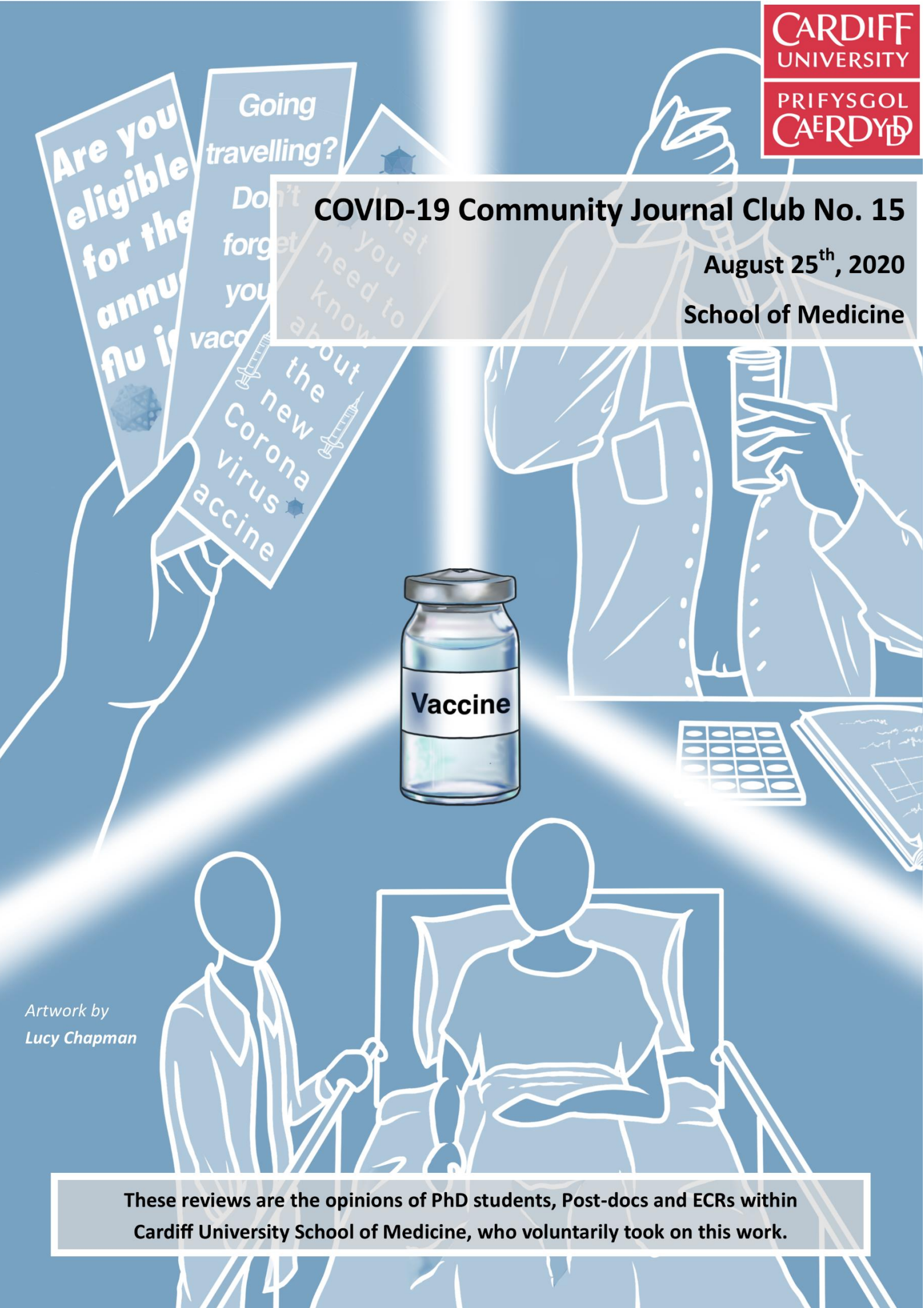

COVID-19 Community Journal Club No. 15

August 25th, 2020

School of Medicine

Artwork by
Lucy Chapman

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

Thank you to:

Elise Moses, Pragati Sabberwal, Sophie Reed, Ruth Jones, Rebecca Bayliss, Ruban Durairaj, Owen Moon, Stephanie Burnell, Camille Rabesahala De Meritens, Kate Liddiard, Sarah Lauder, Patricia Dos Santos Rodrigues, Lorenzo Capitani, Ana Pires, Ellie Pring, Alicia Teijeira Crespo, Stephanie Hanna, Tom Pembroke, Valentina Bart, Sarah Curtis, Freya Shepherd, Stefan Milutinovic, Yuan Chen, Amy Codd, Sarah Gallaway, Shayda Maleki Toyserkani, Sandra Dimonte, Oliver Scourfield and Ariadni Kouzeli for producing these reviews.

Drs Ceri Fielding, Carmen Van den Berg, Luke Davies, Andrew Godkin, Kristin Ladell, Emma Jones and James Matthews for paper selection.

Oliver Scourfield for compiling the digest.

