the transmembrane spike (S) glycoprotein and nucleocapsid (N) protein represent the dominant targets of induced antibodies [24]. Seroreactivity to S protein, specifically subunit 1 (S1) and its receptor binding site (RBD), are highly correlated with neutralising activity [8, 10, 14], and antibodies against the RBD are reported to account for 90% of neutralising activity in convalescent sera [25]. In contrast, antibodies against the internal N protein do not neutralise [8, 14]. Although cross-reactive antibodies, particularly against spike subunit 2 (S2), have been reported in pre-pandemic sera [26], they do not possess appreciable neutralising activity [27]. Notably, SARS-CoV-2 RBD has little sequence homology with those of the seasonal coronaviruses [28].

In addition to neutralising activity, diverse antibody Fc effector functions, including antibody-dependent cellular cytotoxicity and antibody-dependent complement deposition have been induced by experimental vaccination against SARS-CoV-2 and could contribute to antibody-mediated protection [18, 19], as documented for other respiratory viruses [29]. Taken together, measurement of antibodies against RBD or S1 by robust serological assays would be predicted to correlate with protection to most natural exposure to SARS-CoV-2. However, it should be recognised that immunity is not absolute and high dose or prolonged exposure to a pathogen can overwhelm what normally constitutes robust protection [30].

### ***Durability of antibody response to SARS-CoV-2***

Initial reports concerning the persistence of the antibody response warned of extremely rapid waning, particularly following milder COVID-19 infections [7], indicating that protective immunity might be transient. Though decline of specific IgM within a few months of an acute infection is usual [9], return of a primary IgG response to a non-protective baseline within

this time frame is not typical. Many subsequent studies have since reported more stable antibody kinetics in both blood and saliva, reporting detectable neutralising activity against SARS-CoV-2 in the majority during the period of assessment (3–8 months) [8, 9, 13, 14, 31, 32], including in asymptomatic healthcare workers at 4 months post diagnosis [33]. These data are consistent with protective immunity lasting several years for most individuals.

Publications with contrasting estimates of anti-SARS-CoV-2 IgG durability present largely consistent primary data but differ in interpretation. Decay in antibody production after infection or vaccination is not linear and is especially difficult to extrapolate from early time points. Even for long-lived antibody responses, decay half-life within the first few months is often around 30 days and may not reach steady state until about 3 years [34]. Extrapolation of antibody persistence after the first few months is more reliable and sustained production of SARS-CoV-2 specific antibody at around 3 months post infection is predictive of antibody persistence at 5 months [35]. Although individuals that produce higher initial antibody levels also tend to have slower decay rates and longer-lived protection, significant heterogeneity exists [36].

The immune response to vaccines is influenced by many factors [37]. For natural infections, individual variation is further obfuscated by antigen load, which is dependent on infection severity (itself influenced by many factors) and impacts initial antibody titres [12, 25]. Antibody durability is also affected by infection severity and is more variable following mild MERS-CoV infection compared to severe disease [38]. Among mild COVID-19 patients, faster recovery from disease is associated with better sustained antibody [35], highlighting the potential for pathogen- or disease-mediated influence. Following infection, the inflammatory milieu, cellular infiltrate, and pathogen-associated molecular patterns can all influence levels and kinetics of antibody production [39]. Some respiratory viruses, such as RSV (and possibly coronaviruses), appear to directly interfere with development and duration of immunological memory, though protective antibody is still produced [4, 40].

SARS-CoV-2 specific IgA in serum and saliva has been reported to show much more rapid decay than IgG [13], though some individuals maintain stable low levels of specific IgA in sera [31]. Mucosal IgA contributes to protection against respiratory viruses [4, 41], and these dimeric forms of IgA possess enhanced neutralisation activity against SARS-CoV-2 [42]. Antibody prevalence to common coronaviruses was found to be lower in nasal secretions

than in serum, suggesting that systemic IgG responses are also more durable than mucosal IgA for coronaviruses [43]. In contrast, decay rates of IgA from nasal washes have been reported to be similar to the kinetics of serum IgG assessed a year after experimental challenge infections with an endemic coronavirus [44]. IgA and IgG are believed to play complementary roles in protection against viruses, with the former dominant in the upper- and latter dominant in the lower respiratory tract [45]. Though, even in the absence of IgA, serum IgG can access the respiratory tract through the processes of transudation, exudation, and transcytosis (via FcRn) to mediate protection against viruses [46].

