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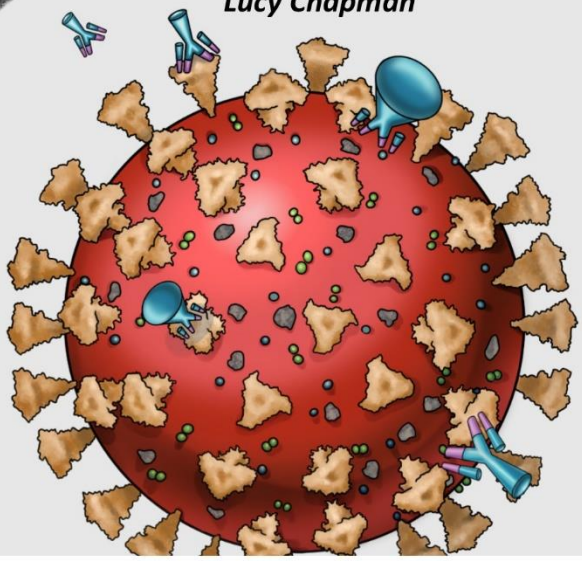

These reviews are the opinions of PhD students, Post-docs and ECRs within Cardiff University School of Medicine, who voluntarily took on this work.

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Special Focus

T Cell and Antibody Responses To SARS-CoV-2: What Do We Know So Far?

Extra Material

Antiviral responses in COVID-19 presented by **Professor Paul Klenerman**

To access this and other British Society of Immunology webinars, go to the following link:

https://www.immunology.org/coronavirus/connect-coronavirus-webinars

Table of Contents Page

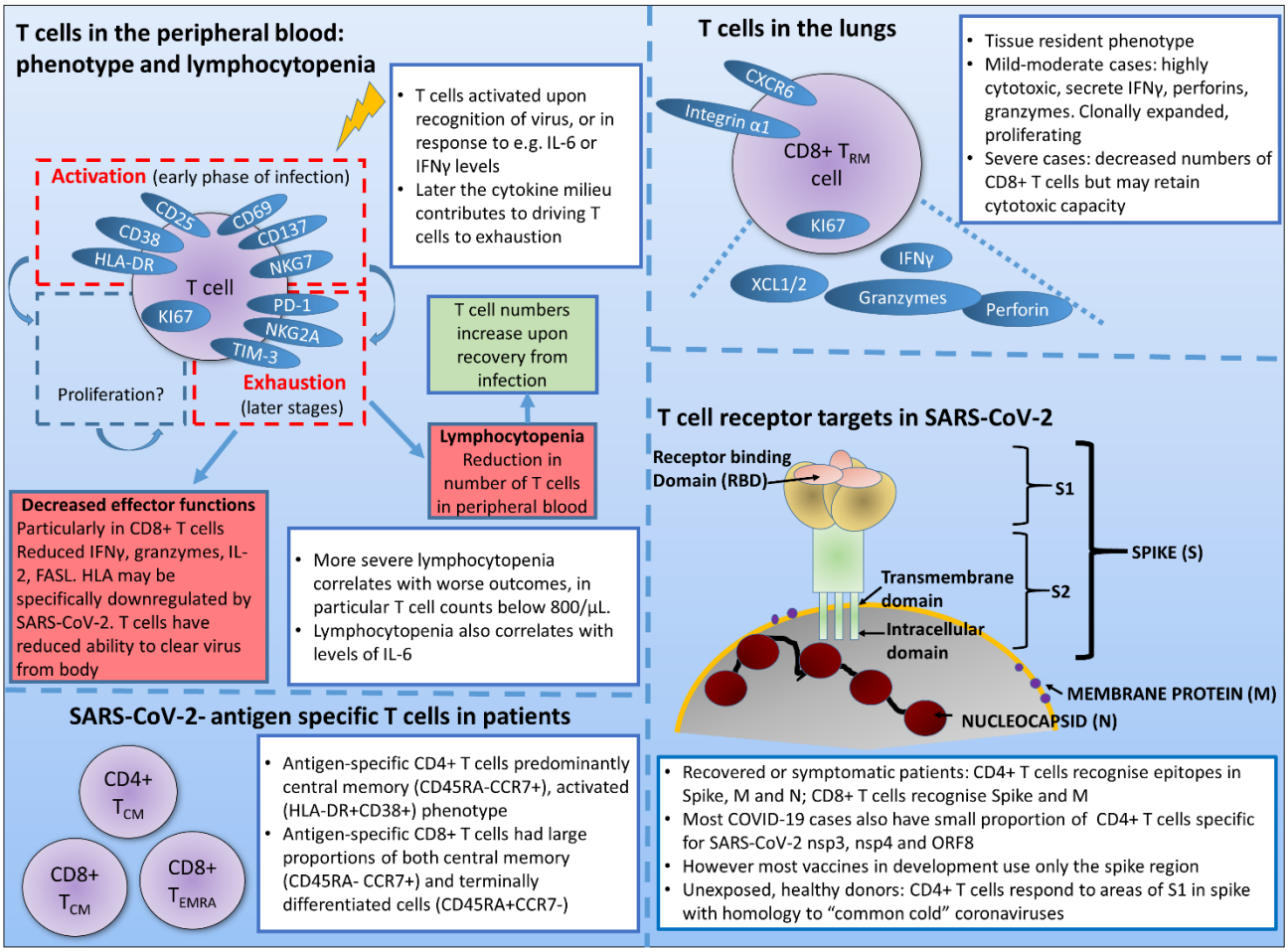
THESE REVIEWS ARE A COLLECTIVE UPDATE ON BOTH T CELL AND ANTIBODY RESPONSES TO SARS-COV-2 USING PAPERS PREVIOUSLY REVIEWED IN THE COMMUNITY JOURNAL CLUB.

THE REVIEWS ALSO CONTAIN REFERENCES OBTAINED BY LITERATURE SEARCH AND MAY CONTAIN PAPERS YET TO BE PEER-REVIEWED.

Emerging Features of The T Cell Response to SARS-COV-2

Stephanie Hanna, Sarah Galloway, Owen Moon, Freya Shepherd, Sandra Dimonte and Amy Codd

Graphical Abstract



Introduction

T cells are a key aspect of the adaptive immune response to viral infection. T cells provide support to the antibody response, directly target infected cells and provide a memory response which offers protection against re-infection.

Dysfunction and dysregulation of the T cell response is emerging as a characteristic of COVID-19 disease.

