COVID-19 Community Journal Club

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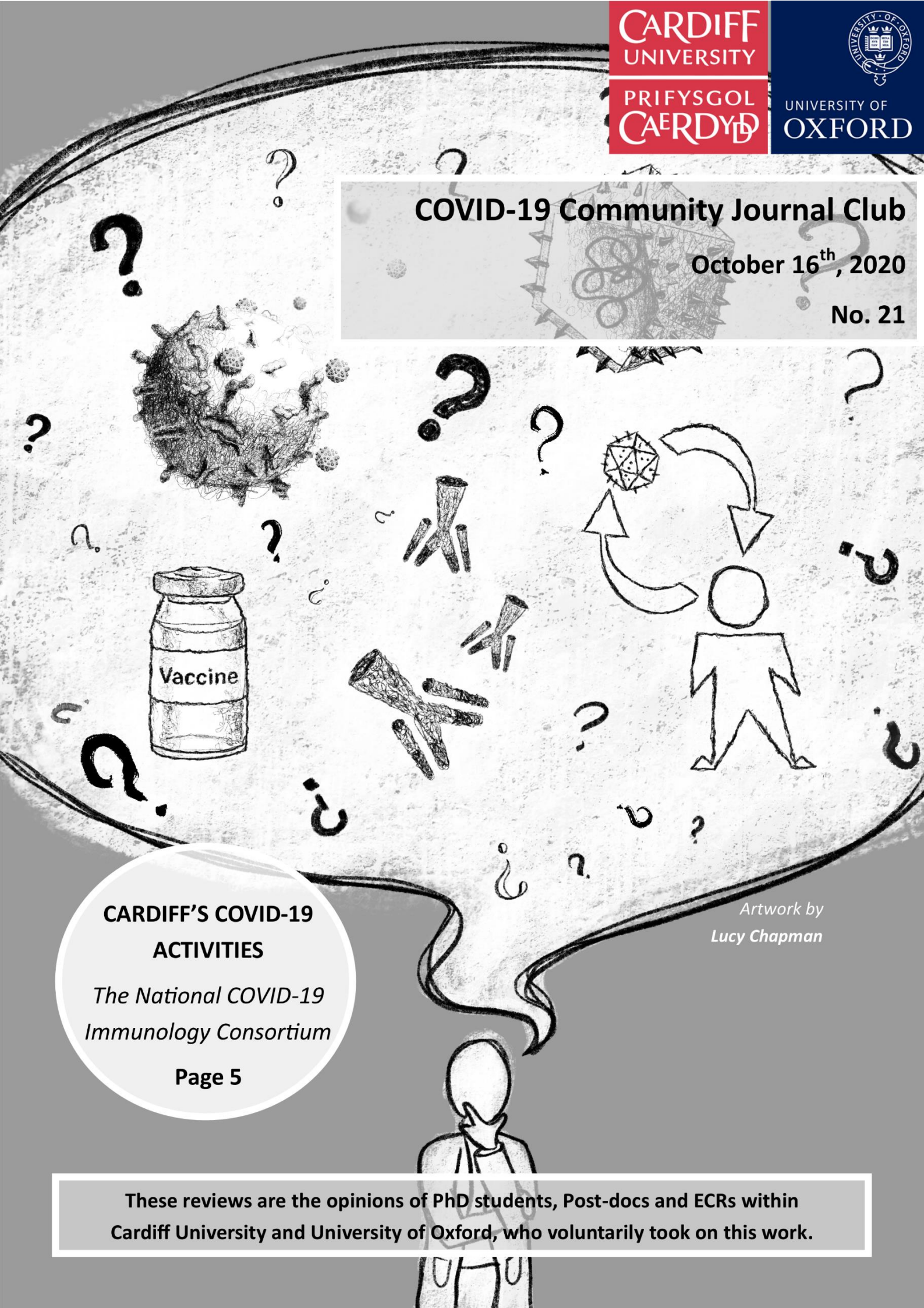

Artwork by
Lucy Chapman

CARDIFF'S COVID-19 ACTIVITIES

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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.

