COVID-19 Community Journal Club

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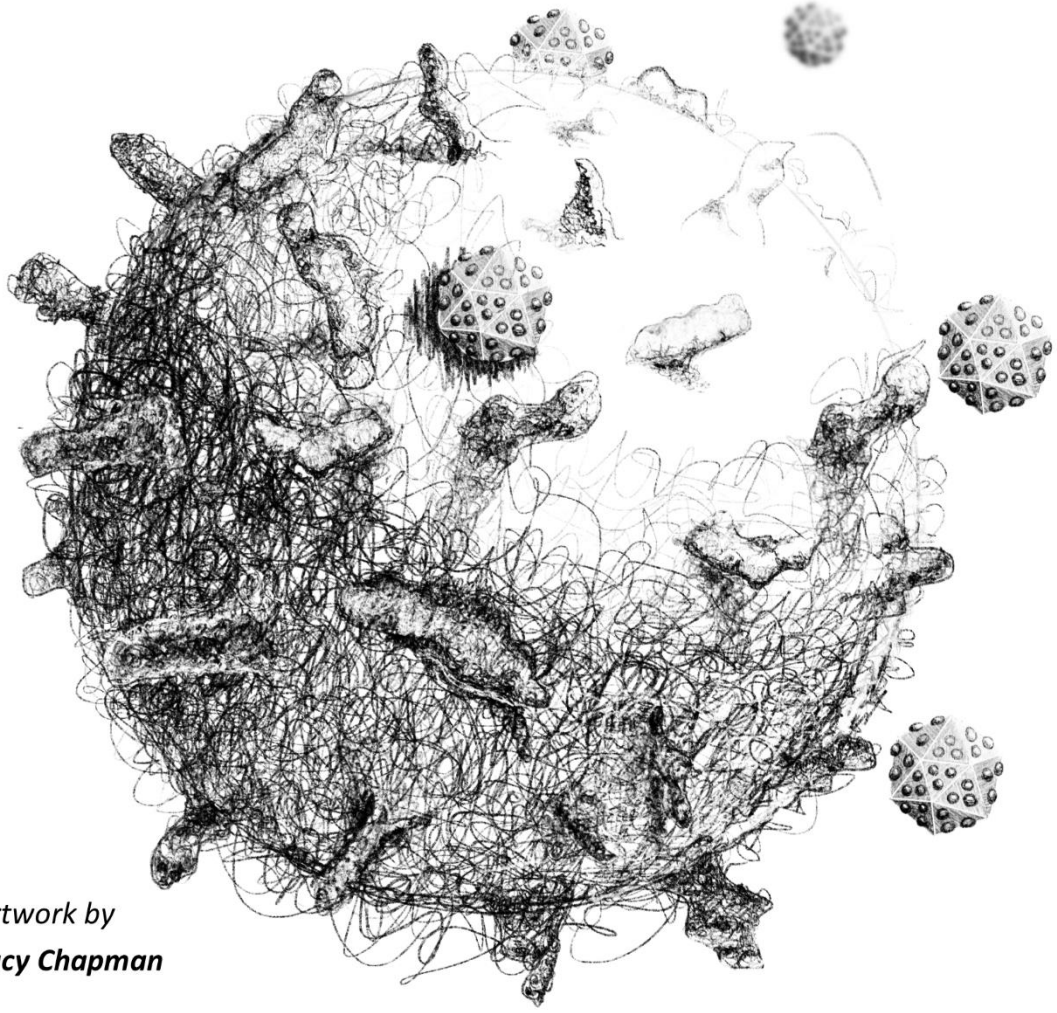

Artwork by
Lucy Chapman

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

Cardiff University Community Journal Club has recently joined forces with University of Oxford's COVID-19 Literature Initiative run by Felix Richter and Professor Katja Simon. Thus, members of both universities have contributed reviews to this edition.

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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 PRE-PRINTS POSTED ONLINE
(in *arXiv, bioRxiv, medRxiv and Research Square) BEFORE* PEER REVIEW.

Antibodies

High titers of multiple antibody isotypes against the SARS-CoV-2 spike receptor-binding domain and nucleoprotein associate with better neutralization

Noval, M.G. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.15.252353

Summary:

Sera from 101 COVID-19 recovered healthcare workers were analysed for their neutralising capacity against both authentic and pseudotyped SARS-CoV-2 virus. 6% had high neutralising titres, with 75% of samples having low neutralisation capacity. Antigen-specific antibodies were identified in response to SARS-CoV-2 infection, with high IgG, IgM and IgA titres against the receptor-binding domain (RBD) and nucleoprotein (N) epitopes of the virus. Neutralising capacity correlated with antibody titres, with the highest neutralising sera having antibodies against the RBD and N epitopes in all antibody isotypes (IgG, IgM, IgA). This suggests a robust antibody response is required for effective virus neutralisation in COVID-19 infection.

Research Highlights :

1. Convalescent sera from 101 SARS-CoV-2 PCR-positive, recovered healthcare workers were tested for virus neutralisation capacity. 75% had low neutralisation, 20% intermediate and 5-6% had high neutralisation of authentic virus. This finding was regardless of time-point of sera collection (day 32-57 post-symptom onset).
2. Both IgG and IgM antibody isotypes had equal responses to RBD and N antigens, whereas IgA was skewed towards RBD epitopes. However, there was a correlation between time post-symptom onset and antigen-specific antibody isotype titres found.
3. Anti-RBD isotype titres correlated positively with neutralisation capacity of authentic SARS-CoV-2, however, IgG titres correlated best with pseudotyped virus neutralisation. Anti-N isotype titres had weaker correlations for neutralisation of both authentic and pseudotyped virus, with the exception of IgG isotypes.
4. The highest neutralising sera had higher titres of IgG, IgM and IgA against both RBD and N antigens. With the strongest correlations between anti-RBD antibodies, and those with the highest titres for all antibody isotypes.
5. 4 of the 6 samples with the highest neutralising capacity tested positive for antibodies against RBD and N epitopes for all isotypes; IgG, IgM, IgA. This suggests having high titres of multiple isotypes of antibodies results in better virus neutralisation.