In the peripheral blood, $CD8^+$ T cells and NK cells from COVID-19 patients have lower percentages of $CD107a^+$, IFN, IL-2^+, granzyme B^+ and TNF^+ cells indicating loss of anti-viral functionality compared to healthy controls (M. Zheng et al., 2020). Most, but not all studies, have reported an exhausted(H. Y. Zheng et al., 2020), terminally differentiated or senescent (Mazzoni et al., 2020) (Mazzoni et al., 2020) T cell phenotype, particularly in $CD8^+$ T cells (M. Zheng et al., 2020). For example, PD-1 (Diao et al., 2020; Yang et al., 2020), Tim-3 (Diao et al., 2020; Zhou et al., 2020) and the inhibitory receptor NKG2A (M. Zheng et al., 2020) have all been used as markers of an exhausted phenotype. In contrast Wilk et al. reported no clear change in CD4+ or CD8+ T cell exhaustion in COVID-19 patients (Wilk et al., 2020). Nevertheless, a combination of exhaustion markers along with patient demographics can be used to distinguish between mild and severe COVID-19 and healthy controls (H. Y. Zheng et al., 2020) (H.-Y. Zheng et al., 2020), and exhaustion markers increase as disease progresses (Diao et al., 2020). The findings of most T cell phenotyping and functional studies suggest that SARS-CoV-2 infection results in aberrant CD4+ T cells functionality, followed by hyper-activation and then exhaustion of cytotoxic CD8+ T cells (Ganji et al., 2020; H. Y. Zheng et al., 2020). SARs-CoV-2 antigen-specific CD4+ T cells predominantly had an activated (HLA-DR+ and CD38+) (Braun et al., 2020) central memory (CD45RA-CCR7+) phenotype (Weiskopf et al., 2020), whilst antigen-specific CD8+ T cells had large proportions of both central memory (CD45RA- CCR7+) and terminally differentiated cells (CD45RA+CCR7-)(Weiskopf et al., 2020). In the lungs of COVID-19 patients CD8+ T cells have a resident memory phenotype, are clonally expanded, and have a cytotoxic, anti-viral phenotype, with high expression of KLRs and granzymes (Chua et al., 2020) (Liao et al., 2020). Interestingly, severe cases had decreased CD8+ T cells in the lung but they showed increased proliferation (Liao et al., 2020).

SARS-CoV-2 Driven Immune Evasion

Studies suggest that the SARS-CoV-2 virus may be downregulating MHC class I and II genes as a mechanism of immune evasion. Further investigation is required in order to confirm this mechanism of action (Ong et al., 2020; Zhang et al., 2020)

Lymphopoenia is associated with severe disease.

T cells have largely been found to be depleted in COVID-19 disease; it has been reported that 43.8 – 80.9 % of patients have an abnormally low level of circulating lymphocytes (lymphocytopenia) on admission (Fei et al., 2020; Lombardi et al., 2020) and several studies have shown a correlation of lymphocytopenia to severe COVID-19 disease. Currently

unpublished work from Anhui Medical university, report that reduced lymphocyte counts upon admission increase both the risk of severe disease and death (Fei et al., 2020). The authors demonstrate that patients with lymphocyte reduction (relative to patients with lymphocyte counts in the normal range) have worse lung (right, left and bilateral) CT scores, respiratory function and elevated indicators of hepatic injury which demonstrates multi-organ injury that they suggest may play a role in the increased risk of death associated with COVID-19 lymphocyte reduction (Fei et al., 2020). As a result of these emerging data, it has been recommended that patients with T cell counts lower than 800/µL may require urgent intervention, and other studies have validated models on a small cohort of patients which indicate lymphocytopenia can be used to stratify patients according to their need of intensive care facilities (Diao et al., 2020; Tan et al., 2020). Liu *et al.* showed that lymphocyte numbers improve upon resolution of viral infection, inferring a direct correlation of SARS-CoV-2 viral infection and immune cell dysregulation (Liu et al., 2020). To conclude, several studies have identified lymphocytopenia as a condition associated with COVID19 whose extent not only increases with disease severity but upon admission can feasibly be used to identify patients who are more likely to require ICU care. Furthermore, the resolution of lymphocyte reduction alongside decline and patient recovery highlight lymphocytopenia as an important facet of COVID-19. Its association with proinflammatory cytokines and the NLR ratio indicates this is an area where research should be focused.

Antigen-specific T Cell Responses

~ 50% of healthy donors had some SARS-CoV-2-reactive $CD4^+$ T cells, which may have been generated in response to “common-cold” coronaviruses HCoV- OC43 and HCoV-NL63 and could cross-react to recognise SARS-CoV-2 (Braun et al., 2020; Grifoni et al., 2020). Given that vaccines currently in development mainly target spike protein and the observed reactivity to M and N proteins by SARS-CoV-2 reactive T cells, this study provides a rational to expand the scope of SARS-CoV-2 vaccines and include M and N proteins as targets.

Early Conclusions and Important Questions Going Forward

There is evidence that T cell responses to SARS-COV-2 are impaired. *It is unclear if there is an early T cell over activation followed by functional exhaustion in the face of overwhelming infection, or does the virus lead to early T cell exhaustion facilitating ongoing infection?*

- Loss of T cell responses to SARS-COV-2 is associated with worse disease.
- Restoring these responses likely represents a therapeutic opportunity.

- Immunity to SARS-COV-2 could arise through prior exposure to other common coronaviruses. *Working out the role of cross-reactive T cells in coronavirus infections will be important for both therapeutics approaches as well as possible diagnostics.*
- T cells may represent an important component of protective immunity induced by infection or vaccination.

References

Braun, J., Loyal, L., Frentsch, M., Wendisch, D., Georg, P., Kurth, F., . . . Thiel, A. (2020). Presence of SARS-CoV-2 reactive T cells in COVID-19 patients and healthy donors. *medRxiv*, https://doi.org/10.1101/2020.04.17.20061440

Chua, R. L., Lukassen, S., Trump, S., Hennig, B. P., Wendisch, D., Pott, F., . . . Eils, R. (2020). Cross-talk between the airway epithelium and activated immune cells defines severity in COVID-19. *medRxiv*, https://doi.org/10.1101/2020.04.29.20084327

Diao, B., Wang, C., Tan, Y., Chen, X., Liu, Y., Ning, L., . . . Chen, Y. (2020). Reduction and Functional Exhaustion of T Cells in Patients With Coronavirus Disease 2019 (COVID-19). *Front Immunol, 11*, 827. https://doi.org/10.3389/fimmu.2020.00827

Fei, J., Fu, L., Li, Y., Xiang, H.-X., Xiang, Y., Li, M.-D., . . . Zhao, H. (2020). Reduction of lymphocyte at early stage elevates severity and death risk of COVID-19 patients: a hospital-based case-cohort study. *medRxiv*, https://doi.org/10.1101/2020.04.02.20050955