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Please direct any comments or queries to Awen Gallimore 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.

Cardiff's COVID-19 Activities

The National COVID-19 Immunology Consortium

Written by Professor Ian Humphreys

Link: https://www.uk-cic.org/

The National COVID-19 Immunology Consortium (CIC) is an unprecedented national collaboration of UK immunologist that aims to answer key questions regarding the role that the immune response plays, either protective or harmful, during SARS-CoV-2 infection. The consortium is supported by £6.5m funding from UKRI and the Medical Research Council. Cardiff University (led by Ian Humphreys and Awen Gallimore) is playing a key role in the consortium, making multiple contributions to the large national research programme:

What constitutes a good immune response to SARS-CoV-2?

Work led by David Price and Kristin Ladell will use a broad range of techniques to perform high-definition analysis of $CD4^+$ and $CD8^+$ T cell responses in patients that experienced a range of COVID19 symptoms, varying from severe to asymptomatic. The aim of these studies is to define the features of a protective T cell responses to SARS-CoV-2. Furthermore, longitudinal analyses are planned in collaboration with laboratories in Germany and Sweden. These studies will allow the investigation of the relationship between primary and memory T cell responses in different patient groups, potentially revealing key immune correlates of protection against COVID-19.

How does complement cause damage in COVID-19?

Paul Morgan and colleagues identified complement activation as a key signature of severe COVID-19. Furthermore, studies in Cardiff have provided exciting clinical data demonstrating that anti-complement therapy dramatically improves symptoms in severe COVID-19 patients. The Morgan laboratory will now work with other leading complement groups in Newcastle (Harris, Kavanagh) and Cambridge (Schwaeble) to provide a comprehensive analysis of the complement system and its activation in COVID-19. These studies will inform our understanding of the roles of complement in driving COVID-19 pathology and illuminate potential targets for anti-complement therapy.

How does SARS-CoV-2 'dodge' the immune system?

To survive, viruses need to evade the cellular immune system of their hosts. The Stanton and Wang laboratories aim to determine how effectively SARS-CoV-2 manipulates the cytotoxic effector functions of NK and CD8 T-cells, and therefore decipher whether such abilities

contribute to viral survival and disease occurrence. They will also systematically screen SARS-CoV-2 genes for the evasion functions responsible and determine how they operate. This is crucial to our understanding of SARS-CoV-2 infection, disease, and pathogenicity. This work has the potential to contribute to predictions of clinical outcome, identification of therapeutic targets that would boost recognition of pathogens by the immune system, and contributing to vaccine strategies, by identifying genes that may be targeted to generate less virulent vaccine candidates.

Collectively, this work will help identify patients most at risk from severe disease, assist doctors in deciding treatments and aid the rapid development of vaccines and novel treatments for severe COVID-19.

News and Views

‘Provocative results’ boost hopes of antibody treatment for COVID-19

Cohen, J. 2020. *Science*

Link: https://doi.org/10.1126/science.abf0591

In this article, John Cohen details the progress that has been made on monoclonal antibody cocktail therapy for COVID-19: REGN-COV2. The antibodies which constitute the cocktail have potent neutralizing capacity and target the receptor binding domain of SARS-CoV-2. To develop such a therapy, antibody genes from recovered COVID-19 or artificially immunized mice were placed into Chinese hamster ovary cells for bulk manufacture.

Preliminary clinical data from Regeneron Pharmaceuticals demonstrated that the cocktail treatment could greatly reduce viral load in seronegative patients; alleviating symptoms at a faster rate. Compared to placebo, this finding was statistically significant. However, the treatment showed minimal efficacy in those who had already developed their own humoral response to the virus. In a statement, the co-founder of Regeneron, George Yancopoulos, stressed how the target population would be those “who had not yet mounted their own immune response” in an effort to turn patients into those who have “already started to effectively fight the virus”.