Impact for COVID-19 research:

- Understanding antibody responses to COVID-19 implicates vaccine design and helps to understand long-lasting memory to the virus. Also, helps understand seroprevalence within the population.

Methodologies:

- Study Type: *Cohort study.*
- Important cell lines/viral models used*: Vero E6 cells for authentic neutralisation assay, ACE2-293T cells for pseudotyped neutralisation assay.*
- Key Techniques: *SARS-CoV-2 RBD and nucleoprotein ELISA's, pseudotyped lentivirus neutralisation assay, authentic SARS-CoV-2 neutralisation assay.*

Limitations:

- No comparison of patient sera neutralising capacity to their disease severity. Would be interesting to know if those patients with the highest neutralising antibodies had milder/ more severe infections.

Pre-COVID-19 humoral immunity to common coronaviruses does not confer cross-protection against SARS-CoV-2

Miyara, M. *et al.* 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.14.20173393

Summary:

By analysing the serum of 76 healthy donors from 2015, Miyara *et al*., demonstrate the widespread presence (ca 8%) in the COVID19-naive population of cross-reactive antibodies against SARS-Cov-2. These antibodies were mainly against Spike, the spike S2 subunit, and nucleocapsid proteins, and no reactivity against the SARS-CoV-2 Receptor Binding Domain was detected in the healthy donors or in pooled intravenous immunoglobulins (IVIG). None of the pre-existing antisera had in vitro neutralizing activity against SARS-Cov-2, possibly because they fail to target RBD. Also, the serum of seven out of eight severe COVID19 patients analysed had pre-existing antibodies against other coronaviruses suggesting that these do not provide protection against severe SARS-Cov-2 infection

Research Highlights:

1. A substantial percentage of the population has pre-existing antibodies against coronavirus
2. Pre-existing antibodies do not bind SARS-CoV-2 RBD
3. Pre-existing antibodies fail to neutralize SARS-Cov-2 in vitro
4. Pre-existing antibodies do not protect against severe COVID19 infection

Impact for COVID-19 research:

- This study shows how IVIG prepared before the SARS-Cov-2 pandemic would not be useful to fight the disease but, in pooled IVIG, even a small amount of convalescent patient's serum is enough to neutralize the virus in vitro.

- The study indicates how the serological tests that look at previous SARS-Cov-2 infection should focus on the anti-RBD antibodies to avoid false positives

Methodologies:

- Study Type: in vitro, patient samples
- Important cell lines/viral models used:
- Key Techniques: *neutralisation assay*

Limitations:

- The study fails to identify which are exactly the antibodies found in the population that are cross-reactive for SARS-Cov-2. This could be done by isolation of individual B cell clones reactive with SARS-CoV-2 antigens. Because of this they cannot exclude that specific clones found in the healthy population could neutralize SARS-CoV-2 and help fight the infection, although they show that none of the pooled healthy donor samples can neutralize the virus in vitro.
- They fail to acknowledge that cross-reactive antibodies from a different coronavirus had been found to be neutralizing (https://www.biorxiv.org/content/10.1101/2020.05.14.095414v1)

T-cells

Ex vivo detection of SARS-CoV-2-specific CD8+ T cells: rapid induction, prolonged contraction, and formation of functional memory

Schulien, I. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.13.249433

Summary:

Schulein *et al.* defined a set of immunodominant CD8+ T-cell epitopes that were targeted in the majority of tested convalescent individuals of a Caucasian cohort after mild SARS-CoV-2 infection. The analyses revealed that a functional immune response is generated by both, pre-existing and newly induced virus-specific CD8+ T cells, irrespective of the targeted viral protein and the HLA restriction. Competent SARS-CoV-2-specific CD8+ T-cell memory is composed of heterogeneous subsets, e.g. T_{CM}, T_{EM1}, T_{EM2} and T_{EM3}, required for a flexible response upon re-infection. The authors also established experimental tools for high resolution ex vivo analyses of SARS-CoV-2-specific CD8+ T cells. This understanding of what underpins a functional memory response is important for vaccine development.

Research Highlights:

1. Immunodominant SARS-CoV-2-specific CD8+ T-cell epitopes are targeted in the majority of individuals with convalescent SARS-CoV-2 infection.
2. MHC class I tetramer analyses revealed the emergence of phenotypically diverse and functionally competent pre-existing and newly induced SARS-CoV-2-specific memory CD8+ T cells that showed similar characteristics to influenza-specific CD8+ T cells.
3. SARS-CoV-2-specific CD8+ T-cell responses are rapidly induced within a week of infection followed by a long contraction phase which outlasts the humoral immune response (upper detection limit of antibodies was 84 days when CD8+ T cells remained detectable at this time and later timepoints).
4. CD8+ T-cell responses might serve as a more precise correlate of antiviral immunity than antibody measurements after convalescence.
5. Majority of identified SARS-CoV-2-specific CD8+ T-cell epitopes that were dominantly targeted in convalescent individuals with mild SARS-CoV-2 infection, show little evidence for cross-recognition in SARS-CoV-2-naïve individuals.

Impact for COVID-19 research:

- Importance for detection of memory and vaccine development

Methodologies:

- Study Type: *Ex-vivo PBMC analysis using MHC I tetramers*
- Key Techniques: *Flow cytometry, CyTOF for differentiation trajectories, mass cytometry*

Limitations:

- Relatively small sampling size of healthy donors and SARS-CoV-2 infected individuals, especially when taking into account their different HLA types.
- Caucasian cohort (although sampled globally).
- Only those with a mild infection were sampled and the parameters for defining a mild infection were not discussed.