Ganji, A., Farahani, I., Khansarinejad, B., Ghazavi, A., & Mosayebi, G. (2020). Increased expression of CD8 marker on T-cells in COVID-19 patients. *Blood Cells Mol Dis, 83*, 102437. https://doi.org/10.1016/j.bcmd.2020.102437

Grifoni, A., Weiskopf, D., Ramirez, S. I., Mateus, J., Dan, J. M., Moderbacher, C. R., . . . Sette, A. (2020). Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell*. https://doi.org/10.1016/j.cell.2020.05.015

Liao, M., Liu, Y., Yuan, J., Wen, Y., Xu, G., Zhao, J., . . . Zhang, Z. (2020). The landscape of lung bronchoalveolar immune cells in COVID-19 revealed by single-cell RNA sequencing. *medRxiv*, https://doi.org/10.1101/2020.02.23.20026690

Liu, B., Han, J., Cheng, X., Yu, L., Zhang, L., Wang, W., . . . Cheng, Z. (2020). Persistent SARS-CoV-2 presence is companied with defects in adaptive immune system in non-severe COVID-19 patients. *medRxiv*, https://doi.org/10.1101/2020.03.26.20044768

Lombardi, A., Trombetta, E., Cattaneo, A., Castelli, V., Palomba, E., Tirone, M., . . . Bandera, A. (2020). Early phases of COVID-19 are characterized by a reduction of lymphocyte populations and the presence of atypical monocytes. *medRxiv*, https://doi.org/10.1101/2020.05.01.20087080

Mazzoni, A., Salvati, L., Maggi, L., Capone, M., Vanni, A., Spinicci, M., . . . Cosmi, L. (2020). Impaired immune cell cytotoxicity in severe COVID-19 is IL-6 dependent. *J Clin Invest*. https://doi.org/10.1172/JCI138554

Ong, E. Z., Chan, Y. F. Z., Leong, W. Y., Lee, N. M. Y., Kalimuddin, S., Haja Mohideen, S. M., . . . Low, J. G. H. (2020). A Dynamic Immune Response Shapes COVID-19 Progression. *Cell Host & Microbe, 27*(6), 879-882.e872. https://doi.org/10.1016/j.chom.2020.03.021

Tan, L., Wang, Q., Zhang, D., Ding, J., Huang, Q., Tang, Y. Q., & Miao, H. (2020). Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. *Signal Transduct Target Ther, 5*(1), 33. https://doi.org/10.1038/s41392-020-0148-4

Weiskopf, D., Schmitz, K. S., Raadsen, M. P., Grifoni, A., Okba, N. M. A., Endeman, H., . . . de Vries, R. D. (2020). Phenotype of SARS-CoV-2-specific T-cells in COVID-19 patients with acute respiratory distress syndrome. *medRxiv*, 2020.2004.2011.20062349. https://doi.org/10.1101/2020.04.11.20062349

Wilk, A. J., Rustagi, A., Zhao, N. Q., Roque, J., Martínez-Colón, G. J., McKechnie, J. L., . . . Blish, C. A. (2020). A single-cell atlas of the peripheral immune response in patients with severe COVID-19. *Nat Med*. https://doi.org/10.1038/s41591-020-0944-y

Yang, X., Dai, T., Zhou, X., Qian, H., Guo, R., Lei, L., . . . Zhang, B. (2020). Analysis of adaptive immune cell populations and phenotypes in the patients infected by SARS-CoV-2. *medRxiv*, 2020.2003.2023.20040675. https://doi.org/10.1101/2020.03.23.20040675

Zhang, B., Zhou, X., Zhu, C., Feng, F., Qiu, Y., Feng, J., . . . Wang, J. (2020). Immune phenotyping based on neutrophil-to-lymphocyte ratio and IgG predicts disease severity and outcome for patients with COVID-19. *medRxiv*, 2020.2003.2012.20035048. https://doi.org/:10.1101/2020.03.12.20035048

Zheng, H. Y., Zhang, M., Yang, C. X., Zhang, N., Wang, X. C., Yang, X. P., . . . Zheng, Y. T. (2020). Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. *Cell Mol Immunol, 17*(5), 541-543. https://doi.org/10.1038/s41423-020-0401-3

Zheng, M., Gao, Y., Wang, G., Song, G., Liu, S., Sun, D., . . . Tian, Z. (2020). Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell Mol Immunol, 17*(5), 533-535. https://doi.org/10.1038/s41423-020-0402-2

Zhou, Y., Fu, B., Zheng, X., Wang, D., Zhao, C., Qi, Y., . . . Wei, H. (2020). Pathogenic T-cells and inflammatory monocytes incite inflammatory storms in severe COVID-19 patients. *National Science Review, 7*(6), 998-1002. https://academic.oup.com/nsr/article/7/6/998/5804736

Antibodies in COVID-19: Specificity and Function

Oliver Scourfield, Sophie Reed, Valentina Bart, Alicia Teijeira Crespo, Ruth Jones, Ellie Pring and Stephanie Burnell.

Introduction

In the case of COVID-19 disease, the production of a robust antibody response is crucial for infection control and long-term protection. Studies of SARS-CoV, MERS-CoV and seasonal coronaviruses as well as both animal infection and patient studies SARS-CoV-2 have informed on the characteristics of an effective antibody response. This review will outline the major aspects of SARS-CoV-2 antibody research to date including kinetics of antibody response, antibody cross-reactivity, neutralising antibodies, long-term immunity and convalescent plasma therapy.

Kinetics of antibody responses to SARS-CoV-2

Multiple studies have sought to investigate antibody responses to SARS-CoV-2 infection. Blood samples collected from COVID-19 patients longitudinally over the course of infection have been used to analyse antiviral antibody kinetics. Zhao *et al.* collected plasma from 173 SARS-CoV-2 patients and found over 40% of patients had antibodies against the virus 1 week after onset, whereas 100% had antibodies 15 days post onset (1). At this time point, 94.3% of the cohort were IgM^+ whereas only 79.8% were IgG^+. Conversely, a study by Long *et al.* discovered that 100% of 285 COVID-19 patients were IgG^+; however, this was 19 days post symptom onset (2). Tan *et al.* sampled blood from 67 COVID-19 patients and found that IgM appeared on day 7, whilst IgG appeared on day 10 after symptom onset, peak titres were on days 28 and 49 respectively (3). Interestingly, weak responders for IgG were shown to have superior viral clearance rates (3). However, the functional capacity of these antibodies to induce a neutralising response, which directly inhibit viral entry, has not been studied here (1-3). Discrepancies between these three studies could be due to blood collection time-points, the subjectivity of symptom onset time within patients and also the sensitivity and antigens used for immunoassays/ELISAs detecting these antibodies. Additionally, the slight differences in antigens used for experimentation may also explain the variation in results as Zhao *et al.* used receptor-binding domain (RBD) of the spike (S) protein whereas both Long *et al.* and Tan *et al.* used the nucleocapsid (N).