The data regarding REGN-COV2 is similar to that from Eli Lilly’s LY-CoV555: a single monoclonal antibody. However, both require more clinical evidence to better understand how these treatments will benefit patients. Both treatments show little change with increased dose. It is understood that Eli Lilly and Regeneron are seeking emergency use authorisation from the FDA.

A few issues are highlighted about Regeneron’s approach to this mode of therapy. With the greatest efficacy seen in those who are seronegative, changes in diagnostics are needed to evaluate a patient’s viral load and immune response at point of care. This would require additional tests, increasing the value of an already extremely expensive therapy. Regeneron’s cocktail has hit the headlines recently for its use in the treatment of President Donald Trump. Cohen further details the President’s treatment, including other antiviral drugs such as remdesivir, in a more recent article: ***Update: Here’s what is known about Trump’s COVID-19 treatment*.**

Journal Reviews

Immune Responses and Clinical Implications

Longitudinal immune profiling reveals key myeloid signatures associated with COVID-19

Mann, E.R. *et al*. 2020. *Science Immunology*

Link: https://doi.org/10.1126/sciimmunol.abd6197

Summary:

Blood from 49 patients stratified by disease severity (FiO_2/ICU admission) was assessed within 7 days and throughout hospitalization. Circulating immune cells and cytokines were assessed, CRP appeared to correlate to multiple immune cell changes. Findings supported by other studies include increased neutrophil to lymphocyte ratio (NLR), cytotoxic $CD8^+$ T-cell response, CRP and IL-6 in COVID-19. Monocyte ($CD14^+CD16^+$) phenotype seemed to be most altered in mild disease and LPS stimulation reduces $COX\text{-}2^+$ and increased percentage of $ki67^+$ cells. The authors propose patients could be stratified on admission by a high NLR and LPS-induced increases to $ki67^+$ monocytes and reduced COX-2 expression.

Research Highlights:

1. **T-cells-** $CD8^+$ T-cells appear active but varies, most patients have higher perforin expression implying cytotoxic activity and overall this was increased before discharge.
2. **B-cells**- were overall decreased but expansion of antibody secreting subset correlating to IgG expression. Expansion of $CD27^-IgD^-$ cells may indicate an impeded B-cell response.
3. **Monocytes-** $CD14^+CD16^+$ monocytes were expanded, especially in mild-disease where CD64 expression and percentage of $TNF\alpha^+$ cells were increased. Increased proportion of $ki67^+$ cells in severe cases even in unstimulated samples.
4. **LPS stimulation of Monocytes-** Increased proportion of $Ki67^+$ monocytes, correlated with CRP and with IL-6, IP-10, IL-10 and Monocyte Chemoattractant Protein-1 (MCP-1). COX-2 was reduced in patients, especially those with severe disease. The percentage of $ki67^+$ cells were higher after recruitment and reduced throughout admission (post-LPS treatment).
5. **Chemokines/Cytokines-** IL-6, IL-10, MCP-1 and IP-10 were higher in patients especially in severe cases and after recruitment but decreased throughout admission.

Impact for COVID-19 research:

- Highlights COVID-19 related changes to the myeloid compartment and changes throughout admission.

- Given that LPS-stimulated monocytes from severe patients have reduced COX2 expression it suggests treatment with NSAIDS could lower COX-2 and have a negative impact on patients.
- Proposed that treatments should focus on reducing neutrophil/monocyte influx at the appropriate time in disease pathology.

Methodologies:

- Study Type: *Cohort study*
- Key Techniques: *Multiplex* bead array assessment of cytokines/chemokines and flow cytometric profiling of systemic immune cells.

Limitations:

- Limited patient number and most healthy controls were frontline workers.
- COVID-19 symptom onset was defined by patients and therefore may not be accurate.