Predictions of SARS-CoV-2 protective antibody lifespan somewhat mirror assessment of SARS-CoV-1 antibody titres, which were initially thought to be relatively short-lived [47]. However, despite lack of re-exposure to this virus, around 90% of individuals had neutralising antibody at 3 years post SARS-CoV-1 infection, and specific IgG has since been measured in some up to 13 years post-infection [48]. As common for antibody responses to other viruses, *Guo et al* reported that after a rapid decline of antibodies in the two years following infection, reduction over subsequent years was much slower [48]. Vaccinology has demonstrated that lifelong protective antibody responses result after inoculation of a repetitive protein antigen, with sufficient quantity and kinetics to reach an antigenic threshold, in combination with an appropriate immunostimulatory response [49]. Natural infection with influenza appears to fulfil these conditions and neutralising antibodies conferring homologous immunity are maintained for life (Fig. 2B); survivors of the 1918 H1N1 influenza pandemic had significantly higher seropositivity and serum-neutralizing activity against an antigenically identical virus than controls born in subsequent years [50].

### ***Evasion of humoral immunity by respiratory viruses: antigenic variation***

Antibody responses to the endemic human coronaviruses (HCoV-HKU1, HCoV-OC43, HCoV-229E, and HCoV-NL63) are often considered transient and short-lived (Fig. 2A). Typically these viruses cause mild respiratory disease and have long circulated between humans [51]. First infection by all four endemic coronaviruses takes place early in childhood and seropositivity plateaus by age 6, remaining near universal in adults [43, 52].

However, human experimental infection studies performed with coronaviruses by *Callow et al* showed that adult volunteers have high baseline antibody levels which are boosted and remain significantly elevated a year after infection [44]. These levels correlate with partial or

total immunity upon homologous re-challenge. Another human experimental infection study by Reed *et al* reported complete protection upon homologous viral re-challenge under similar conditions [2]. In the former study, specific antibodies peaked by day 12 post-infection, quicker than would be expected for a primary response but typical of an anamnestic response. Therefore, challenge studies in adults measure recall responses rather than primary responses, and it is feasible that the baseline levels of antibodies in study volunteers may protect from natural exposure but be overwhelmed by the high inoculum used for challenge. Susceptibility to reinfection may also be elevated in challenge studies: although influenza reinfections of young healthy adults with homologous virus do not commonly occur naturally, they can be achieved after sequential experimental challenge [53].

Several studies of natural infections have shown widespread seasonal coronavirus infections in adults, including reinfections [54, 55]. Importantly, data presented by Galanti *et al* demonstrate that reinfections with the same coronavirus were usually milder in severity or asymptomatic, particularly in adults, indicating a degree of functional immunity remained between infections [54]. Additionally, these studies did not assess the contribution of strain variation to reinfection. Incomplete cross-protection to related strains of endemic coronaviruses has been previously established experimentally and is hypothesised as a significant factor in the epidemiology of infections [2] (Box 1).

### ***Evasion of immunity by respiratory viruses: immunomodulation***

RSV is another respiratory virus commonly associated with reinfection. Its genetic variability is relatively low, particularly in the highly conserved Fusion (F) protein. Neutralising antibodies raised against F protein following infection are associated with protection [4]; thus, antigenic variation is not usually considered to make a significant contribution to reinfection. Instead, virally-mediated immunomodulation is postulated to underlie the short duration of immunity [4, 40]. Although protective antibodies are induced by infection, disturbance of type I and III interferon signalling, antigen presentation and chemokine-induced inflammation are implicated in suppression of long-lived protection against RSV [56], and similar factors may influence development of long-term immunity to SARS-CoV-2 [57].

RSV is increasingly recognised as a major pathogen of those with respiratory comorbidities and elderly adults. Though waning immunity, along with immunosenescence, is believed to

contribute to the increased burden of disease in the elderly, protection resulting from RSV infection is perhaps more robust than widely appreciated. While adults can be reinfected with RSV, disease is typically mild and confined to the upper respiratory tract, with much lower viral loads recovered. Reinfections in young children are also associated with milder disease [58]. Moreover, natural infection with RSV increases neutralising serum antibody responses to protective levels [3]; however, such increases in antibody titre may be short-lived [4].

One study demonstrated that neutralising antibody titres remained above a threshold associated with protection in 19 of 20 volunteers followed for 2 years post-infection, with only 1 reinfection observed in this time [59]. In an experimental re-challenge study using homologous RSV, only 6 of 15 adult volunteers could be reinfected with the same strain within the 2 year study period, and just 3 of 15 individuals were reinfected with the same strain twice [60]. Additionally, higher levels of neutralising antibodies correlated with protection, over half of reinfections were asymptomatic and the duration of viral shedding for homologous reinfections was reduced to 1.7 days from 4.6 days during the initial challenge [60].