Suboptimal SARS-CoV-2-specific CD8+ T-cell response associated with the prominent HLA-A*02:01 phenotype

Habel, J. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.17.20176370

Summary:

Habel *et al.* identified two novel HLA-A*02:01-restricted SARS-CoV-2 epitopes (A2/S_{269} and A2/Orf1ab$_{3183}$) in individuals with COVID-19 using a combination of peptide prediction and *in vitro* peptide stimulation. The authors determined the frequency and activation profiles of the SARS-CoV-2-specific $CD8^+$ T-cell response in acute and convalescent COVID-19 patients and uninfected individuals. The more prominent A2/S_{269} CD8+ T cell population was equivalent to that seen in acute or convalescent patients positive for HLA-A*02:01. However, although the $CD8^+$ T cell population was higher than in uninfected HLA-A*02:01-positive donors, it was lower compared with the populations that are typically generated in response to other viral epitopes from with influenza (A2/M_{158}) and EBV (A2/$BMLF_{1280}$) viruses and were negative to activation markers. These data suggest that HLA*02:01 restricted virus-specific $CD8^+$ T were both at low prevalence and express a less than optimal (for virus elimination) phenotype.

Research Highlights:

1. The authors identified two novel HLA-A*02:01-restricted SARS-CoV-2 epitopes (A2/S_{269} and A2/Orf1ab$_{3183)}$ in individuals with COVID-19
2. $CD4^+$ T Cell responses to SARS-CoV-2 infection is similar to that of other viral infections
3. $CD8^+$ T cells which target SARS-CoV-2 presented by the HLA-A802:01 are lower in number than those of other viral infections and are of a phenotype which is suboptimal for viral clearance
4. Although 'early memory' CD8+ T-cells from convalescent HLA-A*02:01 COVID-19 patients were 5-fold higher than those from pre-pandemic samples, the SARS-CoV-2-specific response was 10-fold lower.

Impact for COVID-19 research:

Understand the immune response to SARS-CoV-2/COVID19

- Although there is a robust CD4+ T Cell response to SARS-CoV-2 infection, the SARS-CoV-2 CD8+ T Cells are suboptimal. This is in line with previous publications by the same research group which found CD4+ T-follicular helper cells and activated CD38+ HLA-DR+ CD8+ T-cells are present in the blood of patients with COVID-19. Although the mechanisms underlying this suboptimal CD8+ T cell response remain unknown, this presents further insight into correlates of protection which currently remain uncertain.
- The fact that this T cell response is compared to two other viruses suggests that SARS-CoV-2 actively controls the observed suboptimal T cell response.

Develop a vaccine for SARS-CoV-2/COVID19

- Although this is not the primary aim of the paper, the findings that CD8+ T cells responses in SARS-CoV-2 infected and convalescent patients are suboptimal leads the authors to conclude that an appropriately designed vaccine might elicit a higher protective CD8+ T cell recall response than that which results from a natural infection.

Methodologies:

- Study Type: *in vitro and ex vivo*
- Important cell lines/viral models used: *PBMCs from SARS-CoV-2 patients and healthy donors. Viral models were SARS-CoV-2, influenza A and Epstein-Barr virus.*
- Key Techniques: *peptide prediction, in vitro peptide stimulation, flow cytometry.*

Limitations:

- Further investigation is needed to identify CD8+ T cell epitopes across many HLA class I alleles and SARS-CoV-2 proteins and better determine the CD8+ T-cell responses and their activation profiles in COVID-19.
- Comparisons of T cell responses would be better in closely related viruses (e.g. SARS-CoV or MERS-CoV), the choice of comparing results with influenza and Epstein-Barr virus is not entirely justified in the paper.
- It is uncertain if these results are representative of CD8+ T cells across other HLA class I alleles; however, the authors do acknowledge this limitation and discuss and justify these shortcomings. Indeed, all the findings presented within this paper are analysed and justified within the context of the broader literature surrounding SARS-CoV-2 and should be commended for this.
- Figure 4 reports that approximately 70% of CD8+ T cells are positive for intracellular granzyme staining in acute and convalescent COVID-19 patients; this appears unusually high and requires further investigation. The authors do rationalise this by suggesting a bystander effect activation; however, this seems important to clarify, although this may be beyond the scope of this publication.
- Minimal evidence reported in the study for the comparison of the SARS-CoV-2 specific T cells restricted to HLA2 in group of patients with different disease outcome (i.e sever vs mild infection) to explore whether the frequency of the activation profile is associated with disease severity.

Vaccines

RNA-Based COVID-19 Vaccine BNT162b2 Selected for a Pivotal Efficacy Study

Walsh, E.E. *et al.* 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.17.20176651

Summary:

The BioNTech/Roche RNA vaccine candidate is currently in phase III trials. In this preprint, the companies present new Phase II data for their RNA vaccine candidates showing that a secreted trimerized SARS-CoV-2 receptor-binding domain (BNT162b1) and prefusion membrane-anchored SARS-CoV-2 full length spike protein (BNT162b2) show high antibody titer production comparable to convalescent samples. However BNT162b2 shows an increased safety profile with less adverse effects in older adults. Neither of the two vaccine candidates seem to be able to improve age-related antibody decrease during vaccination in comparison to young adults. More promising though is that even in the old cohorts, overall antibody titers were comparable or higher than in convalescent patient sera.

