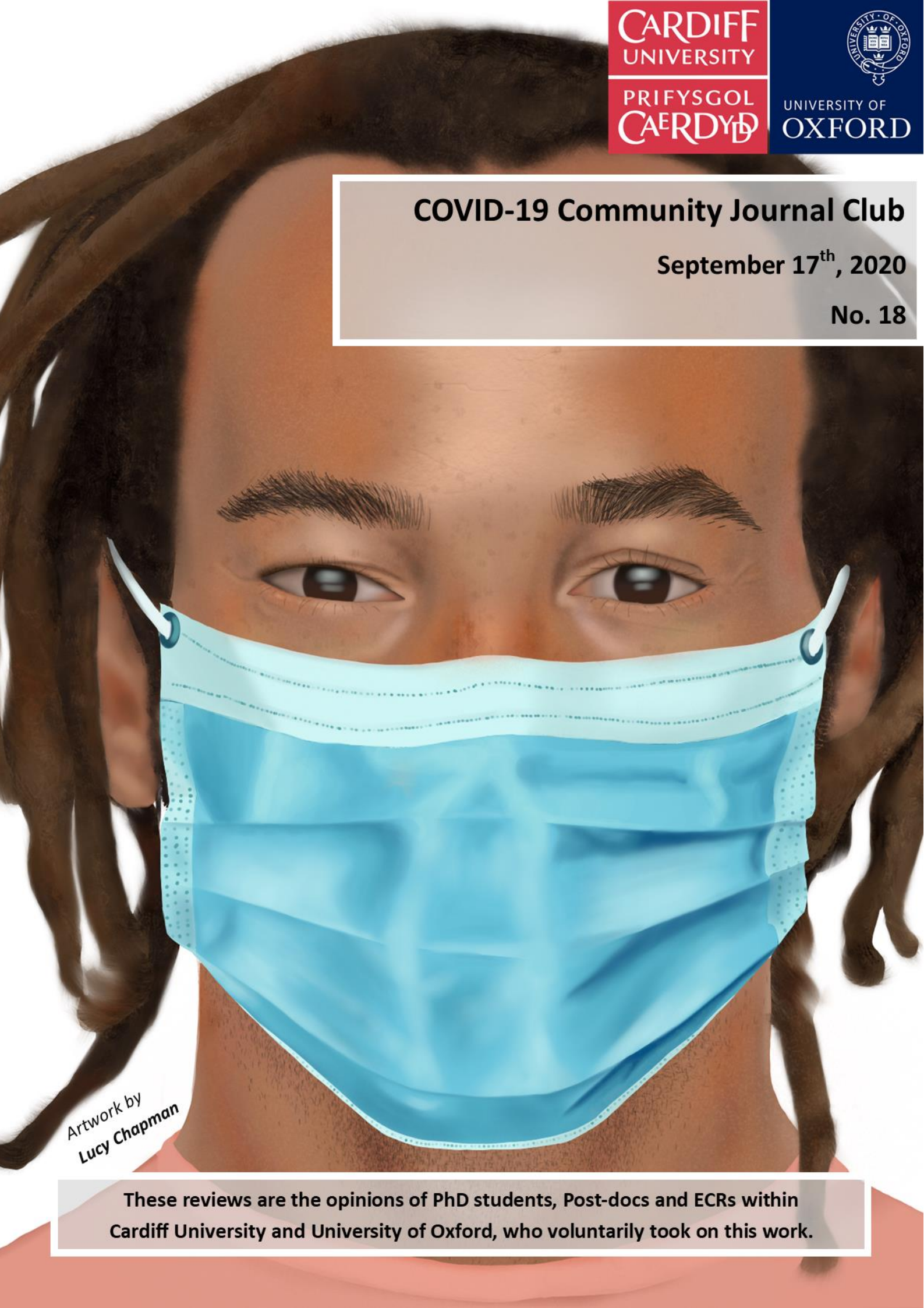
CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD
UNIVERSITY OF OXFORD
COVID-19 Community Journal Club
September 17th, 2020
No. 18
Artwork by
Lucy Chapman
These reviews are the opinions of PhD students, Post-docs and ECRs within Cardiff University and University of Oxford, who voluntarily took on this work.

Thank you to:

Elise Moses, Pragati Sabberwal, Sophie Reed, Ruth Jones, Rebecca Bayliss, Owen Moon, Stephanie Burnell, Kate Liddiard, Sarah Lauder, Lorenzo Capitani, Ana Pires, Ellie Pring, Alicia Teijeira Crespo, Stephanie Hanna, Valentina Bart, Sarah Curtis, Freya Shepherd, Stefan Milutinovic, Yuan Chen, Amy Codd, Sarah Gallaway, Shayda Maleki Toyserkani, Sandra Dimonte, Oliver Scourfield, Ariadni Kouzeli, Fangfang Lu, Arthur Dyer, Dorothee Berthold, Ester Gea-Mallorquí, Aljawharah Alrubayyi, Sara Danielli, Michael Tellier, Julie Mazet, Felix Richter, Max Quastel, Lion Uhl and Athena Cavounidis for producing these reviews.