While antigenic variation does not appear to be a firm requirement for reinfection, it could be that underappreciated antigenic variation, particularly in the more variable attachment glycoprotein (G protein), enhances the ability of RSV to cause repeated infections through life [61]. Importantly, innate immunity has been demonstrated to make a significant contribution to the ability of RSV to reinfect: presence of neutrophilic inflammation at the time of exposure has been demonstrated to be a major determinant to susceptibility to RSV challenge [62], and could contribute to reinfections observed with other respiratory viruses.

### ***Immunology of antibody durability***

Following antigenic stimulation during acute viral infection, extrafollicular clonal expansion of naïve B cells produces a wave of short-lived proliferating plasmablasts and plasma cells that secrete mainly lower-affinity antibodies. Some activated B cells enter lymphoid follicles and initiate germinal centre reactions to generate higher affinity antibody-secreting long-lived plasma cells and memory B cells [63, 64]. Short-lived plasma cells are responsible for the initial high titres of antibody, particularly IgM, but levels wane as this cell population contracts. The average half-life of IgG is around 3 weeks and the rate of antibody decay

slows dramatically as free immunoglobins are removed from circulation and antibody decay kinetics become determined by the remaining longer-lived plasma cell populations.

Once antigen is cleared, protective levels of specific antibody are maintained by non-proliferating long-lived plasma cells that primarily reside in the bone marrow, where they continuously secrete high-affinity antibodies into circulation [65]. A single plasma cell can produce up to  $10^9$  molecules of Ig in a day (~15 ng), and so a relatively small number of long-lived plasma cells could confer protection [66]. For many antibody responses, the rate of decay does not reach a steady state until 2–3 years after antigen exposure, suggestive of lengthy retention of antigen following viral clearance and long-lived plasma cells with a range of intermediate lifespans [67].

Infections with respiratory viruses, including influenza, generate robust plasma cell responses [4, 68]. Comparable circulating plasma cells, which correlate with specific antibody levels, have been observed following SARS-CoV-2 infection [31, 42, 69]. Importantly, SARS-CoV-2 specific plasma cells were found to be present in bone marrow in a majority of donors at 8 months post infection [70]. Additionally, as these cells were present at similar in number to plasma cells specific for contemporary influenza viruses [70], it seems there is no SARS-CoV-2-mediated deficiency in their formation or survival. Plasma cells are also abundant in mucosal tissues and IgA-expressing plasmablasts with mucosal-homing profiles are prevalent in the early circulating plasma cell response to SARS-CoV-2 and RSV infection [4, 42].

However, what determines the longevity of a given antigen-specific plasma cell is still not well understood (Box 2). The magnitudes of B cell activation and T cell help are central concepts in the ‘imprinted lifespan’ model that hypothesises an adequate number of plasma cells must initially enter the long-lived pool in order to sustain antibody production above a protective threshold long-term [49]. Analysis of vaccine responses suggests that antigen type (protein and multivalent/repetitive epitopes) and antigen load are the most important parameters for sustained antibody responses. Most respiratory viruses feature repetitive protein antigen on their surface, including the S protein of SARS-CoV-2, and so antigen load is likely to be an important variable in natural infection.

As well as bone marrow, long-lived plasma cells can also survive for decades in mucosa-associated lymphoid tissue, including IgA forms [67]. Yet, the more rapid decay of mucosal

IgA compared to serum IgG following primary SARS-CoV-2 infection suggests that long-lived mucosal plasma cells may be lesser in number than those in the bone marrow. For now, definitive data on the survival of mucosal long-lived plasma cells and their contribution to long term immunity are lacking.

### ***Contribution of memory B cells and recall responses***

While plasma cells are the source of circulating antibodies, memory B cells direct antibody recall responses against viruses. Memory B cells are mainly generated with T cell help in germinal centre reactions, and robust numbers appear in the circulation in the weeks following respiratory infections [71]. These specific memory B cells are long-lived and reside in the spleen, lymph nodes, or sites of infection, including the lungs [72-74], where they can readily sample antigen. Upon re-exposure to cognate antigen, mouse models suggest that memory B cells can quickly differentiate into plasma cells without requiring additional T cell help [75]. Alternatively, memory B cells can re-enter germinal centres to boost humoral immunity and replenish the memory B cell pool. These combined responses result in rapid production of specific antibodies that encompass higher affinities and wider breadth than a primary response. Memory B cell recall responses can top up waning levels of antibody and replenish long-lived plasma cells upon exposure to virus during subclinical infections of children [64, 76]. Additionally, as memory B cells possess a broader range of specificities than the plasma cell pool, they can provide protection against antigenically variant viruses that can escape neutralisation by pre-existing antibodies in mice [77].

Pre-existing memory B cells in humans can also drive evolution of improved antibody responses by undergoing additional rounds of somatic hypermutation and selection with antigen persistence (Box 3), or upon re-exposure to homologous or antigenically similar viruses [78, 79].