Research Highlights:

1. BNT162b2 RNA vaccine candidate shows slightly better safety profile (less swelling, less redness and injection site pain, less fevers and chills (especially in old adults))
2. Both RNA vaccine candidates elicit antibody responses comparable or even improved to convalescent antibody titers
3. Old individuals (65+) remain having lower antibody titers than young adults but comparable to convalescent

Impact for COVID-19 research:

- This project is crucial for the safety and development of new vaccine candidate against SARS-CoV-2.

Methodologies:

- Study Type: *Clinical Trial Phase I/II*
- Important cell lines/viral models used: *Human test subjects given vaccine candidates, no SARS-CoV-2 was used for this study*
- *Key Techniques: Self-reported health assessment to evaluate side effects, SARS-CoV-2 serum neutralization assay and S1- or RBD-binding IgG Luminex for serological analysis.*

Limitations:

- Not all experimental groups have been well balanced (some are off for sex)
- Small group of placebo (n=3 per subgroup)
- Unclear whether any person has been excluded due to natural COVID infection (and whether they have monitored this).

NVX-CoV2373 vaccine protects cynomolgus macaque upper and lower airways against SARS-CoV-2 challenge

Guebre-Xabier, M. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.18.256578

Summary:

In this short communication, the authors examine the efficacy of Novavax's subunit vaccine candidate NVX-CoV2373 with adjuvant Matrix-M1™ in cynomolgus macaques. Immunisation induced antibody titres that could inhibit hACE2 interaction and neutralise live virus. Responses were greater than those seen in patient samples. Vaccination also protected animals from challenge in both the upper and lower airways. This study adds value to the vaccine candidate, which is entering clinical trials.

Research Highlights:

1. Compared to patient convalescent plasma, prime-boost NVX-CoV2373 elicits greater titres of anti-S IgG, live-virus neutralising antibodies and hACE2-inhibiting antibodies. Responses were between 5-14 fold different.
2. Subgenomic RNA was undetectable in BAL and nasal fluids of immunised macaques 4 days post challenge, indicating no viral replication.
3. Little to no inflammation in the upper and lower airways was observed 7 days post challenge of immunised animals, compared to moderate-severe inflammation observed in placebo controls, including perivascular mononuclear infiltrate within the alveoli.

Impact for COVID-19 research:

- Adds value to previous research on the vaccine candidate, which is entering clinical trials

Methodologies:

- Study Type: *in vitro, in vivo*
- Important cell lines/viral models used: *SARS-CoV-2 (WA-1, 2020) strain, Vero E6 cells for neutralising assays*
- Key Techniques: *anti-spike IgG ELISA, RT-PCR using Envelope sgRNA*

Limitations:

- Conclusions of sgRNA from nasal swabs may be limited by the fact that half of controls also had no detectable sgRNA.

A single dose of an adenovirus-vectored vaccine provides protection against SARS-CoV-2 challenge

Wu, S. *et al*. 2020. *Nature Communications*

Link: https://doi.org/10.1038/s41467-020-17972-1

Summary:

Wu *et al.*, evaluate the protective efficacy of the mucosal vaccination route, in addition to the normal intramuscular vaccination route. Demonstrating that a single mucosal inoculation of Ad5-nCoV can protect the upper and lower respiratory tracts against SARS-CoV-2. For what they study the induction of antibodies, T-cell responses in wild type BALB/c mice (mouse-adapted model) and ferrets.

Research Highlights:

1. Ad5-nCoV induce humoral and cellular immune responses at week 2 p.i
2. Complete protection for the upper and lower respiratory tracts against SARS-CoV-2 infection can be achieved using a single mucosal inoculation of Ad5-nCoV in mice.

Impact for COVID-19 research:

- Although this Ad5-nCov vaccine is already in clinical trials, it shows the benefits of intranasal infection in comparison to intramuscular vaccination route. Highlighting the importance of the vaccination route that should be considered in human clinical trials for the development of vaccines.

Methodologies:

- Study Type: *In vivo Mouse and Ferret model*
- Important cell lines/viral models used: *Virus used is a mouse-adapted SARS-CoV-2/HRB26/human/2020/CHN (HRB26M, GISAID access no. EPI_ISL_459910) which was generated by passaging the human patient isolate in 4-6 week old female mice for 14 passages. Ad5-nCoV consist in a deficient replicative adenovirus which contains a codon optimized version of the full spike protein gene of SARS-CoV-2 based on the Wuhan-Hu-1 strain.*
- Key Techniques: *intramuscular and intranasal vaccination of mice and ferrets with Ad5-nCov-2. Neutralization assay of sera from mice; ELISpot, cellular immune responses in ferrets; cytokine staining of spleen.*

Limitations:

- In this study they used a mouse-adapted SARS-CoV-2 (MASC) which is not the Wuhan strain. Although it's able to infect mouse the mutation MASC contains affect to the RBD section of the spike protein.
- The number of animals used is low. Whilst the n=10 per group as there are different conditions the groups are reduced (from 3-4 mice see Fig.2)
- As mention by the authors, intranasal vaccination and some virus (such as adenovirus) can cause asthma. There is no data about this might have occurred.

The recombinant subunit vaccine RBD-Fc, consisting of SARS-CoV-2 RBD and human IgG Fc as an immunopotentiator, elicits robust neutralizing antibody responses against SARS-CoV-2 infection

Liu, Z. *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-61074/v1

Summary:

The receptor binding domain (RBD) is vital for eliciting SARS-CoV-2 infection. Vaccines that can recognize this region and mount an immune response against it may be of global benefit. 3 immunisations with recombinant subunit vaccine RBD-Fc in mice induced strong RBD-specific antibody responses that were able to block virus-hACE2 interaction, neutralize pseudotyped and live infections and provide protection from challenge. Antibodies induced by RBD-Fc are able to cross-react with related coronaviruses but recognize conformational, rather than linear epitopes. RBD-Fc is one of many potential vaccine candidates that are continuing development.