Host transcriptomic profiling of COVID-19 patients with mild, moderate, and severe clinical outcomes

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

Link: https://doi.org/10.1101/2020.09.28.316604

Summary:

The authors compare transcriptomic profiling of nasopharyngeal tissue of patients with mild, moderate and severe COVID-19 against healthy controls. They report dysregulated immune pathways including cytokine-cytokine receptor signalling, complement and coagulation cascades, JAK-STAT, and TGF-β signaling pathways in all patients increasing with disease severity

Research Highlights:

1. Most immune response related genes are upregulated in all patients, with expression rising with severity
2. ACE2 expression is highest in severe disease
3. Regarding gender differences, several immune relate genes are upregulated in male mild and moderate patients, but not in females

Impact for COVID-19 research:

- Very low. The same dysregulated responses have been reported before.

Methodologies:

- Study type: *In silico*

- Key Techniques: *extraction of RNA form nasopharyngeal swabs, shotgun transcriptome sequencing*

Limitations:

- The patient cohort consisted of only 50 participants; Only 3 patients were in the severe disease group.
- No distinction was made between asymptomatic and mild cases.
- It is unclear at which time point after infection the swabs were taken; thus we can't know whether immune signatures predict disease severity or are caused by it.

T-cells

T cell assays differentiate clinical and subclinical SARS-CoV-2 infections from cross-reactive antiviral responses

Ogbe, A. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.28.20202929

Summary:

Ogbe *et al* developed T cell assays to distinguish SARS-CoV-2 specific responses from cross-reactive responses. Strong ex vivo ELISpot and proliferation responses to multiple antigens (including M, NP and ORF3) were found in those who had been infected by SARS-CoV-2. Unexposed subjects had a more narrow proliferative response to spike antigens only, (likely a cross-reactive response to common cold coronaviruses). Seronegative doctors with high occupational exposure and recent COVID-19 compatible illness had broad T cell responses, indicating that a T cell response to SARS-CoV-2 can be detected after infection even if antibodies cannot. Memory responses to specific non-spike proteins provides a method to distinguish recent infection from pre-existing immunity in exposed populations.

Research Highlights:

1. High magnitude and broad T cell responses to SARS-CoV-2 structural proteins are detected in convalescent subjects by ex vivo ELISPOT and T cell proliferation assays
2. In contrast the T cell proliferation assay demonstrated memory T cell responses only to spike protein in the T cell repertoires of unexposed, SARS-CoV-2 seronegative individuals (but not M, NP, ORF3, ORF6, ORF7 or ORF8).
3. In convalescent patients, T cell responses to either M or NP were correlates of the global response to spike, structural and accessory proteins indicating that an assay to measure responses to M or NP could reflect the global effector T cell response.
4. IFNγ Elispot responses appear to peak a month after symptom onset and then show a trend towards declining
5. Symptoms, particularly self- reported fever, correlated with stronger memory T cell responses in recovery
6. Identified high frequency of proliferating cells and broad targeting of SARS-CoV-2-specific CD4+ and CD8+ T cells in a flow cytometry based assay of central memory T cell responses. This assay appeared more sensitive than ELISpot
7. SARS-CoV-2-specific CD4+ T cells (and to a lesser extent CD8+ T cells) were polyfunctional in recovery.
8. Using a T cell proliferation assay, T cell responses to SARS-CoV-2 proteins with high breadth and magnitude were detected in convalescent doctors that were seronegative, indicating that a memory T cell response may exist in the absence of a T cell response. The most sensitive assays in this case was M and/ or NP-specific T cell responses in the proliferation assay.

9. Significantly higher magnitude of CD4+ but not CD8+ T cells proliferating in response to the M, N, ORF3, 6, 7 and 8 was detected in these doctors.