SARS-CoV-2 neutralising antibodies (nAbs) were detectable from day 10, up to day 60 post symptom onset and peaked between 31-40 days. Samples were taken from 70 COVID-19 patients, up to 60 days post symptom onset and nAbs were detected by a modified

cytopathogenic assay based on live SARS-CoV-2 (4). More specifically, Wu *et al.* found SARS-CoV-2-specific nAbs present between 10-15 days after disease onset and these remained present up to 2 weeks after patient discharge; however, this was studied within a cohort of patients with only mild symptoms (5). Interestingly, many publications have shown significant correlations of higher antibody titres in both older patients and those with more severe disease (1, 3-5). Wu *et al.* also found that roughly 30% of recovered patients had very low nAb titres, whilst older patients and those with high nAb titres had lower lymphocyte counts and higher C reactive protein (CRP) levels (5). Given that the elderly and those severely affected with COVID-19 have higher nAb titres, it is unclear as to why they are less able to clear the disease effectively. Further work is required to address this issue.

Antibody Cross-reactivity between Different Coronavirus Strains

SARS-CoV-2 expresses S glycoprotein homotrimers that are essential for binding the angiotensin-converting enzyme 2 (ACE2) receptor and subsequent viral entry into the host cells (Figure 1). Each S protomer consists of S1 and S2 subunits, with the RBD located within S1. Based on the understanding of related coronaviruses, it is thought that an adaptive immune response, particularly the production of nAbs targeting S protein regions, is essential for recovery and future protection against SARS-CoV-2 (6, 7). Several studies have recently focused on the isolation of protective antibodies from convalescent individuals to understand which epitopes could offer such protection.

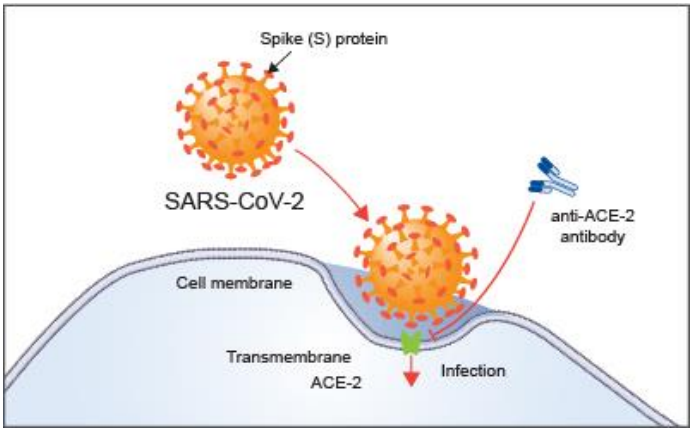


Figure 1. Anti-ACE-2 antibodies inhibit the interaction between the S protein on SARS-CoV-2 and the ACE-2 receptor.

Source: https://www.rndsystems.com/resources/articles/ace-2-sars-receptor-identified

SARS-CoV-2, via its RBD, uses cellular receptor ACE2 to facilitate entry into cells for infection. As this is the main pathway the virus uses to infect cells, most antibodies are developed to inhibit this route (Figure 1). SARS-CoV-2 and SARS-CoV share almost 76% identity in amino acid sequence for the S protein and due to this, cross-reactivity has been reported between viruses (8). Wang *et al.* has shown from solving the crystal structure of SARS-CoV-2 spike protein in complex with the human ACE2 (hACE2), that the binding is similar to SARS-CoV in complex with hACE2 (9).

Lv *et al.* studied the similarities in antibody binding to the S protein between SARS-CoV-2 and SARS-CoV in human and in mouse models and demonstrated that cross reactivity was common in both species (10). Also, Zost *et al.* reported the generation of monoclonal antibodies directed against the SARS-CoV-2 S protein from four infected patients; these had varying degrees of cross-reactivity with SARS-Cov (11). Wrapp *et al.* found that SARS-CoV RBD-directed single domain antibodies cross-reacted with the SARS-CoV-2 RBD and engineered them into a bivalent human IgG1 Fc-fusion (12).

Despite these findings, minimal cross reactivity between SARS-CoV-2 and SARS-CoV has been shown by serological assay in patients (13). Yuan *et al.* have also demonstrated that, although a cross-reactivity might exist for some antibodies between SARS-CoV and SARS-CoV-2, the binding affinity is different, suggesting that there are non-conserved residues in some epitopes (14). In addition, Hachim *et al.* narrowed down the antibody profiles in patients to 15 antigens derived from potential open reading frames of SARS-CoV-2. Eleven of these induced a novel response, as they shared low homology with other human CoVs (15).

Together, these findings suggest that cross-reactivity varies depending on the antibody target as the S1 and S2 domains and the N demonstrated very low cross-reactivity among coronaviruses (SARS-CoV-2, SARS-CoV and MERS-CoV) (13).

Neutralising Antibodies and Spike: Interaction and Therapeutic Potential

Neutralising antibodies that develop in response to infection have potential for use as a therapeutic. In studies that focus on convalescent plasma, SARS-CoV-2 specific nAbs were shown to elicit their neutralising capacity by binding the RBD of the SARS-CoV-2 S protein (16, 17). Crystal structure analysis revealed that this neutralisation is caused by steric hindrance of RBD-ACE2 binding interaction, preventing SARS-CoV-2 binding and entry via the ACE2 receptor on human cells (16, 17). Specifically, Wu *et al*. identified that 18 of the 21 amino acids involved in this interaction were identical to those found in the epitope of a particular monoclonal antibody isolated from a convalescent patient (17).

Additionally, two SARS-CoV-2 specific antibodies identified from a different patient (CA1 and CB6) could neutralise viral activity *in vitro*, with CB6 also protecting rhesus macaques from SARS-CoV-2 infection (18). Again, they were suggested to bind overlapping ACE2 binding epitopes. While both studies only investigated one single patient, similar results were found when applying a rapid antibody discovery platform to the serum of four individuals recovered from SARS-CoV-2 infection. This study detected 386 recombinant SARS-CoV-2 reactive antibodies and classed them into five categories based on their reactivity to S protein subdomains and cross-reactivity to other coronavirus strains (11). Most nAbs mapped to the RBD, with 324 unique amino acid sequences detected.

Across multiple studies, nAbs obtained from patients have been shown to functionally reduce SARS-CoV-2 viral titres *in vivo*. B38 and H4 - monoclonal antibodies obtained from the same patient, were able to therapeutically lower viral load in hACE2 transgenic mice 12 hours after viral challenge (17). Their therapeutic capacity is shown in their complete competition with ACE2 for RBD binding. The two nAbs recognise different epitopes and the authors emphasise the benefit of using both together as an antibody-based therapy for COVID-19 patients.