Please direct any comments or queries to Awen at gallimoream@cardiff.ac.uk

All previous editions of the Community Journal Club can be found at:
https://www.cardiff.ac.uk/news/view/2260179-getting-to-grips-with-covid-19/_recache

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CAUTIONARY NOTE:

SOME REVIEWS ARE OF PAPERS POSTED ONLINE (in *arXiv, bioRxiv and medRxiv)* BEFORE PEER REVIEW.

News and Views

Controversial 'human challenge' trials for COVID-19 vaccines gain support

Cohen, J. 2020. *Science*

Link: https://doi.org/10.1126/science.abd9203

Practical steps towards 'human challenge trials' which test vaccine efficacy by infecting volunteers with the virus are taking place. It is believed that these trials may speed up vaccine development. The advocacy group 1Day Sooner have recruited volunteers and collaborated with researchers to initiate the development of lab grown virus strains needed for these trials. The group organized an open letter signed by 15 Nobel laureates and 100 other prominent researchers, ethicists and philosophers to urge the U.S government to 'undertake immediate preparations for human challenge trials' in young healthy people who are less likely to suffer severe disease from COVID-19. Some points highlighted were:

- Advocacy group 1Day Sooner is showing there are young people willing to take risks for the greater good
- Recruitment of 30,000 volunteers from 140 countries to take part in challenge trials
- Growing and testing lab grown 'challenge strains' needed for the studies has started in the Hill's lab at Oxford University and biotech startup Curative Inc in Los Angeles.
- WHO was split as to whether these trials should take place without a 'rescue' treatment available and they estimate it will take 2 months after a viral strain is made and characterised to launch a challenge study
- Enthusiasm for a challenge trial could grow if a 'rescue' treatment for COVID-19 was available

SARS-CoV-2 T cell immunity: Specificity, function, durability, and role in protection

Altmann, D.M. and Boyton, R.J. 2020. *Science Immunology*

Link: https://doi.org/10.1126/sciimmunol.abd6160

Taking into account numerous studies, the authors give an overview about different aspects of T cells immunity in SARS-CoV2-2 infection and emphasize the emerging need for T cell data to determine immunity levels in the population. Below are summaries of highlighted field:

Lymphopenia

Lymphopenia is often observed in severely ill COVID-19 patients and has been interpreted more so as sequestration of T cells to sites of infection than a plain reduction of T cells. There is however no clear evidence for displacement of T cells from the periphery to the lungs. Furthermore, studies have shown that the BAL fluid of severe COVID-19 cases with the most prominent lymphopenia contain lower levels of CD8 T cells which makes simple sequestration unlikely.

T cell subsets

Most infected people (except for maybe the most severe cases) show a robust T cell response that is categorised as broadly T_H1 with CD4 responses slightly dominant over CD8. Most T cells show an activated phenotype, but some studies also report increased expression of exhaustion markers such as PD1. It is important to find out if there are viral adaptations that cause a chronic activation profile and if this is hindering the formation of long-term T cell memory.

Despite many detailed studies of T cell subsets and their function, it remains unclear which subsets are more likely to be involved in protective or pathogenic immunity. While CD4 and CD8 T cells can be detected in most patients (CD4 more common), responses seem to be larger and cover more epitopes in more severe patients. A simple reason for this could be that a longer exposure to a higher viral load leads to priming of more T cells. Another, less trivial reason could be that big T cells responses may contribute to immunopathogenesis, such as lung damage.

Mapping epitopes

It has been a high priority to map SARS-CoV-2 epitopes targeted by T cells. Despite many difficulties and big diversity in approaches and cohorts, some common results are emerging. According to a number of studies, the most abundant responses are to spike proteins, followed by either membrane antigen or nucleocapsid. However, the antigen hierarchy in SARS-CoV-2 is more equally spread across antigens than in previously known coronaviruses. Limited available data suggest that there are some epitopes commonly recognized between individuals and between studies in different countries. These epitopes have to be further characterised in terms of presentation by specific HLA I and HLA II alleles. Findings from this

research will be useful for vaccine development, design of diagnostic tests of T cell immunity and determining COVID-19 risks in populations with specific HLA polymorphisms.

Cross-reactivity

Antibody studies have shown that there is cross-reactive immunity to some antigens of distantly related human coronaviruses and a significant proportion of blood donations obtained before the COVID-19 outbreak cross-react with SARS-CoV-2 S and M peptide pools. Also people with immune memory for SARS-CoV-1 have cross-reactive responses to SARS-CoV-2. It needs to be determined if this affects protection from disease and if this could be the reason for decreased susceptibility in school-age children because they are regularly exposed to common cold coronaviruses.

Correlation of B and T cell responses

Generally, people infected with SARS-CoV-2 make an antibody and a T cell response that are correlated in size, but T cell and B cell recognition can also become uncoupled. This is either because a mild infection elicited only T cell responses without a detectable antibody response or because the antibody response was transient and already decreased below detection limit while T cell memory is still robust. There are indeed people who lack an antibody response (and may never have been tested PCR+) but show strong T cell immunity against SARS-CoV-2. If measuring T cell immunity is more reliable than measuring antibody, commercial T cell testing kits might be of great value for diagnostic labs and in order to determine immunity levels in the population.