Anamnestic responses have been observed with SARS-CoV-2 following re-challenge of rhesus macaques 35 days after initial infection, resulting in further elevated neutralising antibody titres within 7 days [19]. In humans, S-specific memory B cells are very rare in unexposed individuals but appear in appreciable numbers as early as 2 weeks after SARS-CoV-2 infection [80]. Numbers of SARS-CoV-2 specific memory B cells steadily increase over the following months and are still present more than 6 months after initial infection, indicating that this B cell memory to SARS-CoV-2 is likely long-lasting [31, 32]. S- and

RBD-specific memory B cells are increased in hospitalised cases compared with non-hospitalised [31], highlighting the importance of antigen load in strength of humoral responses. While one group found SARS-CoV-1 patients lacked peripheral memory B cell responses at 6-year follow up [81], another found memory B cells capable of producing neutralising antibodies in an individual 10 years after a SARS-CoV-1 infection [82]. Memory B cells, or their progeny, can be sustained for life: specific memory B cells, capable of producing potent neutralising antibodies, have been observed in individuals 90 years on from influenza infection [50]. Such long-term persistence may require periodic re-stimulation through encounter with antigenically similar viruses, or antigen-independent means [83].

Thus far, only circulating memory B cell responses to SARS-CoV-2 have been well-studied. Outcome of RSV infection in human challenge is not influenced by circulating memory B cell frequencies [4]. Instead, it is likely that faster responding respiratory tract-resident memory B cells, are more relevant to protection against RSV and SARS-CoV-2, as reported for influenza [73]

#### ***Contribution and durability of T cell immunity***

Antigen-specific effector T cells are vital components of the immune response to respiratory viral infections, and early T cell responses during COVID-19 are correlated with rapid viral clearance and reduced disease severity [84, 85]. Subsets of CD4<sup>+</sup> T cells coordinate innate and adaptive immunity, among numerous roles, these cells support generation of high affinity antibodies and long-lived plasma cells and memory B cells. Cytolytic CD8<sup>+</sup> cells directly kill virally infected cells and play a critical role in mediating viral clearance following infection. After initiation of human infection, antigen specific T cells undergo clonal expansion, peaking around 10 days later [86]. Upon successful clearance of a pathogen, these effector T cell populations contract, but both CD4<sup>+</sup> and CD8<sup>+</sup> long-lived memory T cells (T<sub>M</sub>) are maintained in lymphoid organs, the peripheral circulation, and within tissue. The T<sub>M</sub> classification covers a broad continuum of cell subsets with diverse immediate effector functions, turnover, and location [87].

Robust SARS-CoV-2 circulating CD4<sup>+</sup> and CD8<sup>+</sup> T<sub>M</sub> responses are present in the majority of convalescent individuals, irrespective of severity [88-90], and these T<sub>M</sub> populations are maintained for over 6–8 months post infection [31, 91]. Most CD4<sup>+</sup> T<sub>M</sub> possess the classical antiviral T<sub>H1</sub> phenotype or a T<sub>FH</sub> phenotype [92]. Recall T<sub>FH</sub> responses can further

enhance available T cell help to specific B cells in germinal centres and promote the generation of potent and lasting antibody responses.

Expanded pools of specific  $T_M$  cells undergo recall responses that are more rapid, stronger, and better tailored. While infections with respiratory viruses are usually confined to the respiratory tract, data from mice shows that  $T_M$  cells can be recruited from the circulation or lymphoid tissues to a site of infection by inflammation [93]. Prior to trafficking,  $T_M$  cells typically undergo proliferation for several days. This intrinsic delay in the  $T_M$  recall response, in contrast to the instantaneous activity of antibodies that can achieve ‘sterilising’ immunity, means that circulating T cell mediated immunity has historically been side-lined in consideration of correlates of protection.

Nonetheless, pre-existing numbers of specific circulating  $CD4^+$  and  $CD8^+$  memory T cells correlate with reduced disease severity for influenza infections in humans [94, 95]. Furthermore, T cell responses are directed at different and more varied targets than those of neutralising antibodies. Notably, studies assessing T cell contribution to protection have measured cross-reactive T cell immunity to viral strains for which the donor lacked specific antibody. T cell responses might also have critical importance where antibody responses are insufficient for protection; consistent with this, depletion of  $CD8^+$  cells prior to SARS-CoV-2 rechallenge partially abrogates protective immunity in rhesus macaques that possessed sub-protective antibody titres [18]. Prior studies in mice have also suggested important roles for specific  $CD8^+$  T cells in protection from SARS-CoV-1 [96]. As  $CD4^+$  and  $CD8^+$   $T_M$  cell populations specific to n, em, rane, non-structural, and N proteins, as well as S protein are generated following SARS-CoV-2 infection [31], T cells could also be important to exert protection against escape mutants that may be generated by the selective pressure of neutralising S protein specific antibodies.