Research Highlights:

1. Immunisation induced strong IgG responses that were RBD-specific and able to neutralise both pseudotyped and live SARS-CoV-2 infection. Antisera was able to significantly block RBD-hACE2 interaction and S-mediated cell-cell fusion.
2. hACE2 transgenic mice immunised with RBD-Fc were protected from infection, with no detectable viral RNA in the lungs seen 4 days post challenge.
3. RBD-Fc can induce significant cross-neutralising IgG responses to pseudotyped SARS-CoV and bat-SARSr-CoV virus.
4. Insertion of single natural mutations in the RBD of SARS-CoV-2 had no effect on the neutralising capacity of mouse antisera.
5. 4 linear epitopes from SARS-CoV-2 RBD that could greatly bind mouse antisera were unable to block antisera neutralizing activity and couldn't effectively inhibit pseudotyped infection with SARS-CoV-2, indicating that neutralizing antibodies induced by RBD-Fc recognize conformational epitopes.

Impact for COVID-19 research:

- Characteristics of antibody responses (neutralising capacity, hACE2 blocking) are similar to other vaccine candidates, indicating a consensus. The inclusion of epitope recognition will further guide vaccine designs.

Methodologies:

- Study Type: *in vitro, in vivo*
- Important cell lines/viral models used: *hACE2 transgenic mouse model, multiple cell lines used.*
- Key Techniques: *ELISAs for RBD-Fc interaction, subcutaneous injection for vaccination, challenge intranasally*

Limitations:

- Some figures are incorrectly referred to in text
- Does not greatly consider other domains of SARS-CoV-2 (e.g. S2, which is needed for virus fusion)

Sampling SARS-CoV-2 proteomes for predicted CD8 T-cell epitopes as a tool for understanding immunogenic breadth and rationale vaccine design

Hare, J. *et al.* 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.15.250647

Summary:

In silico prediction was applied to 287 SARS-CoV-2 proteomes to maximise global peptide coverage. The proteome sequences came from South America, Asia, Europe and USA. 11,267 unique peptides binding to a range of HLA-A, HLA-B and HLA-C alleles were identified. The largest protein 'contributors' of peptides were the spike and ORF1ab proteins. However, other studies have carried out similar analysis, in which novel decision-making pathways for comprehensive evaluation and prioritization of potential epitopes were developed (Yarmarkovich *et al*., 2020, Smith *et al*., 2020.)

Research Highlights:

1. Peptide prediction from 8 SARS-CoV-2 proteomes (of 287 analysed) gave 100% epitope coverage in the sample population.
2. Peptide conservation between SARS-CoV-2 proteomes is higher compared to HIV, where peptide prediction from 83 proteomes would be required to reach population coverage (in a previous analysis). The degree of conservation in the SARS-CoV-2 proteome is encouraging for vaccine development.
3. T cell peptide selection for vaccine development could achieve high population coverage by focusing on specific regions within the spike glycoprotein (residues 319-865) and ORF1ab protein (residues 2750-3250 and 4500-5500).

Impact for COVID-19 research:

- Limited impact. Identifying peptides with maximum population coverage across multiple SARS-CoV-2 proteomes is beneficial for global therapeutic efforts however further evaluation of this particular set of peptides is necessary.

Methodologies:

- Study Type: *in-silico*
- Key Techniques: *NetMHCpan-4.1 peptide prediction*

Limitations:

- It is not possible, when using *in-silico* prediction alone, to determine if the resulting peptides will be presented endogenously
- Peptide immunogenicity was not tested
- While the predicted peptide results are provided in the supplementary materials, the predicted corresponding HLA allele(s) is not provided.

Virology

In plain sight: the role of alpha-1-antitrypsin in COVID-19 pathogenesis and therapeutics

Oguntuyo, K.Y. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.14.248880

Summary:

The authors explore endogenous and exogenous proteases activity in SARS-CoV-2 pathogenesis. SARS-CoV-2 naïve serum exhibits significant inhibition of SARS-CoV-2 entry through protease inhibitors alpha-1-antitrypsin (AAT) and alpha-2-macroglobulin (A2M). AAT inhibition of protease-mediated SARS-CoV-2 entry in vitro occurs at lower concertation of what is present in serum and bronchoalveolar tissues, suggesting that AAT activity is physiologically relevant. In addition, AAT-deficient individuals display manifestations associated with risk factors linked to SARS-CoV-2 severity. Collectively, the study suggests that the anti-inflammatory and regulatory functions of AAT have implications for SARS-CoV-2 pathogenicity, SARS-CoV-2 tissue restriction, convalescent plasma therapies, and potentially AAT therapy.

Research Highlights:

1. Sera from patients not exposed to SARS-CoV-2 was capable of neutralizing SARS-CoV-2 pseudoviruses.
2. AAT is neutralizing factor regulating protease-mediated SARS-CoV-2 entry in SARS-CoV-2 naïve serum.
3. AAT-deficiency is associated with clinical manifestations linked to risk factors in SARS-CoV-2 pathogenicity.

Impact for COVID-19 research:

- The data suggest that AAT may may affect SARS-CoV-2 pathogenicity and could be a potential therapeutic target.

Methodologies:

- Study Type: *in vitro, SARS-CoV-2 pseudoviruses*
- Important cell lines/viral models used*: Vero-CCL81, parental 293T, and isogenic 293T cells*
- Key Techniques: *VSVΔG pseudotyped particles and neutralization assays, Plaque reduction neutralization titration (PRNT)*

Limitations:

- While the authors link the AAT activity to the SARS-CoV-2 pathogenesis, minimal data was presented in the level of AAT activity in serum of COVID-19 patients with different disease outcomes.