Cytokines

Numerous studies are in agreement that T cell responses to SARS-CoV-2 are overwhelmingly T_H1 and sometimes show a polyfunctional phenotype ($IFN\gamma^+$, $IL2^+$, $TNF\alpha^+$) that might be beneficial because severely ill ICU patience show a reduction in IFNγ and a shift to T_H2 responses. Considering this, it is clear that a successful vaccine should be able to elicit a protective cytokine profile, while not stimulating a TH2 response. While a lot of research has been focussing on cytokine storms as biomarkers for severity, it appears that this burst of cytokines is more likely to come from innate immunity rather than T cells.

In summary, a deeper understanding of T cell immunology will be indispensable for deciphering the pathogenesis resulting from COVID-19, especially considering that there are now many people suffering from long term damage caused by chronic, so-called “long-COVID”. While there was much focus on analysing antibody responses at the start of the pandemic, it is now clear that we also need to focus on T cells.

Activation of TLR7 and Innate Immunity as an Efficient Method Against COVID-19 Pandemic: Imiquimod as a Potential Therapy

Poulas, P. *et al*. 2020. *Frontiers in Immunology*

Link: https://doi.org/10.3389/fimmu.2020.01373

Poulas *et al*. discuss the concept of using Imiquimod (IQ) as a potential anti-viral drug against SARS-CoV-2. IQ is a synthetic immune modulator that triggers the innate immune system by binding to receptors on the cell surface, particularly TLR7. The ability of IQ to stimulate innate immunity highlights its potential as an anti-viral treatment. IQ-induced activation of TLR activates downstream signalling cascades that eventually promote synthesis of types I & III interferons (IFNs) and a range of other pro- inflammatory cytokines. For example, IQ is a potent TLR7 agonist and can induce IFNα, interleukin (IL)-6, IL-12 and tumour necrosis factor alpha (TNFα). These cytokines further stimulate and activate immune cells, including T-cells, NK cells & macrophages, and promote proliferation and differentiation of B-lymphocytes. IQ-induced activation of innate immunity allows it to indirectly activate the cellular arm of acquired immunity and stimulate T-helper cells type 1 (Th1) to produce IFNγ. In contrast, IQ suppresses the humoral arm of adaptive immune response through inhibition of Th2 cytokines (such as IL-4 and IL-5). IQ can further modify the immune response through activation of Langerhans' cells. Activated Langerhans' cells migrate to regional lymph nodes where they enhance antigen presentation to T-cells.

IQ is the only member of the imidazoquinolines family that is approved for clinical use due to its well-documented anti-viral/tumour properties. Topical IQ, in the form of 5% cream (Aldara), is approved for the treatment of genital/perianal warts. When applied topically, IQ is well-accepted with limited side-effects. It has a half-life of ~30h. Additionally, there have been a small number of studies that suggest systematic administration of IQ as an anti-viral treatment against HPV and HIV. Consequently, Poulas *et al.* propose repurposing/repositioning of IQ through systemic administration using compounding suppositories; each containing 6.25 mg of IQ. They express that they have clear evidence that IQ offers sufficient stimulation of innate and acquired immunity to help eliminate SARS-CoV-2 during early phases of infection. Therefore, Poulas *et al*. propose a clinical trial to assess IQ as a potential anti-SARS-CoV-2 drug.

Reviews

T cell responses in patients with COVID-19

Chen, Z. and Wherry, E.J. 2020. *Nature Reviews Immunology*

Link: https://doi.org/10.1038/s41577-020-0402-6

Summary:

Chen & Wherry discuss in detail the data available to date (both published & preprint) on the role of T cell responses following SARS-CoV-2 infection. The authors focus on some key aspects of the T cell response that are emerging; Lymphopenia, the $CD8^+$ T cell response, the $CD4^+$ T cell response, T cell differentiation, T cell memory and the strength of the T cell response.

Review Highlights:

- Reports suggest that severe Covid-19 patients exhibit either an insufficient or an extensive T cell response, resulting in either virus-mediated pathology or T-cell driven immunopathology.
- Lymphopenia is a hallmark of severe Covid-19, with reports of it affecting $CD4^+$ T Cells, B cells, NK Cells and preferentially $CD8^+$ T cells. Unlike other respiratory infections that exhibit transient lymphopenia, SARS-CoV-2 infection appears to induce a more persistent effect.
- Numerous studies have demonstrated an alteration in both the activation and differentiation of $CD8^+$ T cells during severe Covid-19, with increased expression of the inhibitory receptors, PD1, TIM-3, LAG3, CTLA4, NKG2A and CD39 reported. It is emerging that there appears to be heterogeneity in the $CD8^+$ response to SARS-CoV-2 and this may enable the identification of distinct patient immunotypes. Furthermore, studies from patients who have recovered from severe Covid-19 develop a memory population of SARS-CoV-2 specific $CD8^+$ T cells.
- Similar to the $CD8^+$ response, there is evidence of altered activation, suggesting exhaustion in $CD4^+$ T cells from patients with severe Covid-19. One report suggests that greater activation in patients $CD4^+$ T cells than the $CD8^+$ population defines a worse clinical outcome. Furthermore, a strong $CCR6^+$ $CD4^+$ signature may indicate a role for TH17 driven immunopathology.
- Severe Covid-19 appears to skew the T cell response towards either a hyper activation or an ineffective terminally differentiated state. High levels of systemic cytokines and chemokines (IL-6, CXCL8, CXCL9, CXCL10) and delayed or defective interferon responses are reported.