Impact for COVID-19 research:

- The study provides an insight to different ex-vivo assays to measure T cell responses and show for the first time an optimized method to distinguish between SARS-CoV-2 specific T cell responses from pre-existing cross-reactive responses to other coronaviruses
- Suggest central memory T cell responses to M and NP in the proliferation assay as the best marker of SARS-CoV-2 specific response.
- Lays the groundwork for assessing T cell responses to SARS-CoV-2 vaccines

Methodologies:

- Key Techniques:
    1. IFN-γ enzyme-linked immunospot (ELISpot) assay
    2. Intracellular cytokine stimulation (ICS) assay
    3. Proliferation assay using CellTrace® Violet
    4. Flow cytometry analysis
    5. Standardised ELISA
    6. Lactate measurements
    7. SARS-CoV-2 pseudotype micro-neutralisation assay
- Assays were validated between multiple groups
- Large cohorts were used

Limitations:

- The correlation data for T cell responses in M or NP protein and other structural and accessory parts included donors with low responses which may limit the conclusion.
- There are isolated examples of seronegative 2020 controls which appear to have a SARS-CoV-2 specific response

Antibodies

SARS-CoV-2 antibody responses in patients with aggressive haematological malignancies

O'Nions, J. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.29.20202846

Summary:

This manuscript looks into whether patients with aggressive hematological malignancies are at a higher risk due to factors including malignancy and anti-cancer treatments resulting in immunological dysfunction. The results show that these patients are capable of producing antibodies and neutralizing responses against S1 and N SARS-CoV-2 antigens, although time to seroconversion was consistently longer when compared to the general population. This has important implications for patient management including self-isolation, modification to therapies and understanding responses to vaccines.

Research Highlights:

1. 8/10 patients had detectable IgG responses to S1 and N. Out of those that did not produce IgG responses, one had clinical diagnosis of COVID-19, but was PCR negative, the other did not receive COVID-19 modifying treatment
2. Production of anti-N IgG appears delayed with only 50% seroconverted by day 28 compared to 90% in healthy individuals
3. 6/8 seropositive patients exhibited neutralizing activity
4. Clinical severity correlated with magnitude of antibody response in all but two patients

Impact for COVID-19 research:

- The first longitudinal study of serological responses to SARS-CoV-2 in adult patients undergoing anticancer treatments for hematological malignancies. Further studies are required, but this indicates that these patients may have delayed seroconversion upon COVID-19 infection, but still have the ability to produce antibodies.

Methodologies:

- Study Type: *Cohort Study*

Limitations:

- Small number of patients (10)
- Highly variable responses within the cohort
- Patients had different malignancies and treatments, larger study is required to look at each of these individually

LY-CoV555, a rapidly isolated potent neutralizing antibody, provides protection in a non-human primate model of SARS-CoV-2 infection

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

Link: https://doi.org/10.1101/2020.09.30.318972

Summary:

Using the sera from a convalescent patient, Jones *et al*. were able to use high-throughput screening and sequence analysis to identify a lead monoclonal antibody to take forward for clinical development. LY-CoV555 competes with ACE2 for the RBD of SARS-CoV-2, and demonstrated superior neutralising capacity to antibodies with similar binding sides. This superiority is perhaps due to LY-CoV555 being able to recognise the RBD in both the up and down conformation. Prophylactic treatment with LY-CoV555 in a non-human primate model demonstrated protection in both the upper and lower respiratory tracts. This data supports the progression of LY-CoV555 in clinical studies.

Research Highlights:

1. High-throughput screening of PBMCs from a convalescent COVID-19 patient, obtained 20 days post symptom onset, identified 440 unique antibodies which mostly belonged to VH3 gene family (57%).
2. 175 successfully cloned and expressed antibodies possessed broad epitope diversity, with only 10% competing with viral receptor ACE2. Those which competed demonstrated greater neutralisation capacity using a pseudotyped lentivirus reporter assay.
3. The most successful candidate LY-CoV555 binds to SARS-CoV-2 RBD via an epitope that overlaps with ACE2-binding and has a unique mode of epitope recognition, as it is accessible in both the up and down conformations of RBD.
4. Prophylactic treatment of rhesus macaques (day -1) with LY-CoV555 caused significant reduction in SARS-CoV-2 viral load and active replication at all doses by day 3 in BAL, nasal and throat swabs. Viral load was also decreased in the lungs, at day 6. Maximal protection was seen with 2.5 mg/kg and above.
5. LY-CoV555 elicited sustained serum concentrations in a dose-dependent manner, which correlated with reductions in viral load.