- Minimal evidence was reported for impact of variant AAT genotypes on the relative severity of COVID-19.
- While the authors link the AAT activity to the SARS-CoV-2 pathogenesis, no data was presented in the level of AAT activity in serum of COVID-19 patients with different disease outcomes.
- Further studies could investigate biologically contribution of AAT in tissue restriction in SARS-CoV-2 infection.

IFITM proteins promote SARS-CoV-2 infection of human lung cells

Bozzo, C.P. *et al.* 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.18.255935

Summary:

Bozzo *et al.* describe the role for the IFITMs in the context of ß-coronaviruses and find their involvement in viral entry. IFITMs have been long described as a family of host restriction factors (RF) for many viral infections, restricting viral replication by affecting fusion capacity. Here, the authors show that overexpression of these RF do impair SARS-CoV-2 infection, but not infectivity when expressed in producer cells. However, at endogenous levels the three best characterised members of the IFITM family (IFITM1, IFITM2 and IFITM3) boost SARS-CoV-2 infection in human lung cells. In particular, IFITM2 expression appeared critical for SARS-CoV-2 infection. The authors suggest that SARS-CoV-2 hijacks IFITMs for replication and spread.

Research Highlights:

1. Overexpression of IFITMs impairs SARS-CoV-2 infection by impairing SARS-CoV-2 S-mediated membrane fusion
2. IFITMs are expressed in primary human lung cells and induced by type I IFN
3. Silencing of endogenous IFITM expression severely impaired SARS-CoV-2 infection, making IFITMs as infection cofactors for SARS-CoV-2 in human cell lines.
4. IFITM2 had the strongest effect and its silencing reduced infectious production by 4 orders of magnitude.

Impact for COVID-19 research:

- Understanding the role of host factors in viral entry: IFITMs are thought to affect cell membrane rigidity and to interfere with viral fusion. Instead of restricting infection, here the authors describe that IFITMs may act as a cofactor for infection, replication and spread.

Methodologies:

- Study Type: *In vitro*

- Important cell lines/viral models used: *Vesicular- Stomatitis-Virus (VSV) containing a luciferase reporter gene instead of the open reading frame for its Glycoprotein (VSV(luc)ΔG) pseudotyped with the SARS-CoV-2 S protein; human epithelial lung cancer cell line Calu-3.*
- Key Techniques: *siRNA knock-down (KD), fusion assay with HIV-1 particles containing β-lactamase-Vpr but with the virions containing the SARS-CoV-2 S instead of the HIV-1 Env protei, proximity ligation assays (PLA).*

Limitations:

- There is no discussion on the different IFITMs localization and their role on impairing viral infection.
- Infectivity from producer cells silenced from IFITMs or with its endogenous levels may also be informative of the possible dual role as a cofactor and possible restriction factor when overexpressed.

SRSF protein kinases 1 and 2 are essential host factors for human coronaviruses including SARS-CoV-2

Heaton, B.E. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.14.251207

Summary:

The authors performed a CRISPR/Cas9 screen in A549 cells overexpressing ACE2 to find new host factors involved in SARS-CoV-2 infection. Even though the screening was sub-optimal, the authors found that the host kinases SRPK1 and SRPK2 are important for SARS-CoV-2 virus production. They show that these two kinases phosphorylate in vitro the viral nucleocapsid protein. Inhibition of SRPK1 and SRPK2 by small molecules inhibitors decrease SARS-CoV-2 and coronavirus 229E viral production in vitro (immortalized cell lines and primary human lung cells).

Research Highlights:

1. Host kinases SRPK1 and SRPK2 are important for SARS-CoV-2 virus production
2. SRPK1 and SRPK2 phosphorylates the virus nucleocapsid protein.
3. Inhibition of SRPK1 and SRPK2 by small molecule inhibitors decreased viral production of SARS-CoV-2 and coronavirus 229E in vitro.

Impact for COVID-19 research:

- Evidence that the host kinases SRPK1 and SRPK2 are important for SARS-CoV-2 virus production. This is potentially mediated by the phosphorylation by these two kinases of the viral nucleocapsid protein.

- Inhibition of SRPK1 and SRPK2 decrease SARS-CoV-2 virus production in immortalized cells and in primary human lung cells. Small molecules inhibitors of SRPK1 and SRPK2 are available but none of them have been currently approved by the FDA for therapeutic purposes.

Methodologies:

- Study Type: *in vitro*
- Important cell lines/viral models used: *Cell lines: A549 cell line overexpressing ACE2, Calu-3 cell line, hUH7 cell line, and primary type II pneumocytes. Viral models (coronavirus): SARS-CoV-2 and 229E.*
- Key Techniques: *CRISPR/Cas9 screen.*

Limitations:

- No direct confirmation that inhibition of SRPK1 and SRPK2 decreases the phosphorylation of the viral nucleocapsid (N) protein (potential kinases redundancy for example).
- No direct evidence that dephosphorylation of the viral N protein is responsible for the decreased SARS-CoV-2 virus production.
- No in vivo evidence that inhibition of SRPK1 and SRPK2 decrease SARS-CoV-2 virus production (only performed in vitro).
- CRISPR/Cas9 screening in A549 cells overexpressing ACE2 is sub-optimal due to limited cytotoxicity of SARS-CoV-2 in this cell line.
- Reliant on pharmacologic kinase inhibition that is fairly toxic

SARS-CoV-2 ORF9c Is a Membrane-Associated Protein that Suppresses Antiviral Responses in Cells

Dominguez-Andres, A. *et al.* 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.18.256776

Summary:

This study characterised the SARS-CoV-2 ORF9c protein as a highly unstable protein and showed it can interact with membrane-associated proteins distributed throughout the membrane compartments in A549 cells. Cells expressing only SARS-CoV-2 ORF9c protein had altered proteome and transcriptome, including induced IL-6 signaling, downregulated interferon signaling, complement signaling, and antigen presentation. Remarkably, the transcriptome and ubiquitinome changes upon ORF9c protein expression resemble the full viral infection reported previously. Moreover, Inhibition of ERAD/proteasome could partially restore the cellular changes caused by ORF9c protein expression. This study indicates that ORFs encoding “accessory” proteins could be of importance in affecting host cell response.