Journal Reviews

Vaccines

First-in-Human Trial of a SARS-CoV-2 Recombinant Spike Protein Nanoparticle Vaccine

Keech, C. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.05.20168435

Summary:

Following positive data from mice and baboons, Keech *et al*. report day-35 primary data from a randomised, placebo-controlled phase 1 clinical trial using the vaccine candidate NVX-CoV2373. NVX-CoV2373 is a recombinant nanoparticle constructed with trimeric spike glycoproteins of SARS-CoV-2. NVX was given at either 5 or 25 µg and in some was supplemented with 50 µg adjuvant (Matrix-M). The authors report a safe and tolerable vaccine that induces antibody responses which exceed those from convalescent plasma. T-cell responses are Th1 biased and adjuvant allows for dose sparing. NXV-CoV2373 is a promising candidate for the long-term protection of individuals from COVID-19.

Research Highlights:

1. Immunisation with 50 µg Matrix-M induced vast anti-spike IgG and neutralising antibody titres that were similar at both doses of vaccine (5 µg or 25 µg). By day 35, geometric mean titres of IgG were ~100-fold greater than those receiving vaccine alone.
2. Mean antibody titres exceeded those from convalescent serum by 4-fold (neutralising) to 6-fold (anti-spike IgG). Single vaccination induced levels comparable to convalescent serum from asymptomatic individuals.
3. A strong correlation between anti-spike IgG and neutralising antibody titres in those vaccinated with adjuvant (r=0.9466) was similar to that from convalescent sera.
4. Antigen-specific CD4+ T-cell responses to NVX-CoV2373/Matrix-M were predominately of Th1 phenotype (TNF-α, IFN-γ, and IL-2 production).
5. NVX-CoV2373 was well tolerated with mild reactogenicity, of short duration (≤ 2 days), recorded. Second vaccination resulted in greater reactogenicity, both local and systemic but there was no serious adverse events.

Impact for COVID-19 research:

- Positive data from a vaccine candidate will allow further development in future trials.

Methodologies:

- Study Type: *Phase 1 Clinical Trial (randomised, placebo-controlled), 131 healthy adults, 2 intramuscular injections 21 days apart*
- Key Techniques: *Spike protein serum IgG ELISA, SARS-CoV-2 Microneutralisation assay, polyfunctional CD4+ intracellular cytokine staining*

Limitations:

- Study only included healthy and young (<60) adults so safety and benefit of vaccine in most the vulnerable is unknown. Will be pursued in future work.
- T-cell responses were only measured from 16 participants.

Replication-Competent Vesicular Stomatitis Virus Vaccine Vector Protects against SARS-CoV-2 Mediated Pathogenesis in Mice

Case, J.B. *et al*. 2020. *Cell Host & Microbe*

Link: https://doi.org/10.1016/j.chom.2020.07.018

Summary:

Case *et al.* reports the efficacy of a Vesicular-Stomatitis virus (VSV), modified to contain the spike gene of SARS-CoV-2, as a vaccine strategy to induce protective immunity against SARS-CoV-2 in a mouse model of infection. The VSV vaccine approach offers an advantage over the adenovirus-vector (AdV) vaccines currently in human trials, as pre-existing immunity to VSV is negligible. Comparatively, much greater antibody titres are generated in response to VSV than AdV vaccination in preclinical testing. This study offers a promising proof-of-principle, but extensive further work is needed to determine its efficacy and safety profile.

Research Highlights:

1. VSV-SARS-CoV-2 vaccination induces high titre neutralizing Ab's, especially with a prime-boost strategy. Neutralizing Ab titres are reported to be higher than with other vaccine candidates during preclinical testing.
2. Passive transfer of sera from prime-boost vaccinated mice afforded protection to naïve mice from subsequent SARS-CoV-2 infection.
3. Following VSV-SARS-CoV-2 prime-boost vaccination, the levels of infectious virus at 4 dpi was negligible in the lungs and lung pathology at 8 dpi was minimal.
4. Prime-boost vaccination significantly reduces some pulmonary pro-inflammatory cytokine expression (INFβ, IFNλ, IL-6, and IL-1β) but not all (TNFα, IFNγ).

Impact for COVID-19 research:

- Whilst this is an early pre-clinical study, with a number of limitations, it does highlight the potential of VSV as a vaccine platform for SARS-CoV-2.

Methodologies:

- Study Type*: In-Vivo Mouse Model*
- Important cell lines/viral models used: Use of a replication-competent VSV that expresses a modified form of the SARS-CoV-2 spike (VSV-eGFP-SARS-CoV-2) as a vaccine approach.
- Key Techniques: Mouse model of SARS-Cov-2 infection, *in situ* hybridization of SARS-CoV-2, *in vitro* serum neutralization assays.