The functional capacity of SARS-CoV-2-specific nAbs has also been shown by Cao *et al*. Using single cell RNA sequencing, they identified 14 potential candidates from 8,559 antigen-binding IgG1+ clonotypes (19). The most potent nAb, BD-368-2, greatly reduced viral load in SARS-CoV-2-infected hACE2-transgenic mice, when given either therapeutically or prophylactically. Cryo-EM analysis also revealed that the epitope of BD-368-2 overlapped with the ACE2 binding site (19). While this study also identified a range of S1/S2 non-RBD binding antibodies, those did not possess neutralising ability (19). In line with this, another study suggested that the most potent nAbs were most competitive with ACE2 in binding to RBD (16).

Collectively, these studies demonstrate the potential therapeutic use of SARS-CoV-2-specific nAbs, either from convalescent plasma or made recombinantly as mAbs or mAb derivatives, which block viral interaction with ACE2, inhibiting viral entry and infection in the host. However, while these studies suggest the importance of antibody binding to RBD epitopes for neutralisation, this might not be the only route. In one of the aforementioned studies, only two of the four identified nAbs bound to the RBD; the target epitopes of the other two antibodies have not been investigated (17). Further, when screening serum samples of six SARS-CoV-2 infected patients, nAbs were shown to target two distinct S region peptide pools. Specifically, peptide S21 P2 contained a highly conserved fusion peptide that was identified as a potential pan-coronavirus epitope (20). Antibodies targeting this region have been suggested to block virus/target cell fusion. In contrast, peptide S14 P5, which in prefusion conformation, locates proximal to, but not within the RBD, was SARS-CoV-2 specific and could

possibly interfere with SARS-CoV-2/ACE2 interaction (20). Depletion of antibodies targeting these peptides significantly reduced the ability to neutralise infection with a SARS-CoV-2 pseudo-typed lentivirus.

Data described here clearly point toward a protective role of antibodies that interfere with ACE2 binding. It is essential at this point to compare the epitopes of antibodies identified across different studies to determine their overlap and identify the best nAb that could be utilised in the development of therapeutics and prophylactics.

Looking beyond the S protein, antibody responses have been detected to a wide range of SARS-CoV-2 antigens. ORF3b and ORF8, which are among the most unique genes of SARS-CoV-2, induce a humoral response in SARS-CoV-2 patients (15). While it is currently unclear whether these antibodies confer protection, they could play an important role in disease detection. Currently, S and N regions are used by most commercially available antibody tests; however, single test detection of N or S antibodies at early time points were associated with high false negative rates. In contrast, the combination of at least N + ORF3b + ORF8 was shown to be highly sensitive and specific in early diagnosis (15).

Most of the studies detailed here are limited by small sample sizes and do not assess antibody responses at different time points during and after infection. It will be important to understand what drives the production of nAbs and whether they confer long-term protection against SARS-CoV-2 infection.

Antibody Responses to SARS-CoV-2 Vaccines

Vaccine candidate studies in animal models have also delivered useful data on nAb responses. Models give an insight into to the immune protection initiated by vaccines and how it may protect the host against SARS-CoV-2 infection. Quinlan *et al.* conducted an immunisation study in rats using the SARS-CoV-2 RBD and found a robust neutralising response. Sera collected from the rats on day 40 following immunisation was able to potently neutralise retroviruses pseudotyped with the SARS-CoV-2 S protein *in vitro* (21). Using a similar approach, Ravichandran *et al.* immunised rabbits with various SARS-CoV-2 S protein antigens and also utilised a pseudovirus neutralisation assay to find that 3 out of 4 antigens generated strong nAbs in rabbit sera (22). Smith *et al.* created a DNA vaccine candidate 'INO-4800', which targets the SARS-CoV-2 S protein. In both mice and guinea pigs immunised with INO-4800, functional antibodies that neutralised the S protein-ACE2 interaction were generated with high antibody titres found within the lungs (23). These studies show that various vaccine candidates produce functional nAbs against SARS-CoV-2, which may provide protection against COVID-19 infection.

Do antibodies provide long-term immunity to SARS-CoV-2?

Recent reviews have highlighted the importance of determining whether SARS-CoV-2 patients have long-term protective immunity against disease, both informing vaccination trials and public health interventions. As we are in the relatively early stages in SARS-CoV-2 pandemic, it is unknown how long any protective antibody response against reinfection might last in humans.

Preliminary studies in rhesus macaque models suggest a limited protective immunity against SARS-CoV-2 reinfection. Published work by Chandrashekar *et al.* showed in nine rhesus macaques re-challenged with SARS-CoV-2 35 days after the initial infection, a reduced viral load, increased virus specific antibody titres and reduced clinical symptoms compared to naïve controls (24). These findings are supported by unpublished work by Bao *et al.* who observed that rhesus macaques appeared protected from subsequent SARS-CoV-2 infection when re-challenged 28 days after the initial infection (25). Though the sample number was limited (n=2), SARS-CoV-2 was not detected in nasal swabs after the re-challenge and little pathological evidence of infection was observed. Limitations to both studies are the small sample size and the short duration between SARS-CoV-2 challenges. In future studies it would be interesting to determine if protective antibody immunity is maintained over years.

Further predictions/insights about the potential long-term immunity against SARS-CoV-have been gleaned from other members of the coronavirus family, such as seasonal coronaviruses and the more severe SARS-CoV and MERS-CoV. In 1990, Callow *et al.* inoculated 15 volunteers with the seasonal coronavirus 229E, of which 10 became infected. After 1 year, the antibody response was greatly reduced and no longer sufficient to prevent re-infection with 229E in 6 of the 9 returning, originally infected subjects (26). Further to this, a recent pre-print by Edridge *et al.* determined re-infection rates of four seasonal coronaviruses over a 35-year period. For most viruses, reinfections occurred every 3 years, dependent on the level of the re-exposure in addition to lingering protective immunity (27). Though this work has not yet been peer reviewed, it does support findings from Kissler *et al.* who adapted seasonal coronavirus modelling to estimate SARS-CoV-2 protective immunity may last approximately 45 weeks (28). Additionally, this approach suggested the level of public health intervention that may be required based on the length of protective immune response against SARS-CoV-2. Kellam *et al.* and Huang *et al.* recently reviewed long-term antibody responses to SARS-CoV and MERS-CoV. Broadly speaking, minimal virus specific antibody titres were measured after 2-3 years and individuals with a more severe disease tended to have higher antibody titres (29, 30). Kellam *et al.* also measured seasonal coronavirus reinfection rates and highlighted that an antibody response did not confer protection from reinfection (30). Current evidence suggests that re-infection with SARS-CoV-2 is possible, as long-term immunity is unlikely to

last more than 2/3 years. However, this work does support a vaccine-based approach to controlling SARS-CoV-2 transmission, though regular boosters may be required.