Protection mediated by circulating specific  $T_M$  cells can be very long-lasting;  $T_M$  populations are well established to persist for over 50 years in response to smallpox vaccination of volunteers with vaccinia virus [97]. Although data for other viruses is sparse, it is likely that  $T_M$  responses to respiratory viruses are also likely to be long-lived. Circulating  $T_M$  cells specific for respiratory viruses are found in the elderly, albeit in low numbers for RSV [98]. Boosting of these specific  $T_M$  populations through reinfection or vaccination likely plays a role in lifelong maintenance. However, even in the absence of antigenic-boosting,  $CD4^+$  and

CD8<sup>+</sup> T<sub>M</sub> responses targeting the SARS-CoV-1 coronavirus were present in individuals at 11- and 17-years post-infection [90, 99] and so far the kinetics of T<sub>M</sub> cell responses to SARS-CoV-2 appear similar [31].

### ***Role of tissue-resident T cell immunity***

For some respiratory infections, such as RSV, T<sub>M</sub> cells are not close correlates of protection [100]. Instead, non-circulating tissue resident T memory (T<sub>RM</sub>) are more important. T<sub>RM</sub> possess distinct surface markers and transcriptional profiles and represent the frontline of T cell immunity due to their ability to mount quick immune responses in situ. While there is not sufficient evidence to suggest T<sub>RM</sub> can confer sterilizing immunity, both CD4<sup>+</sup> and CD8<sup>+</sup> T<sub>RM</sub> are associated with optimal protection of humans from rechallenge with many respiratory viruses, including RSV and influenza [100, 101]. Additionally, CD4<sup>+</sup> airway T<sub>RM</sub> mediate protection against SARS-CoV-1 and MERS-CoV [102]. T<sub>RM</sub> also appear to contribute to protection against SARS-CoV-2; rhesus macaques depleted of CD8<sup>+</sup> T cells showed differences in viral load in the upper respiratory tract after just one day post-infection, indicative of CD8<sup>+</sup> T<sub>RM</sub>-mediated activity [18].

T<sub>RM</sub> populations in the lung have been observed to survive for over a year in humans [103], but whether these cells can exhibit real-time persistence, like T<sub>M</sub> populations, or play a role in lifelong immunity is yet to be demonstrated. Experiments in mice have shown that CD4<sup>+</sup> T<sub>RM</sub> subsets persist in the lung after influenza infection, but survival of CD8<sup>+</sup> T<sub>RM</sub> cells is dependent on inflammation and so relatively short-lived [104, 105]. Tellingly, T<sub>RM</sub> cell-mediated cross-reactive immunity to influenza is lost at around 5 months post murine infection [105]. However, as specific T<sub>RM</sub> populations are amplified following recall responses, these cells may persist for longer periods following repeated exposures to antigen [106]. Therefore, CD8<sup>+</sup> T<sub>RM</sub> cells, may be particularly important in reducing disease severity during frequent recurrent respiratory infections which show weaker associations with antibody-mediated protection [100].

### **Concluding remarks and future perspectives**

Much remains to be learned to understand the durability of protective immune responses following respiratory infections. Certain viral infections, including influenza, result in neutralising antibodies and circulating specific memory B and T cell populations can persist for many decades. For other viruses, such as RSV, repeated infections do not seem to be

explained by antigenic variation, suggestive of significant waning immunity. Nevertheless, until old age, repeated infections by homologous viruses are nearly always milder in nature indicating that functional immunity is formed. It is possible that, over time, most children will be infected with SARS-CoV-2 in early life and that primary infections in adulthood will not commonly occur. As it is these primary adult infections (and especially, infections in older adults) that result in serious disease, COVID-19 will likely become a generally mild disease similar to that seen with endemic human coronaviruses.

The relative inefficiency of the ability of IgG to protect in the upper respiratory tract and delay of systemic T cell responses may underlie the apparent difficulty in preventing mild upper respiratory infections after one exposure to some respiratory viruses. Viral-mediated suppression of long-lived protection against is implicated in RSV infections and suggested for coronaviruses. However, specific plasma cells within bone marrow, as well as circulating memory B and T cells, and antibody are all present in the majority around 8 months after SARS-CoV-2 infection, demonstrative of a robust immune response.