Limitations:

- The mouse models used include a number of manipulations following vaccination to enable SARS-CoV-2 infection. The feasibility of VSV as a vaccine strategy should be repeated solely with transgenic hACE2 expressing mice and non-human primates that reproduce the clinical observations of Covid-19, to determine efficacy.
- No clinical scoring was given following SARS-CoV-2 infection of mice, i.e. weight-loss etc.
- The study only examines the generation of Ab responses. T cell responses should be examined.
- By the authors own admission, the use of VSV as a vaccine has the potential for reactogenicity and neuronal infection, and that manipulation of the current VSV-eGFP-SARS-CoV-2 will be required should this arise.

Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques

Mercado, N.B. *et al*. 2020. *Nature*

Link: https://doi.org/10.1038/s41586-020-2607-z

Summary:

Single immunisation of a vaccine would greatly benefit global effort to combat COVID-19. The authors here immunised rhesus macaques with one of seven Ad26 vector-based vaccines which express SARS-CoV-2 S variants. Vaccination induced vast antibody titres with high neutralising capacity. T-cell responses were Th1 biased. Animals were protected from challenge 6 weeks post immunisation, particularly those given variant Ad26-S.PP, who had no viral load in BAL or nasal swabs. It is possible that single dose vaccination is sufficient to protect individuals from SARS-CoV-2, in which the response is dominated by neutralising antibodies (NAbs).

Research Highlights:

1. 4 weeks post immunisation, RBD-binding antibodies, and Abs with neutralising and various Fc effector functions were observed, as well as predominantly Th1 biased T-cell responses.
2. NAb titres inversely correlated with peak sgRNA levels in BAL and NS following challenge at week 6, and were most enriched in the completely protected animals suggesting they are primarily responsible for SARS-CoV-2 protection
3. Of all vaccine candidates, AD26-S.PP demonstrated the greatest immune responses and protection. In animals not vaccinated with AD26-S.PP, challenge induced high pseudovirus NAb, CD4+ and CD8+ T cell responses 14 days post challenge.

Impact for COVID-19 research:

- Positive data from a vaccine candidate, which is now entering clinical trials

Methodologies:

- Study Type: *in vivo, in vitro*
- Key Techniques: *Subgenomic mRNA assay, Systems serology, ELISPOT, neutralization assays using both pseudotyped and live virus*

Limitations:

- The longevity of Nab responses was limited to 6 weeks. Understand that future trials with longer duration are planned.
- Would be nice to compare single dose vs. booster regimen in the same study.

Diagnostics

Simply saliva: stability of SARS-CoV-2 detection negates the need for expensive collection devices

Ott, I. M. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.03.20165233

Summary:

Saliva has previously been shown to be a sensitive source of SARS-CoV-2 RNA but the current FDA-approved method of saliva-based mass testing is dependent on specialized, expensive collection tubes that contain buffers for stabilization and inactivation of the virus. The authors show evidence that SARS-CoV-2 RNA is stable at a variety of temperatures and for prolonged periods of time, when stores in sterile tubes without the use of such buffers. Data presented also suggested of little viral replication within stored saliva samples. Cost-effective saliva testing may benefit countries as they relieve their social restrictions, with less demand for expensive buffers.

Research Highlights:

1. SARS-CoV-2 RNA from saliva remained stable following freeze/thaw from -80 °C (Ct -1.058), being left at room temperature (~19 °C) for 5 days (Ct -0.960) and 3 days at 30 °C (Ct +0.973). Detection remained stable for up to 25 days at room temperature.
2. SARS-CoV-2 RNA in saliva samples remained stable for long periods of storage: -80 °C – 92 days, 4 °C – 21 days, 30 °C – 16 days.
3. SARS-CoV-2 in saliva demonstrated low infectivity. Whilst 11.6% of cells inoculated had increased SARS-CoV-2 RNA 72 hours later, plaque assays using these lysates resulted in no visual plaque forming units.

Impact for COVID-19 research:

- Paper cites and is supportive of other studies demonstrating the stability of SARS-CoV-2 in saliva, but also highlights others that disagree.
- May benefit mass testing, especially in countries with less resources.

Methodologies:

- Study Type: *in vitro*
- Important cell lines/viral models used: *Vero-E6 cells for infectivity studies.*
- Key Techniques: *RT-PCR using* 2019-nCoV N1 primer probe set*, Plaque assays.*

Limitations:

- Low number of samples used to draw conclusions.
- Samples only collected from one hospital – do collect from both inpatients and healthcare workers but do not discriminate between the two in their findings.
- Variation in the number of times samples were tested (some twice, others 4-5 times) was not explained.