The use of convalescent plasma as a therapy

Convalescent plasma for the treatment of infectious disease has been used to successfully reduce mortality in a variety of viral epidemics, including the 1918 influenza pandemic, 2003 SARS and 2009 influenza epidemics (31). During the current COVID-19 pandemic, several studies have investigated convalescent plasma (CP) transfusions as a treatment option based on past success in a variety of viral infections and the lack of current effective antiviral therapies. CP obtained from patients that have recovered from COVID-19 and developed humoral immunity against the virus, contains a large quantity of nAbs capable of neutralising the virus and removing it from the circulation (32). This requires donors that have high levels of nAbs. Studies on MERS-CoV and SARS-CoV infections have shown that the neutralising antibody titre should exceed 1:80 and that levels of nAbs start to decrease 4 months following recovery, reaching undetectable levels at 36 months (33-35). Table 1 shows several studies investigating the use of CP in COVID-19 patients, indicating that all studies have used the donor plasma within the 4-month time window and generally used similar titres, although this information is not always provided. There is currently no consensus on the amount of CP that should be administered with some studies administering on a patient-by-patient basis rather than a one-dose fits all regime. All patients were treated successfully and improved or recovered from infection.

Until Li et al. carried out the first randomised clinical trial looking at the use of CP therapy for patients with COVID-19 in China, all other investigations contained only case studies on 2-10 patients (36-41). Although this large study found no statistically significant differences between patients treated with CP and the control group, there were clear trends indicating that patients treated with CP demonstrated more favourable outcomes. This was the case for several secondary end points including 28 day mortality (15.7% with CP compared to 24.0% control) and rate of discharge (51% with CP compared to 36% control) (36). It is important to note that this study was terminated early, with only half the planned number of patients enrolled, due to containment of the disease. This resulted in an underpowered study and could therefore explain the lack of significance.

Table 1: Information to show the CP treatment regimen and outcome of several COVID-19 studies

Author	Plasma Donor	Titre	# of Patients	Administration	Recovery?	Ref
Li	Fully recovered, discharged >2 weeks	>1:640	103	4-13 ml/kg of recipient body weight	All patients recovered, only 2 experienced adverse events within hours of transfusion	(36)
Duan	3 week post onset of illness, 4 day post discharge	>1:640	10	1 dose of 200 ml	All recovered	(37)
Zhang			4	900 ml of CP transfused in 3 batches	significantly reduced viral load within 10 days	(38)
				200 ml	Recovered	
				8 transfusions of CP (total 2,400 ml) over 26 day period	viral load reduced PCR day 5 negative	
				300 ml	PCR negative by day 21	
Shen	Asymptomatic for at least 10 days	>1:1000	5	2 transfusions of 200-250 ml	Of the 5 patients, 3 discharged and 2 are in stable condition	(39)
Ahn	Recovered from COVID-19 for 18 days.		2	2 transfusions (500 ml total)	Resolution of lung infiltrates. Negative after day 26	(40)
				2 transfusions (500 ml total)	Leukocytosis and lymphopenia immediately recovered, bilateral infiltration on chest X-ray improved by day 9	
Ye	Afebrile status for at least 3 days, negative for 2 consecutive PCR tests and 3 weeks following disease onset		6	3 transfusions (600 ml total)	Negative PCR by 10 day	(41)
				2 transfusions (400 ml total)	Alleviation of respiratory distress. Negative swabs	
				3 transfusions (600 ml total)	Complete resolution of consolation by CT by day 10	
				200 ml	Negative throat swab	
				200 ml	Negative throat swab	
				200 ml	Relief of symptoms, radiologic changes after 3 days	

A further explanation for the lack of statistical significance could be treatment time-point. The median time between symptom onset and the treatment of patients with CP was 30 days; however, multiple studies have demonstrated that patients treated within 14 days of infection showed significantly improved outcome (37, 42). In the study conducted by Duan *et al.* all patients treated with CP before day 14 of illness showed a rapid increase of lymphocyte counts, a decrease in CRP and absorption of lung lesions; however, those patients that received CP after 14 days of illness demonstrated a much reduced level of improvement (37).

This study showed that CP therapy was well tolerated, resulted in an increase or maintenance of high level nAbs and a disappearance of viremia within 7 days, with no adverse side effects observed. These results are in agreement with the study carried out by Shen *et al.* where CP transfusion of critically ill patients between 10 and 22 days after admission resulted in increases in nAb titres in addition to normalisation of body temperature, decreased SOFA score; viral loads decreased and became negative within 12 days (39).

Another reason for discrepancy between studies could be the age of the patients. Patients in the Duan *et al.* study had a median age of 52.5 years, similarly the age range in the Shen *et al.* study was 36-65, whereas the median age in the Li *et al.* study was 70 years. Mortality due to COVID-19 is much higher in the elderly, this therefore could have skewed the data and resulted in reduced significance (36, 37, 39).

As with all therapies, there are potential risks involved when administering CP treatment, such as the transmission of residual virus from donor to patient. In the Duan *et al.* study, this was mitigated by applying methylene blue photochemistry to inactivate any virus that may have remained in the donor plasma (37, 43). No other study included here specified how they addressed this risk, stating only that they obtained the plasma by plasmapheresis or apheresis.

Although the only randomised clinical trial to date has not yielded statistically significant differences between patients administered CP and controls, 91.3% of patients with severe disease showed clinical improvement at 28 days with intervention compared to only 68.2% in the control group, in addition to a reduction in mortality and rate of discharged as mentioned earlier (36). Several patients in these studies also received a variety of other drugs including anti-viral treatments and corticosteroids, which could have contributed to the recovery of patients, interfered with immune response or synergised with the therapeutic effect of CP (37). This highlights important limitations of the study that need to be addressed, whilst also indicating a potential role for CP in the treatment of COVID-19 patients with severe disease. Further work is now required with larger cohorts examining differences in age, sex and drug combinations, in addition to determining the optimal doses and time points for CP administration.

Concluding Remarks

In COVID-19 patients, studies have demonstrated that a proportion of antibodies are neutralising and most compete with virus binding to the ACE2 receptor on human cells. Other antibody targets include ORF3b and ORF8, which are among the most unique genes of SARS-CoV-2, as well as some unidentified targets, which have been shown to induce humoral

responses in patients. Some of the antibodies targeting unidentified targets on SARS-CoV-2, were effective at controlling infection. However, the overall consensus is that competing with the SARS-CoV-2 Spike protein interaction with ACE2 is key to neutralising the infection. Antibody cross-reactivity between SARS-CoV and SARS-CoV-2 viruses has been identified in patients. Antibody cross-reactivity between species has also been identified, but is relatively low and the binding affinity of the antibodies differs between species.