Research Highlights:

1. SARS-CoV-2 ORF9c is identified as the first human coronavirus protein with a putative transmembrane domain and is highly unstable, undergoing proteasome-dependent degradation.
2. A549 cells transfected with only SARS-CoV-2 ORF9c protein showed induced IL-6 signaling and downregulated interferon signaling, complement, and antigen presentation-associated pathways.
3. The transcriptome and ubiquitinome changes upon SARS-CoV-2 ORF9c protein expression showed similarity with those upon full viral infection reported previously.

Impact for COVID-19 research:

- This study is focused on a less well-studied viral protein and provided important insights into how SARS-CoV-2 ORF9c contributes to host cell responses.
- This research doesn't have direct translatability, but provides new directions for therapeutics development if the effects of ORF9c protein are further validated in primary lung cells or animal models.

Methodologies:

- Study Type: *in vitro*
- Important cell lines/viral models used: *A549 cells*
- Key Techniques: *liquid chromatography tandem mass spectrometry (LC-MS/MS), label free quantification (LFQ) and tandem mass tag (TMT) mass spectrometry analysis, RNA-seq analysis, Pathway and network analysis*

Limitations:

- The study is mainly conducted in A549 cells. Further validation in primary human lung cells or animal models is needed to strengthen the conclusions in this paper.
- The function of proteasome-dependent degradation of ORF9c protein in regulating the host cell responses is not yet clear and therefore more mechanistic studies is needed to support their inhibitor experiments.

SARS-CoV-2 ORF9b Antagonizes 1 Type I and III Interferons by Targeting Multiple components of RIG-I/MDA-5-MAVS, TLR3-TRIF, and cGAS-STING Signaling Pathways

Han, L. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.16.252973

Summary:

Han *et al.* report a new role for SARS-CoV-2 accessory protein ORF9b as a contributor to viral immune evasion by the suppression of type I and III IFN production. ORF9b targets multiple

molecules of the RIG-I/MDA-5-MAVS, TLR3-TRIF, and cGAS-STING signalling pathways to suppress IFN production. Immunoprecipitation results show ORF9b interaction with RIG-I, MDA5, TBK1, TRIF and STING, but not IRF3. ORF9b localizes mainly in mitochondria and together with MAVS, and shows partial colocalization with RIG-I, MDA5, TBK1, TRIF and STING. ORF9b mechanism of action to inhibit IFN production occurs by impairment of TBK1 and IRF3 phosphorylation, and consequent impairment of IRF3 nuclear translocation.

Research Highlights:

1. SARS-CoV-2 ORF9b accessory protein impairs antiviral immunity by suppressing type I and III IFN production
2. ORF9b interacts with multiple molecules of the RIG-I/MDA-5-MAVS, TLR3-TRIF, and cGAS-STING signalling pathways
3. Suppression of IFN response is through the impairment of TBK1 phosphorylation, IRF3 phosphorylation and IRF3 translocation.

Impact for COVID-19 research:

- This preprint describes a new role for SARS-CoV-2 ORF9b on impairing antiviral immunity, and it may be helpful to find drugs that can target this accessory protein. This protein was already described to affect IFN response in SARS-CoV by enhancing MAVS proteasomal degradation, but not for RIG-I and MDA-5, TBK1, TRIF, and STING pathways.

Methodologies:

- Study Type: *In vitro*
- Important cell lines/viral models used*: Study performed on HeLa, HEK293, or HEK293 using ORF9b from SARS-CoV-2 Wuhan-Hu-1 strain (NC_045512.2), VSV-enhanced green fluorescent protein (eGFP) and SeV*
- Key Techniques: *Luciferase assays, Immunoprecipitation and Confocal microscopy.*

Limitations:

- The paper lacks an initial rational on their investigation for ORF9b
- There is no indication of the number of independent observations per experiment nor number of independent experiments in the figure legends.
- Co-localization with MAVS is exactly the same, so it can be signal leakage (they don't show the controls).
- The infection is in cells transfected with ORF9b and so the levels may be different to those caused by SARS-CoV-2 infection, also, its role on viral infection is evaluated after Sendai virus infection as a surrogate for SARS-CoV-2. The authors discuss this as lack of adequate facilities.
- Localization of a transfected protein may be misleading after transfection as it may induce a stronger protein production and bias the results.
- The fine tune of IFN responses at early or late infection is crucial for the pathogenicity and the development of the disease, the authors only state that IFN are critical to avoid pathogenicity.
- The discussion is disorganized.
- Notes - Line 17 page 26, the authors wrote ORF8b instead of ORF9b. Line 13 page 28, the authors wrote ORF6b instead of ORF9b.

Lipid Metabolism

Altered Lipid Profile is a Risk Factor for the Progression and recurrence of COVID-19: From Two Retrospective Cohorts

Jin, H *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-60159/v1

Summary:

Metabolic abnormalities such as obesity are major risk factors for poor disease outcome of SARS-CoV-2 which may be associated with basic dyslipidemia of patients. Using two retrospective datasets from Wuhan and Chengdu, Jin *et al*. provide evidence that lower LDL cholesterol is associated with an increase probability of viral recurrence, this being defined as patients testing positive after initial hospital discharge. Furthermore, the study show that triglyceride dyslipidemia is associated to increased mortality. Interestingly, and in contrast to published studies, Jin *et al*. did not find any survival benefit of statin treated patients in comparison to non-statin controls. In summary, the paper strengthens the point of dyslipidemia being a central contributor to poor SARS-CoV-2 disease outcome, however highlighting partially other factors than previous studies.