Antibodies and T-cells

Neutralizing and binding antibody kinetics of COVID-19 patients during hospital and convalescent phases

Yao, X-Y. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.07.18.20156810

Summary:

Authors obtained serial blood samples from 34 COVID-19 patients to determine the lifetime of neutralising (Nab), total (Tab), IgM, IgG and IgA antibodies in parallel. Peak titres and times were determined and from this, the authors estimate the median times for each antibody to become seronegative – IgM (4.59 months), IgA (7.78 months) and IgG (42.72 months). Authors suggest that humoral immunity against COVID-19 may persist for some time.

Research Highlights:

1. Seroconversion occurred in all patients and antibody reversion was only observed for IgM in three patients.
2. IgM increased most rapidly after onset of illness followed by Tab, IgG and Nab. IgA titres increased the slowest.
3. IgM and IgA reached peak levels at similar times (~20 days) followed by IgG (~25 days).
4. IgM had the highest peak titre followed by Nab, Tab, IgG and IgA.
5. IgM levels declined fastest with a half-life of ~10 days, followed by IgA and IgG (~51 days and ~177 days respectively).

Impact for COVID-19 research:

- *The duration of IgG is estimated which is important for epidemiological control strategies and vaccination studies*

Methodologies:

- Study Type: *Cohort Study*
- Key Techniques: *Antibody binding and neutralisation*

Limitations:

- Small samples size of 34
- No asymptomatic cases and only 4 severe cases. The cases were not split; therefore, kinetics of severe cases may be masked by the mild cases.
- No consistency between patients in time point and number of blood collection

Distinct Early Serological Signatures Track with SARS-CoV-2 Survival

Atyeo, C. *et al*. 2020. *Immunity*

Link: https://doi.org/10.1016/j.immuni.2020.07.020

Summary:

Early in-depth profiling of SARS-CoV-2 antibodies, comparing 32 patients who recovered from COVID-19 and 30 who died. Patients were all sampled within 20 days of symptom onset and were from two independent hospitals. Patient antibody responses were heterogeneous but those who recovered had a more SARS-CoV-2 spike-protein (S) antibody response, whereas those who died had a more nucleocapsid (N) focused response. S:N antibody ratio could be informative for predict patient outcome, understanding disease progression and vaccine design/trials.

Research Highlights:

1. In-depth profiling of anti-SARS-CoV-2 antibodies, using Luminex multiplex assay.
2. Similar levels of neutralizing antibodies in both convalescent and deceased patients.
3. While heterogeneous, there were differences in antibody responses between groups highlighted by a co-correlate network.
4. Patients who recovered seemed to have more spike-related antibody (S) response rather than nucleocapsid specific response (N).
5. S:N ratio was also seen to be higher in convalescent patients in an independent cohort.

Impact for COVID-19 research:

- Early research suggesting that there is a more beneficial antibody response.
- Potential clinical benefit, **if** S:N antibody ratio is validated in larger cohort and is independent of age related changes to the immune system.

Methodologies:

- Study Type: Cohort Study
- Key Techniques: Custom multiplexed *Luminex assay was used to assess antibody characteristics.*

Limitations:

- Some overlap in S:N ratios in recovered/deceased patients attributed to heterogeneous antibody response and a number of confounding variables including varying treatments. .
- The deceased group were on average older, >70% of each cohort were of white ethnicity, and predominantly male (>66.6%) therefore do not know if the S:N applies to all COVID-19 patients.

Kinetics of viral clearance and antibody production across age groups in SARS-CoV-2 infected 2 children

Bahar, B. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.06.20162446

Summary:

The study contributes to a better understanding of the timing of viral clearance and antibody production in children with COVID-19. Although there is emerging data regarding timing of viral clearance and immunological response in adults with COVID-19, there is little data published in the paediatric population. A lack of knowledge regarding factors affecting time-to-seropositivity is observed in both paediatric and adult patients and this study starts to address this gap in knowledge.

Research Highlights:

1. Median duration of viral shedding was 19.5 days and RT-PCR negative result from a previously positive result was 25 days.
2. The 6 to 15 years cohort demonstrated a longer period until viral clearance was achieved and confirmed by RT-PCR, which was on average greater in females within this age range.
3. Median time to seropositivity (from RT-PCR positive result) was 18 days while median time to reach adequate levels of neutralizing antibodies was 36 days.
4. Only 17 of 33 patients demonstrated neutralizing antibodies, suggesting that some patients may not mount significant immune responses to infection.
5. Demonstrated that IgG class antibodies directed against S1 and S2 glycoproteins could be detected in blood samples of children prior to viral clearance.

Impact for COVID-19 research:

- The study's strength is the inclusion of patients from multiple paediatric ages, which allowed comparison between age groups and sex. Results of serial testing in paediatric patients exclusively is rarely reported.
- This research is unlikely to benefit clinicians greatly.

Methodologies:

- Study Type: *Cohort study*
- Key Techniques: *GenMark ePlex SARS-CoV-2 Test, DiaSorin Molecular Simplexa™ COVID-19 Direct Assay System, Cepheid Xpert® Xpress-SARS116 CoV-2, Seegene Allplex™ 2019-nCoV RT-PCR Assay and DiaSorin Liaison XL SARS-CoV-2 IgG S1/S2 assay.*

Limitations:

- The study was carried out in retrospect and data was not collected at defined time intervals.
- By the authors own admission the serologic assay used in the study had 94.3% positive and 100% negative agreement with a comparative enzyme-linked 296 immunosorbent assay.