Neutralising antibodies were shown to peak between 31-40 days post symptom onset. Significant correlations have been made between antibody levels and patient infection control. Higher antibody titres were observed in older patients and those with severe disease but it is still unclear why they struggle to clear the infection.

The potential for effective vaccination against SARS-CoV-2 has also been investigated as neutralising antibodies have been produced in response to virus proteins. Animal studies have revealed that robust neutralisation responses occur to immunisation using the SARS-CoV-2 RBD in rats and SARS-CoV-2 S protein in rabbits. Furthermore, a DNA vaccine candidate 'INO-4800' was also able to induce neutralising antibodies in animal models.

As we are in the early stages of the COVID-19 pandemic, it is unknown how long protective antibodies are retained post-infection. In rhesus macaque studies, limited protective immunity against SARS-CoV-2 has been observed at 28 days and 35 days post-infection. Studies from SARS-CoV, MERS-CoV and seasonal coronaviruses found neutralising antibody responses post-infection which were greatly reduced at 1 year and seasonal re-infections typically occurred every 3 years. Consequently, it is believed that re-infection with SARS-CoV-2 is possible as long-term immunity may be limited to 2/3 years.

CP has been used as a therapy to successfully treat a number of viral infections in the past where limited treatment options are available. The large quantity of neutralising antibody present in recovered patients can be used in severe COVID-19 cases to help remove virus from the circulation. As nAb titres decrease from about 4 months following recovery from SARS-CoV and MERS-CoV, all studies used donor plasma 4 months post-SARS-CoV-2. There is no consensus on the exact amount of CP to be used or the timing of therapy. Some studies suggest that administration within 14 days of disease onset is crucial to improvement of patient outcome. A lack of improvement with CP therapy in some studies may have been due to the time-point of treatment and the age of the patients recruited into the studies. There is an indication that CP therapy reduces disease severity and improves outcome, but more data is needed to understand whether this difference is significant.

Overall, studies have demonstrated that antibody responses, in particular neutralising antibodies, are crucial to infection control. Our understanding of this is key to informing on vaccination to develop long term immunity and therapeutic strategies such as convalescent plasma and recombinant neutralising antibodies.

References

1. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clinical Infectious Diseases. 2020. https://doi.org/10.1093/cid/ciaa344

2. Long Q-X, Liu B-Z, Deng H-J, Wu G-C, Deng K, Chen Y-K, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nature medicine. 2020;26(6):845-8. https://doi.org/10.1038/s41591-020-0897-1

3. Tan W, Lu Y, Zhang J, Wang J, Dan Y, Tan Z, et al. Viral Kinetics and Antibody Responses in Patients with COVID-19. medRxiv. 2020:2020.03.24.20042382. https://doi.org/10.1101/2020.03.24.20042382

4. Wang X, Guo X, Xin Q, Pan Y, Li J, Chu Y, et al. Neutralizing Antibodies Responses to SARS-CoV-2 in COVID-19 Inpatients and Convalescent Patients. medRxiv. 2020:2020.04.15.20065623. https://doi.org/10.1101/2020.04.15.20065623

5. Wu F, Wang A, Liu M, Wang Q, Chen J, Xia S, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. medRxiv. 2020:2020.03.30.20047365. https://doi.org/10.1101/2020.03.30.20047365

6. Li CK-f, Xu X. Host Immune Responses to SARS Coronavirus in Humans. Molecular Biology of the SARS-Coronavirus. 2009:259-78. https://doi.org/10.1007/978-3-642-03683-5_16

7. Liang Y, Wang M-L, Chien C-S, Yarmishyn AA, Yang Y-P, Lai W-Y, et al. Highlight of Immune Pathogenic Response and Hematopathologic Effect in SARS-CoV, MERS-CoV, and SARS-Cov-2 Infection. Front Immunol. 2020;11:1022-. https://doi.org/10.3389/fimmu.2020.01022

8. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nature communications. 2020;11(1):1620.
https://doi.org/10.1038/s41467-020-15562-9

9. Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, et al. Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. Cell. 2020;181(4):894-904.e9.
https://doi.org/10.1016/j.cell.2020.03.045

10. Lv H, Wu NC, Tsang OT-Y, Yuan M, Perera RAPM, Leung WS, et al. Cross-reactive Antibody Response between SARS-CoV-2 and SARS-CoV Infections. Cell reports. 2020;31(9):107725.
https://doi.org/10.1016/j.celrep.2020.107725

11. Zost S, Gilchuk P, Chen R, Case JB, Reidy J, Trivette A, et al. Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. bioRxiv; 2020.
https://doi.org/10.1101/2020.05.12.091462

12. Wrapp D, De Vlieger D, Corbett KS, Torres GM, Wang N, Van Breedam W, et al. Structural Basis for Potent Neutralization of Betacoronaviruses by Single-Domain Camelid Antibodies. Cell. 2020;181(6):1436-41.
https://doi.org/10.1016/j.cell.2020.04.031

13. Khan S, Nakajima R, Jain A, de Assis RR, Jasinskas A, Obiero JM, et al. Analysis of Serologic Cross-Reactivity Between Common Human Coronaviruses and SARS-CoV-2 Using Coronavirus Antigen Microarray. bioRxiv. 2020:2020.03.24.006544.
https://doi.org/10.1101/2020.03.24.006544

14. Yuan M, Wu NC, Zhu X, Lee C-CD, So RTY, Lv H, et al. A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. Science (New York, NY). 2020;368(6491):630-3.
https://doi.org/10.1126/science.abb7269

15. Hachim A, Kavian N, Cohen CA, Chin AW, Chu DK, Mok CKP, et al. Beyond the Spike: identification of viral targets of the antibody response to SARS-CoV-2 in COVID-19 patients. medRxiv. 2020:2020.04.30.20085670.
https://doi.org/10.1101/2020.04.30.20085670

16. Ju B, Zhang Q, Ge J, Wang R, Sun J, Ge X, et al. Human neutralizing antibodies elicited by SARS-CoV-2 infection. Nature. 2020. https://doi.org/10.1038/s41586-020-2380-z

17. Wu Y, Wang F, Shen C, Peng W, Li D, Zhao C, et al. A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. Science (New York, NY). 2020;368(6496):1274-8. https://doi.org/10.1126/science.abc2241

18. Shi R, Shan C, Duan X, Chen Z, Liu P, Song J, et al. A human neutralizing antibody targets the receptor binding site of SARS-CoV-2. Nature. 2020. https://doi.org/10.1038/s41586-020-2381-y

19. Cao Y, Su B, Guo X, Sun W, Deng Y, Bao L, et al. Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. Cell. 2020. https://doi.org/10.1016/j.cell.2020.05.025