While certain factors of what determines the magnitude and longevity of immune responses, such as antigen load, are known, but the full picture remains elusive. This is exemplified by the significant heterogeneity between individuals in all immune responses to SARS-CoV-2. Following infection, facets of immunity can be discordant in their responses and durability. A sizeable proportion of individuals lack CD8<sup>+</sup> T<sub>M</sub> cell responses but possess specific antibody around 6 months after mild SARS-CoV-2, while in a minority CD8<sup>+</sup> T<sub>M</sub> cells are maintained but antibody is undetectable. Certainly, the often-offered hypothesis that antibody to SARS-CoV-2 rapidly wanes, while circulating T<sub>M</sub> responses are maintained, does not fit data obtained during the COVID-19 pandemic.

SARS-CoV-2 reinfections with original variants are currently rare (Box 1), consistent with measurements of sustained systemic immunity so far [20]. The longevity of protective responses in the mucosal compartment remains a major gap in understanding of immunity. Mucosal antibody and resident cells have been shown to mediate protection for respiratory viruses, but the limited data available suggests these responses may be less long-lived than systemic equivalents, especially in absence of antigen.

Continued investigation of SARS-CoV-2 responses provides an extraordinary opportunity to further understand of the durability of adaptive and mucosal immunity, and their relative contributions to long-term protection from respiratory infection (see *Outstanding Questions*). It is to be hoped that the lessons learnt from intensive global research effort into COVID-19 will lead to new vaccines and treatments that will finally lessen the global toll of respiratory viral infections.

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## Figure Legends

### **Fig. 1: Immunity upon re-exposure to a virus in the respiratory tract.**

(1) Mucosal antibodies (predominantly dimeric IgA), produced constitutively by plasma cells resident in the respiratory tract, can efficiently neutralise virus. (2) Systemic antibodies, (mostly high affinity IgG) are constantly produced by long-lived plasma cells in the bone marrow. These antibodies can move from the blood into the respiratory tract, through transcytosis and transudation, to neutralise virus, as well mediating as other Fc-dependent antiviral effector functions. (3) Tissue resident CD4<sup>+</sup> and CD8<sup>+</sup> T cell populations are rapidly activated to mediate immune-coordination and antiviral activities in situ. (4) Viral antigens from the respiratory tract transit, freely in lymph or carried by dendritic cells, to secondary lymphoid organs. Here, long-lived recirculating memory T and B cell populations are activated and mount rapid recall responses. (5) Once large numbers of effector cells have proliferated from memory T cell precursors, they home to the respiratory infection site. (6) While most memory B cells proliferate into antibody producing plasma cells, some re-enter germinal centre reactions to replenish the memory B cell pool. Through affinity maturation and somatic hypermutation, germinal centre reactions evolve the potency and breadth of the antibody response. (7) Newly created antibody secreting plasma cells traffic to sites including the bone marrow and mucosa-associated lymphoid tissue where they can reside long-term.

### **Fig. 2: Contributions of adaptive immune responses to protection against respiratory viruses in durable and transient responses.**

**A.** In a transient immune response, levels of neutralising antibodies are lower but typically still initially provide sterilising immunity against homologous virus. Subsequent waning reduces levels below the threshold for sterilising immunity within a few years. In the absence of sterilising immunity, T memory and T resident memory cell populations, though smaller than following a robust response, can contribute functional protection to lessen disease severity upon reinfection with either homologous or variant virus.

**B.** In a durable immune response, high levels of neutralising antibodies mediate sterilising immunity that prevents reinfection with homologous virus. Despite rapid waning over the initial months, antibodies decay slowly thereafter, maintained at a protective level for many years by long-lived plasma cells. Recirculating memory T cells are also long-lived and contribute functional protection which can lessen disease severity caused by viral variants

that escape antibody-mediated immunity. Rapid responses of tissue resident memory cells ( $T_{RM}$ ) contribute robust immunity in the months following initial infection; however, these populations are currently thought to be relatively short lived in the absence of repeated stimulation.

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**Box 1: Antigenic variability of coronaviruses and SARS-CoV-2 variants**

While coronaviruses possess proofreading capacity which corrects many errors that arise during replication, different co-circulating genetic clusters of HCoV-NL63, HCoV-OC43 and HCoV-HKU1 exist and HCoV-OC43 and HCoV-229E display continuous genetic drift [51]. Hence, rather than inherently transient immunity, strain heterogeneity and insufficient cross-protection may be key determinants of susceptibility to reinfection, as observed with serotypes of rhinovirus [107]. Coronaviruses have non-segmented genomes, so cannot achieve the extremely high rates of recombination produced by independent assortment, which leads to large antigenic shifts in viruses such as influenza A [108]. However, coronaviruses (including SARS-CoV-2) are capable of recombination and it plays an important role in their evolution. At the present time, SARS-CoV-2 shows much lower genetic diversity than the endemic coronaviruses, which have accumulated genomic variation over a long period of time, up to 1000 years in the case of HCoV-NL63 [51, 109].