- Does not provide any evidence if the duration of viral shedding is related to infectivity and transmission.

Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications

Mathew, D. *et al.* 2020. *Science*

Link: https://doi.org/10.1126/science.abc8511

Summary:

Mathew *et al.* studied a group of 125 COVID-19 patients and 37 healthy controls, using high dimensional flow cytometry to perform deep immune profiling of T and B cell subsets in their peripheral blood. By performing an integrated analysis of 200 immune and 50 clinical features, they demonstrate the heterogeneity of the immune response in patients hospitalised with COVID-19 and identify three immune signatures associated with poor clinical trajectories versus improving health.

Research Highlights:

1. Many COVID-19 patients displayed robust $CD8^+$ cell and/or $CD4^+$ cell activation and proliferation and plasmablast responses, however 20% of patients had minimal detectable response compared to controls
2. Identification of three immunotypes: 1. patients with robust activation and proliferation of $CD4^+$ cells, relative lack of cTfh, modest activation of TEMRA-like; highly activated or exhausted $CD8^+$ cells; signature of T-bet^+ plasmablasts. 2. $Tbet^{bright}$ effector-like $CD8^+$ cell responses, less robust $CD4^+$ cell responses, and $Ki67^+$ plasmablasts and memory B cells; 3. an immunotype suggesting a failure of immune activation, by largely lacking detectable lymphocyte response to infection.
3. COVID-19 patients presented a robust plasmablast response, although it was not correlated with blood activated cTfh responses.
4. Lymphopenia preferential for $CD8^+$ cells was observed in many patients, although a stable $CD8^+$ and $CD4^+$ cell activation and plasmablast responses was observed during COVID-19.
5. Building of an integrated computational model that connects patient immune response phenotype to disease severity, by integrating 200 immune features with extensive clinical data, disease severity scores and temporal changes.

Impact for COVID-19 research:

This work helps to better understand the immune response to SARS-CoV-2, as well as correlate with clinical symptoms and the pathogenesis of the disease. It is also one of the first studies performing deep immune profiling on a large COVID-19 patient cohort.

Methodologies:

- Study Type: Clinical cohort study
- Key Techniques: *High dimensional flow cytometry (27-parameter flow cytometry) and Luminex*

Limitations:

- It is not clear which patients were included in the Luminex analysis, as well as in the follow up study on day 7.
- Not clear how long after recovery from COVID-19 patients were sampled.
- Other immune cell types and circulating inflammatory mediators could be included in the computational model.

SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls

Le Bert, N. 2020. *Nature*

Link: https://doi.org/ 10.1038/s41586-020-2550-z

Summary:

Patients recovered from SARS (2003) were found to have long-lasting memory T-cells with cross-reactivity to SARS-CoV-2 nucleoprotein (NP). SARS-CoV-2 specific T-cells were also discovered in patients with no history of SARS, COVID-19 or known contact with patients. These healthy donors had T-cells specific to both NP and non-structural SARS-CoV-2 epitopes. These epitopes showed low homology to common cold human coronaviruses, but were conserved amongst animal betacoronaviruses. This suggests betacoronavirus infection can induce long-term T-cell immunity against SARS-CoV-2 infection.

Research Highlights:

1. All COVID-19 recovered patients had IFNγ T-cell responses against SARS-CoV-2 NP peptides, but few had T-cell responses against non-structural peptides (NSP7, NSP13) conserved between betacoronaviruses.
2. COVID-19 recovered patients T-cells recognised epitopes which are previously described CD4 and CD8 T-cell epitopes from the NP region of SARS-CoV-1. This suggests a T-cell cross-reactivity between SARS-CoV-1 and SARS-CoV-2.
3. SARS (2003) recovered patients were found to still have IFNγ T-cell responses to SARS-COV-1 NP epitopes (17 years after infection), but not to non-structural epitopes of the virus. These patients T-cells also expanded in response to SARS-CoV-2 NP peptides, inferring a cross-reactivity. This also suggests COVID-19 patients will have a long-lasting T-cell immunity.

4. SARS-CoV-2 specific IFNγ T-cell responses were found in 19 out of 37 healthy donors, reacting to both NP and non-structural peptides. Some of the epitopes which these T-cells reacted to had minimal homology to other common cold coronaviruses.
5. Further characterization of specific unexposed donors identified T-cells reactive to the same NP epitopes which were also found to react with T-cells in COVID-19 and SARS recovered patients. 3 healthy individuals had SARS-CoV-2 reactive CD4 and CD8 T-cells which efficiently expanded *in vitro*, some of which were found to be effector memory differentiated.

Impact for COVID-19 research:

- These findings suggest COVID-19 patients will have long-lasting T-cell immunity to SARS-CoV-2 and that there is cross-protection between betacoronaviruses. This has implications for public health strategies and vaccine design.