20. Poh CM, Carissimo G, Wang B, Amrun SN, Lee CY-P, Chee RS-L, et al. Two linear epitopes on the SARS-CoV-2 spike protein that elicit neutralising antibodies in COVID-19 patients. Nature communications. 2020;11(1):2806. https://doi.org/10.1038/s41467-020-16638-2

21. Quinlan BD, Mou H, Zhang L, Guo Y, He W, Ojha A, et al. The SARS-CoV-2 receptor-binding domain elicits a potent neutralizing response without antibody-dependent enhancement. bioRxiv. 2020:2020.04.10.036418. https://doi.org/10.1101/2020.04.10.036418

22. Ravichandran S, Coyle EM, Klenow L, Tang J, Grubbs G, Liu S, et al. Antibody repertoire induced by SARS-CoV-2 spike protein immunogens. bioRxiv. 2020:2020.05.12.091918. https://doi.org/10.1101/2020.05.12.091918

23. Smith TRF, Patel A, Ramos S, Elwood D, Zhu X, Yan J, et al. Immunogenicity of a DNA vaccine candidate for COVID-19. Nature communications. 2020;11(1):2601. https://doi.org/10.1038/s41467-020-16505-0

24. Chandrashekar A, Liu J, Martinot AJ, McMahan K, Mercado NB, Peter L, et al. SARS-CoV-2 infection protects against rechallenge in rhesus macaques. Science (New York, NY). 2020:eabc4776.
https://doi.org/10.1126/science.abc4776

25. Bao L, Deng W, Gao H, Xiao C, Liu J, Xue J, et al. Lack of Reinfection in Rhesus Macaques Infected with SARS-CoV-2. bioRxiv. 2020:2020.03.13.990226.
https://doi.org/10.1101/2020.03.13.990226

26. Callow KA, Parry HF, Sergeant M, Tyrrell DA. The time course of the immune response to experimental coronavirus infection of man. Epidemiol Infect. 1990;105(2):435-46.
https://doi.org/10.1017/s0950268800048019

27. Edridge AW, Kaczorowska JM, Hoste AC, Bakker M, Klein M, Jebbink MF, et al. Coronavirus protective immunity is short-lasting. medRxiv. 2020:2020.05.11.20086439.
https://doi.org/10.1101/2020.05.11.20086439

28. Kissler SM, Tedijanto C, Goldstein E, Grad YH, Lipsitch M. Projecting the transmission dynamics of SARS-CoV-2 through the postpandemic period. Science (New York, NY). 2020;368(6493):860-8.
https://doi.org/10.1126/science.abb5793

29. Huang AT, Garcia-Carreras B, Hitchings MDT, Yang B, Katzelnick L, Rattigan SM, et al. A systematic review of antibody mediated immunity to coronaviruses: antibody kinetics, correlates of protection, and association of antibody responses with severity of disease. medRxiv. 2020:2020.04.14.20065771.
https://doi.org/10.1101/2020.04.14.20065771

30. Kellam P, Barclay W. The dynamics of humoral immune responses following SARS-CoV-2 infection and the potential for reinfection. Journal of General Virology. 2020.
https://doi.org/10.1099/jgv.0.001439

31. Casadevall A, Joyner MJ, Pirofski L-A. A Randomized Trial of Convalescent Plasma for COVID-19—Potentially Hopeful Signals. JAMA. 2020.
https://doi.org/10.1001/jama.2020.10218

32. Marano G, Vaglio S, Pupella S, Facco G, Catalano L, Liumbruno GM, et al. Convalescent plasma: new evidence for an old therapeutic tool? Blood Transfus. 2016;14(2):152-7. https://doi.org/10.2450/2015.0131-15

33. Ko J-H, Seok H, Cho SY, Ha YE, Baek JY, Kim SH, et al. Challenges of convalescent plasma infusion therapy in Middle East respiratory coronavirus infection: a single centre experience. Antivir Ther. 2018;23(7):617-22. https://doi.org/10.3851/imp3243

34. Cao WC, Liu W, Zhang PH, Zhang F, Richardus JH. Disappearance of antibodies to SARS-associated coronavirus after recovery. The New England journal of medicine. 2007;357(11):1162-3. https://doi.org/10.1056/nejmc070348

35. Arabi YM, Hajeer AH, Luke T, Raviprakash K, Balkhy H, Johani S, et al. Feasibility of Using Convalescent Plasma Immunotherapy for MERS-CoV Infection, Saudi Arabia. Emerging infectious diseases. 2016;22(9):1554-61. https://doi.org/10.3201/eid2209.151164

36. Li L, Zhang W, Hu Y, Tong X, Zheng S, Yang J, et al. Effect of Convalescent Plasma Therapy on Time to Clinical Improvement in Patients With Severe and Life-threatening COVID-19: A Randomized Clinical Trial. JAMA. 2020. https://doi.org/10.1001/jama.2020.10044

37. Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proceedings of the National Academy of Sciences. 2020;117(17):9490-6. https://doi.org/10.1073/pnas.2004168117

38. Zhang B, Liu S, Tan T, Huang W, Dong Y, Chen L, et al. Treatment With Convalescent Plasma for Critically Ill Patients With Severe Acute Respiratory Syndrome Coronavirus 2 Infection. Chest. 2020. https://doi.org/10.1016/j.chest.2020.03.039

39. Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, et al. Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma. JAMA. 2020;323(16):1582-9. https://doi.org/10.1001/jama.2020.4783

40. Ahn JY, Sohn Y, Lee SH, Cho Y, Hyun JH, Baek YJ, et al. Use of Convalescent Plasma Therapy in Two COVID-19 Patients with Acute Respiratory Distress Syndrome in Korea. J Korean Med Sci. 2020;35(14). https://doi.org/10.3346/jkms.2020.35.e149

41. Ye M, Fu D, Ren Y, Wang F, Wang D, Zhang F, et al. Treatment with convalescent plasma for COVID-19 patients in Wuhan, China. Journal of Medical Virology.n/a(n/a). https://doi.org/10.1002/jmv.25882

42. Cheng Y, Wong R, Soo YOY, Wong WS, Lee CK, Ng MHL, et al. Use of convalescent plasma therapy in SARS patients in Hong Kong. European Journal of Clinical Microbiology and Infectious Diseases. 2005;24(1):44-6. https://doi.org/10.1007/s10096-004-1271-9

43. Eickmann M, Gravemann U, Handke W, Tolksdorf F, Reichenberg S, Müller TH, et al. Inactivation of Ebola virus and Middle East respiratory syndrome coronavirus in platelet concentrates and plasma by ultraviolet C light and methylene blue plus visible light, respectively. Transfusion. 2018;58(9):2202-7. https://doi.org/10.1111/trf.14652