Impact for COVID-19 research:

- The remarkable timeline of 52 days between serum collection to GMP manufacturing of LY-CoV555 has resulted in the first in-human trials for COVID-19 therapy using monoclonal antibodies.
- The availability of a prophylactic/therapeutic before a viable vaccine is of great benefit to those severely infected with SARS-CoV-2.

Methodologies:

- Study Type: *in silico, in vitro, in vivo*
- Important cell lines/viral models used: *Vero E6 cells for neutralisation, SARS-CoV-2 clinical isolate (USA/WA/1/2020) and Italy-INMI1 isolate*
- Key Techniques: *Single cell screening and sequencing, high-throughput antibody production, Surface Plasmon Resonance, Biolayer interferometry, Hydrogen-deuterium exchange, Cryo-EM, X-ray crystallography, multiple neutralisation assays, non-human primate challenge.*

Limitations:

- Would've been nice to have seen a treatment group alongside prophylactic as it may have more clinical value.

Virology

Structure and Drug Binding of the SARS-CoV-2 Envelope Protein in Phospholipid Bilayers

Hong, M. *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-77124/v1

Summary:

The envelope (E) transmembrane protein which forms a cation-selective channel across the endoplasmatic reticulum Golgi intermediate compartment. Mei Hong et al study in particular the structure of the E transmembrane domain of SARS-CoV-2 bind to hexamethylene amiloride (HMA) and amantadine (AMT). This study reports the 2.4 Å structure determined using solid-state nuclear magnetic resonance (NMR) spectroscopy. A detailed structure of this virus protein would help to identify the mechanism by which this protein mediates the budding and release of progeny viruses and activates the host inflammasome. Also, they report how the E protein binds to HMA and AMT by measuring the pentamer chemical shifts, suggesting that E is a potential antiviral and vaccine target.

Research Highlights:

1. HMA binds dynamically intercalating shallowly into the N-terminal lumen where it exchanges between helices and inhibits cation conduction by steric occlusion of the pore
2. This membrane-bound ETM structure suggests that small-molecule E inhibitors should bind with high affinity to both the acidic E8 and the polar N15 in order to occlude the N-terminal entrance of the protein. Thus, small-molecule drugs should ideally be targeted and delivered to the ERGIC and Golgi of host cells to maximally inhibit SARS-CoV-2 E
3. The protonation equilibria of this loose ring of E8 quintet, together with the anionic lipids in the ERGIC membrane, may regulate the ion selectivity of ETM at the channel entrance
4. SARS-CoV-2 E forms a structurally robust but bipartite channel whose N- and C-terminal halves can interact with drugs, ions and other viral and host proteins semi=-independently.

Impact for COVID-19 research:

- Although this research do not alter our view of the disease and might not suppose a direct benefit for clinicians, the establishment of the E structure permits the design of new E inhibitors as antivirals drugs against SARS-CoV-2.

Methodologies:

- Study Type: *crystallography, in silico*
- Key Techniques: *Structure was determined in phospholipid bilayers using solid-state NMR spectroscopy. Protein was incorporated into DMPC:DMPG membranes for drug*

binding studies. ETM was previously expressed in E.Coli using a His6-SUMO fusion tag and purified by nickel affinity column chromatography and reverse-phase HPLC. NMR spectral were processed in the TopSpin software while chemical shifts were assigned in Sparky and TALOS-N to calculate torsion angles. Other software used was SIMPSON for simulation of the ^{13}C-^{19}F REDOR curves.

Limitations:

- The only structure represented in the study is the closed state of SARS-CoV-2 E. If it possible might be worth it have a structure for its open state to study the different changes the channel might suffer.