In the ongoing COVID-19 pandemic, reinfection with divergent or antigenically-drifted variants, commonly seen in influenza [108], is at present quite rare [109] but may become more frequent as immunity builds in the population and becomes a driving force favouring variant emergence. New variants are now emerging worldwide that may have a greater propensity for reinfection. At the time of writing (March 2021) three variants of concern have received particular attention, namely B.1.1.7, B.1.451, and P1; colloquially known as “Kent”/ “UK”, “South Africa”, and “Brazil” variants, respectively [1]. These variants possess extensive mutations in key S protein and RBD sites, and consequential impacts on transmissibility, mortality, and immune escape have been reported [110, 111], [1]. The polyclonal nature of adaptive immunity raises neutralising antibodies to numerous epitopes on S protein. Although this makes immune evasion extremely difficult for a virus, it is not unachievable, particularly during prolonged selective pressure. Irrespective of immune evasion, increased infectivity of viral variants, as reported for B.1.1.7, could increase the titre of neutralising antibodies required for protection and shorten the duration of effective immunity.

**Box 2: Plasma cell longevity**

Long-term survival of plasma cells is supported by niches in bone marrow or other sites of high CXCL12 expression, where IL-6, BAFF (B cell activating factor), and APRIL (A proliferation inducing ligand) play key roles. These niches also rely on cellular support from eosinophils and T regulatory cells ( $T_{reg}$ ) and can be disturbed by systemic inflammation.

Extended T cell help enhances formation of long-lived plasma cells [112]. Accordingly, germinal centre reactions are believed to be important in the generation of long-lived plasma cells and it appears that the longer a B cell resides in a germinal centre, the greater the chance its progeny will enter the long-lived plasma cell pool [113]. Consequently, the absence of germinal centre formation observed in some post-mortems following fatal COVID-19 has fuelled fears that protective antibody could be short-lived [114]. Furthermore, despite sometimes possessing high neutralising potency, initial antibodies produced in response to COVID-19 show minimal somatic mutation [32, 115], reflective of predominantly extrafollicular responses which mainly generate short-lived plasma cells.

Rather than an aberrant process, an initial dominant extrafollicular response to a viral infection may represent a normal response to ensure maximal early antibody production in the face of dangerous pathogenic inflammation [64]. Indeed, pathogen-associated molecular pattern molecules (PAMPs) have previously been reported to drive B cells to extrafollicularly proliferate into short-lived plasma cells instead of joining the slower germinal centre responses which produce many memory B cells and long-lived plasma cells [39]. Consistent with this, one study demonstrated that patients who recovered from SARS-CoV-2 infection more quickly possessed slower antibody decay kinetics [35], suggesting a reduced contribution of extrafollicular responses to total antibody when infection was controlled early.

It is reasonable to postulate that even in severe infections, a shift towards germinal centre responses would occur upon dampening of inflammatory signals during convalescence. Certainly, robust germinal centre formation is observed in rhesus-macaques following SARS-CoV-2 infection [116]. Moreover, neutralising antibodies, including those against RBD, show greater avidity over time, consistent with ongoing affinity maturation in germinal centres [117]. Additionally, robust expansion of follicular helper T cells has been reported, indicative of plentiful T cell help to support germinal reactions [31]. As the underlying kinetics of the S-specific IgG response in COVID-19 patients reported thus far are consistent with long-term

survival of plasma cells [31, 70, 80], persistence of long-lived plasma cells would be expected far beyond the 8 months currently assessed [70].

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**Box 3: Evolution of humoral responses during and following infection**

While initial antibodies produced by an individual infected with SARS-CoV-2 show minimal somatic mutation [32, 115], specific memory B cells display clonal turnover over the course of 6 months post-infection [32]. As a result, these latter memory B cells are capable of expressing antibodies that possess greater potency and antigenic breadth [32]. Such evolved antibody responses could be important for long-term protection by conferring neutralising activities at lower titres, as well as further limiting the potential of mutation-mediated immune escape by SARS-CoV-2 [118]. Though these processes are predominantly antigen-dependent, antigen is present during viral infection and can persist for months after recovery [32].

Shedding of SARS-CoV-2 RNA is commonly detectable from the upper and lower respiratory tracts and stool for several weeks and even months post-infection [119], likely representing clearance of inactive viral material rather than active virions. SARS-CoV-2 components have been observed in widely disseminated tissues [120], including the gut of asymptomatic individuals 3 months after infection [32]. Even following clearance of virus, antigen can persist for extended periods on follicular dendritic cells in antibody complexes.