Research Highlights:

1. Statins were not found to impact on in-hospital death or re-positive SARS-CoV-2 RNA detection once patients are discharged.
2. After multivariable regression, triglyceride dyslipidemia are associated with mortality
3. LDL-C dyslipidemia may be a biomarker for potential recurrence of SARS-CoV-2 positive testing.
4. Lipid levels negatively correlate with inflammation markers (e.g. CRP), but positively correlate with tissue damaging markers (e.g. ALT) indicating that low lipid levels in SARS-CoV-2 patients may be due to inflammation rather than organ damage.

Impact for COVID-19 research:

- That dyslipidemia correlates with SARS-CoV-2 infection and disease severity has been extensively demonstrated over the past months. The study looks for the first time at testing recurrence (testing positive after testing twice negative in hospital over 3 days).
- The most direct impact was that statin therapy did not seem to change mortality or recurrence of the pathogen, with this contrasting a study of Zhang *et al*.

Methodologies:

- Study Type: *Retrospective clinical study*
- Important cell lines/viral models used: *Patient cohort with SARS-CoV-2-positive individuals*

- Key Techniques: *Biochemical measurements using H7600 autoanalyzer, historic patient records*

Limitations:

- As also highlighted by the authors, for a definite conclusion on the effects of lipid-lower drugs, it would need to be performed as a prospective randomized clinical trial
- The number of statin taking individuals are too low (25 and 47 in Chengdu and Wuhan cohort, respectively)
- Though excluded from this study, individuals who have been previously on lipid-lowering medication and their disease progression would have been insightful to see whether statins or other drugs can preemptively reduce risk of poor disease outcome.
- The paper uses clinical measurements as readout (though practical) it does not allow the same resolution of lipids in patient serum
- The phenomena of recurrence is difficult as most individuals probably did not overcome the disease in the first place. It remains unclear if recurrent patients were only tested when they had symptoms again or have been tested on a regular basis past their initial discharge.
- Using patients history (in terms of dyslipidemia) would have been useful to stratify patients who are dyslipidemic due to the infection versus those who have underlying dyslipidemia due to metabolic abnormalities

Comparisons to Influenza

Specific Dynamic Variations in the Peripheral Blood Lymphocyte Subsets in COVID-19 and Severe Influenza A Patients: A Retrospective Observational Study

Zhang, F. *et al*. 2020. *medRxiv*
Link: https://doi.org/10.1101/2020.08.15.20175455

Summary:
Single centre retrospective clinical cases analysis of severe, non-severe COVID-19 and, separately, severe Influenza A, in comparison to healthy donors. Some useful clinical and laboratory parameter comparisons to Influenza A are made, although a more detailed prospective analysis is described in a preprint from Mudd *et al*. (2020). This study largely supports current data regarding the impact of differing COVID-19 disease severity on lymphocyte subsets and extends knowledge about recovery from COVID-19 disease in severe and non-severe patients.

Research Highlights:

1. The severe COVID-19 and Influenza A cohorts had a significantly higher median age than non-severe COVID-19 or healthy donors, respectively. All severe COVID-19 or Influenza patients had fever and cough symptoms. Dyspnea occurred in 77% of severe Influenza patients compared to severe COVID-19 patients. This differential symptom should be investigated in a larger cohort.
2. All disease cohorts had lower white blood cell (WBC) counts than healthy donors. Notably, the WBC counts were significantly lower in severe COVID-19 disease compared to severe Influenza A.
3. Both severe Influenza A and COVID-19 patients had lower $CD4^+$ and $CD8^+$ T cells than healthy controls, however, in non-severe COVID-19 patients, only $CD8^+$ T cells significantly differed from controls.
4. NK cells did not differ COVID-19 patients (severe or non-severe) compared to healthy donors. This contrasts to studies/preprints where NK cells were comparable to healthy controls in asymptomatic or discharged, compared to severe or deceased patients, respectively (Carsetti *et al*., 2020 Odak *et al*., 2020, Xu *et al*., 2020).
5. Non-severe patients' overall T cell counts recovered more quickly, after 3 weeks compared to 4 weeks for severe patients. $CD8^+$ T cells remained lower in severe patients, compared to non-severe, after 4 weeks.

Impact for COVID-19 research:

- It is reasonably well established that lymphocyte depletion tends to correlate with disease severity, although this study adds to the currently limited findings on recovery of lymphocyte subsets in COVID-19 disease over time
- The COVID-19/ Influenza A comparison is timely coming into the Autumn/ Winter flu season and could aid disease management decision making through understanding of

the differential impact of either disease on lymphocyte subsets. More analysis is required however.

Methodologies:

- Study Type: *Retrospective study of clinical data*
- Key Techniques: *COVID-19 or Influenza disease severity were respectively determined using People's Republic of China National Health Commission guidelines (the use of globally applied WHO guidelines would have made this study more generalisable).*
- *Leucocyte subsets were enumerated and analysed by flow cytometry (antibody information not provided) and T cell subsets were analysed using a Four Colour Human CD3/CD8CD45/CD4 flow kit.*

Limitations:

- Only basic phenotyping of T, B and NK cells was carried out, so this study doesn't provide in-depth insight into functional responses of T cells.
- The authors note that since the study analysed n=99 patients from a single centre and relied on retrospective data, this could introduce bias and reduce generalisability. Also, there were inconsistencies in the time periods between illness onset and hospital admission, which might confound analysis of immune response dynamics.