SARS-CoV-2 cell entry gene ACE2 expression in immune cells that infiltrate the placenta in infection-associated preterm birth

Lye, P. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.27.20201590

Summary:

During pregnancy COVID19 infection is associated with preterm birth, whereas neonatal infection is rare. Lye et al investigate potential routes SARS-CoV-2 could reach the placenta and vertical transmission. Placenta's from pregnancies with Chorioamnionitis (ChA) were found to have elevated levels of ACE2 mRNA due to activation of maternal peripheral macrophages and neutrophils in the presence of intrauterine bacterial infection. These ACE2 expressing cells could potentially import SAR-CoV-2 to placental tissues and increase the risk of vertical transmission.

Research Highlights:

1. ACE2 mRNA expression significantly increased (P<0.01) in pregnancies with ChA. CCL2 also significantly increased.
2. Treatment of placental explants with LPS increases cytokine expression (IL6, IL8 and TNF alpha mRNA) and in turn increased ACE2 mRNA expression.
3. ACE2 protein was not elevated in placentas of preterm ChA pregnancies but a significant increase was seen in preterm placentas than placentas at term.
4. ACE2 localised to syncytiotraphoblasts in fetal blood vessel and M1/M2 macrophage and neutrophils within villous stroma.
5. Increased numbers of M1 macrophages and neutrophils were present in ChA placentas.

Impact for COVID-19 research:

- Peripheral immune cells including granulocytes and monocytes express ACE2 mRNA and protein in mothers. In COVID19 positive pregnancies with ChA complications infected mothers ACE2 immune cells have potential to traffic SARS-CoV-2 to the placenta and increase the risk of vertical transmission.

Methodologies:

- Study Type: *Ex Vivo*
- Key Techniques: *Placental Ex-plant model*

Limitations:

- Small numbers (n=12)
- No case studies (in vivo) evidence

Functional Landscape of SARS-CoV-2 Cellular Restriction

Martin-Sancho, L. *et al.* 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.09.29.319566

Summary:

Martin-Sancho *et al.* identify 65 interferon-stimulated genes (ISG) that mediate the host cell restriction to SARS-CoV-2 infection by a large-scale gain-of-function analysis. These ISGs include endosomal factors inhibiting viral entry, nucleic acid binding proteins supressing RNA synthesis and the majority fall into ER or Golgi resident proteins that inhibit viral translation, regulate lipid membrane composition and vesicle transport. Among the 65 ISGs, BST2 was identified, a known restriction factor that tethers newly synthesized viruses to the cell plasma membrane and impairs viral release. Here, the authors describe BST2 as a restriction factor for SARS-CoV-2 and found that it is counteracted by the accessory protein ORF7a, whose expression rescues viral release.

Research Highlights:

1. Identification of antiviral restriction factors through a large-scale analysis: a subset of 65 ISGs restricts SARS-CoV-2 viral replication
2. ISGs controlling viral entry have impact on endo-lysosomal function, probably impairing low-pH dependent entry
3. ISGs inhibiting RNA replication detect viral RNA and prevent its active replication
4. ER- and Golgi resident ISGs inhibit late stage replication
5. BST2 inhibits SARS-CoV-2 viral release and it is counteracted by ORF7a, that is incorporated in the viral particle

Impact for COVID-19 research:

- This study does not only have a strong impact on restriction factors for SARS-CoV-2 but to any viral infection

Methodologies:

- Study Type: *in vitro.*
- Important cell lines/viral models used: *SARS-CoV-2 USA-WA1/2020, isolated from an oropharyngeal swab from a patient with a respiratory illness who developed clinical disease (COVID-19) in January 2020 in Washington, USA, was obtained from BEI Resources (NR-52281)*
- Key Techniques: *lentiviral transduction, RNA-seq, CRISPR/Cas9, immunofluorescence*

Limitations:

- Assays are conducted in cell lines expressing ISGs by viral transduction and their expression might differ to natural endogenous levels of the proteins. Also, the levels of upregulation in their expression after SARS-CoV-2 infection as ISGs is not addressed.
- Some of the results are a bit vague since as it is a large-scale screen the authors don't investigate all the 65 ISGs.