Other respiratory viruses, including RSV, also exhibit prolonged viral shedding. Persistent RSV antigen is found associated with lymphocytes in the airway a month after challenge inoculation [100], and ongoing production of plasma cells persists for up to a month after [68]. Therefore, continued memory B cell-mediated evolution of antibody responses would be expected during the first weeks and months following viral infection.

Memory B cells themselves may provide a correlate of protection, even in the absence of pre-existing antibodies, particularly against infections that have a slow course of disease. Indeed, circulating memory B cells capable of producing potent SARS-CoV-2 neutralising antibodies are found in individuals that lack robust serum antibody titres [11]. As COVID-19 infection follows a relatively slow path for an acute disease, with hospital admission around 2 weeks post onset and death after 3 weeks, evolved memory cell responses might meaningfully contribute to protection.

**Box 4: Current immunological issues in the COVID-19 pandemic**

Host responses evidently contribute to *Pathology and disease in COVID-19*. Identifying (and inhibiting) the factors driving COVID-19 while maintaining long term immune memory remains a priority. Similarly, understanding the contribution of immune responses to the diverse prolonged *Sequelae* of COVID-19 ('Long COVID') is an urgent priority.

*Duration of immunity* is uncertain following either vaccination or natural infection. However, it seems that levels of antibody and B cell responses reach a relatively stable protective level for many months following an expected initial early contraction. The role and duration of T cell mediated immunity is less certain but also appears to be robust for at least 8 months. Factors such as age and COVID-19 severity seem to influence protective duration.

*Reinfections with a homologous variant* appear rare though can occur. The reasons for reinfection need investigation but, as immunity wanes, more frequent reinfections are to be expected. Immunity induced by vaccination or natural infection appears to reduce disease severity, but effects on viral *Transmission* may not be so great. Preliminary evidence indicates that there is both a reduced frequency of asymptomatic infection and a decrease in viral load in those with existing immunity; it is expected that this will decrease community transmission. Asymptomatic infections with other respiratory viruses, such as RSV and influenza, occur at high frequency but are considered less likely to contribute to spread. Overall, there is little data that conclusively demonstrates the importance of asymptomatic infection in viral transmission.

*Booster vaccination*, especially targeted to "at-risk" groups, appears beneficial and is expected to boost the level, range, and duration of protection.

Defining strong *Correlates of protection* (CoP) is essential in the development of vaccines, for public policy and in tacking variants. Neutralising antibody in the blood (and antibody binding to the receptor binding domain of S) are currently the most predictive CoPs; however, other aspects of immunity (e.g., mucosal antibody responses and T cells) need further study.

*Variants of SARS-CoV-2* continue to emerge, increasingly under immune pressure (Box 1). Close monitoring of immune evasion by viral variants is essential and may necessitate modification of vaccines. *Future vaccines* should also be designed to stimulate mucosal

immunity: induction of local immune responses in the respiratory mucosa is expected to have a greater effect on local viral replication and in onward transmission of SARS-CoV-2.

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## Resources

I) Public Health England (2021). SARS-CoV-2 variants of concern and variants under investigation in England: technical briefing 7.

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**Outstanding questions**

- What is the lifespan and range of protective antibody and cellular memory responses following SARS-CoV-2 infection? Are mucosal antibodies and tissue-resident T cells important in long-term protection?
- Can memory T cells protect against SARS-CoV-2 disease or interrupt transmission: either in the absence of antibody or upon viral escape of existing antibodies?
- What factors influence the durability of antibody and memory B/T cell responses and do they differ? What is the role of T cells in sustaining antibody and memory responses?
- To what extent does pathogen and disease-mediated immunomodulation contribute to reinfection by respiratory viruses? Is the contribution of antigenic variation underappreciated for some viruses?
- Are cases of infection despite vaccination sufficiently common and severe to warrant vaccine modification or widespread use of booster vaccinations?
- Can greater understanding of immunopathology guide us to better therapeutics for acute disease and sequelae?
- How can immunology inform the development of improved vaccines?

**Highlights**

- Neutralising antibody from long-lived plasma cells, supported by memory B and T cells, can provide lifelong protection against severe disease caused by many respiratory viruses. However, antigenic viral variation can undermine such immunity.
- Viral immunomodulation of memory responses may contribute to reinfections by certain respiratory viruses, though reinfections of healthy adults with homologous viruses are uncommon and usually mild in severity.
- Robust systemic antibody, B cell and T cell responses are mounted upon SARS-CoV-2 infection in almost all individuals and these facets of immune memory are sustained for over 8 months post-infection.
- Mucosal immunity, provided mainly by IgA and tissue-resident T cells, is associated with rapid and potent protection against respiratory infection. However, the durability of these responses has not been established.
- Studies of systemic antibody and cellular responses suggest that protection against severe disease caused by non-variant SARS-CoV-2 may be long-lasting in most individuals.