Methodologies:

- Study Type: *Cohort study*
- Key Techniques: *IFNγ ELISpot assay, intracellular cytokine staining (ICS)*

Limitations:

- T-cells against spike protein epitopes not studied here.
- T-cells were sampled from patient PBMCs, not directly in the area of infection (e.g. BAL).

Metabolism and Innate Responses

Elevated Glucose Levels Favor SARS-CoV-2 Infection and Monocyte Response through a HIF-1α/Glycolysis-Dependent Axis

Codo, A.C. 2020. *Cell Metabolism*

Link: https://doi.org/10.1016/j.cmet.2020.07.007

Summary:

Diabetic individuals are prone to the severe form of SARS-CoV-2 with uncontrolled blood glucose levels associated with worse prognosis. The authors suggest that increased blood glucose promotes viral replication and pro-inflammatory cytokine production by monocytes which drives T cell dysfunction and potentially damages lung epithelial cells. The authors propose that targeting the mtROS/HIF-1α/glycolysis axis could be beneficial to treat SARS-CoV-2 infection.

Research Highlights:

1. SARS-CoV-2 could infect human monocytes *ex vivo*. Infected monocytes showed increased ACE2 expression and increased production of IFNα, β, and λ and pro-inflammatory TNF-α, IL-1β and IL-6
2. *In vitro*, glucose increased viral load, ACE2, and IL-1β expression in CoV-2-infected monocytes in a dose-dependent manner
3. Monocytes increased glycolysis in response to SARS-CoV-2 but not human influenza A H1N1 virus or respiratory syncytial virus.
4. Inhibition of glucose flux blocked viral replication in CoV-2-infected monocytes and reduced levels of inflammatory cytokines
5. Blood monocytes from severe patients had increased HIF-1α levels and HIF-1α inhibition *ex vivo* blocked viral replication, AEC2 expression and pro-inflammatory cytokine production by monocytes
6. Media conditioned from CoV-2 infected monocytes impaired CD4 and CD8 T cell activation, increased PD-1 expression in CD4 cells and induced apoptosis of A549 pulmonary epithelial cells

Impact for COVID-19 research:

- This research does not necessary alter our view of SARS-CoV-2 but provides additional insight. If findings are confirmed *in vivo*, there is a potential for clinical application.

Methodologies:

- Study Type: e.g. *in vitro/ex vivo*
- Important cell lines/viral models used: use of primary human monocytes, lymphocytes and A549 pulmonary epithelial cells, HIAE-02 SARS-CoV-2/SP02/human/2020/BRA (GenBank: MT126808.1) virus

- Key Techniques:
    - analysis of publicly available single-cell RNA sequencing (RNA-seq) data from bronchoalveolar lavage of mild and severe COVID-19 patients
    - ex vivo culture and assays of human monocytes, lymphocytes and SARS-CoV-2 virus

Limitations:

- While the authors show that SARS-CoV-2 can infect monocytes *ex vivo*, it is not clear whether and to which extent the virus does infect monocytes *in vivo*.
- The study also only investigates metabolic changes in monocytes and not other cell types
- The study focusses on high glucose environments, if diabetes is well controlled and blood sugar levels are not high, is the susceptibility of patients comparable to healthy individuals?

Mouse Model of SARS-CoV-2

Comparison of Transgenic and Adenovirus hACE2 Mouse Models for SARS-CoV-2 Infection

Rathnasinghe, R. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.07.06.19006

Summary:

Rathnasinghe, R *et al.* compare viral replication and morbidity in the K18 transgenic hACE2 model head-to-head with adenovirus-mediated delivery of hACE2 to the lung. Although this study do not differ with the already published data, it gives a general view of the advantages and disadvantages of using different mouse models. This study provides useful information in deciding which model to apply to specific research hypothesis. Depending on which mouse model, the knock in (K18-hACE2) or the Ad-hACE2 (BALB/C and B6), is considered, the outcome might vary.

Research Highlights:

1. K18-hACE2 and Ad-hACE2 murine model support SARS-CoV-2 viral replication.
2. K18-hACE2 model result in more severe disease
3. K18-hACE2 showed high viral titers in brain although no sign of it in the Ad-hACE2 models

Impact for COVID-19 research:

- According to the results obtained, mouse model is of highly importance as depending on which type of animal model is used in the study this might affect the results obtained.

Methodologies:

- Study Type: *in vivo.*
- Important cell lines/viral models used*: **Virus:** non-replicating E1/E3 deleted viral vector based on Ad5 without antigen or expressing the ACE2 receptor under a CMV promoter. **Mice**: B6 and Balb/C mice and the knock in B6-K18-hACE2 mice.*
- Key Techniques: *Virus administration via intranasally (i.n) with the indicated dose (pfu) of virus. Mice were euthanized by CO2 and pentobarbital injection. H&E and hACE2 staining for adenovirus-mediated delivery of hACE2.*

Limitations:

- In this report they consider pfu for infection which means that the number of viral particle might differ (usually is higher than the pfu).
- They use CO2 to sacrifice the animals. CO2 might cause damage to the lungs which may alter the IHC stain.

- No records of the differences in their immune system. An important aspect to be considered is how the immune system responds to a virus infection and how similar it is to human immune response.