Drs Ceri Fielding, Luke Davies, Andrew Godkin, Kristin Ladell, Emma Jones, James Matthews, Lion Uhl, Fabian Fischer, Sara Danielli, Ewoud Compeer and Felix Richter for paper and preprint selection.

Drs Katja Simon, Lynn Dustin, Fadi Issa, Petros Ligoxygakis, Quentin Sattentau for counter-checking preprint summaries.

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

Table of Contents

Page

Antibodies

T-cells

Immune Responses

Genomic and Transcriptomic Analysis

Vaccines

IFN-α Therapy

Cardiology

SARS-CoV-2 infects human pluripotent stem cell-derived cardiomyocytes, impairing electrical and mechanical function **24**

Marchiano, S. *et al*. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.30.274464

CAUTIONARY NOTE:

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

Antibodies

A comprehensive analysis of recovered COVID-19 patients and dynamic trend in antibodies over 3 months using ELISA and CLIA methods

Dehgani-Mobaraki, P. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.31.20184838

Summary:

This study investigated the antibody response and duration of COVID-19 patients over a three-month period. Patients were invited for serological tests spaced at least 2 weeks apart and the authors used ELISA and CLIA to determine antibody levels.

Research Highlights:

1. The mean duration of viral shedding: 20.13 ± 6.17 days (median of 21 days)
2. Comorbidities present included hypertension, diabetes, coronary heart disease and asthma
3. Most common symptoms were fever, altered sense of smell and taste, muscle pain and sore throat
4. Antibody trends were similar at both time points

Methodologies:

- Study Type: *Cohort Study*
- Key Techniques: *ELISA and CLIA*

Limitations:

- Small sample size of only 30 patients
- Only one follow up time
- Follow up time was not consistent between patients
- A mean value of 53 days, does not equate to a follow up of three months
- Results are very different between methods
- Did not investigate the relationship between antibody titre and neutralizing activity

Cross-reactive antibody responses against SARS-CoV-2 and seasonal common cold coronaviruses

Klompus, S. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.01.20182220

Summary:

Using sera from recovered COVID-19 patients and unexposed individuals, the authors were able to demonstrate cross-reactive IgG responses to linear epitopes from a phase-displayed antigen library against SARS-CoV-2, other epidemic strains and seasonal coronaviruses. Abundant antibody responses to seasonal coronaviruses were found in both groups, although particular peptides could only be bound by recovered sera. Peptides that elicit cross-reactive responses are typically within the spike and nucleocapsid, which may have implications for vaccine design. Vast inter-individual variability of responses towards SARS-CoV-2 peptides in recovered COVID-19 patients was also found.

Research Highlights:

1. Broad antibody responses against peptides of seasonal hCoVs were seen in up to 75% of samples. Binding to particular peptides were comparable between recovered and unexposed individuals.
2. Sera from recovered COVID-19 patients bound to SARS-CoV-1 and SARS-CoV-2 peptides more significantly than in unexposed individuals. However, no convergence of responses towards a particular SARS-CoV-2 peptide was found, demonstrating substantial inter-individual variability between samples.
3. Particular epitopes from seasonal strains (e.g. hCOV-OC43) were more significantly bound by recovered COVID-19 sera however limited cross-reactivity towards alpha-coronaviruses was seen.
4. Peptides that induced cross-reactive responses were typically found within similar regions of the nucleocapsid and spike, particularly the S2 region. Vaccines that only use RBD of spike may induce responses which are unable to cross-react as well.

Impact for COVID-19 research:

- Cross-reactivity has multiple implications for vaccine design, diagnostic tools and long-term protection. The authors cite multiple studies that have showed similar trends to themselves.

Methodologies:

- Study Type: *in vitro, in silico*
- Key Techniques: *Phase immunoprecipitation sequencing (PhIP-Seq) library with 64 amino acid peptides (20 amino acid overlap)*

Limitations:

- Linear peptides do not reflect the complex 3D organisation of viral proteins. May demonstrate why few response towards peptides in RBD were seen.

- By authors own admission, PhIP-Seq has its own limitations with regards to lacking of post-translational modifications to peptides, particularly glycosylation.
- Only mild recovered patients were included. It would be good to see how responses differ due to severity.
- Authors also acknowledge the neutralising capacity of these interactions were not determined

Rapid evaluation of neutralizing antibodies in COVID-19 patients

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

Link: https://doi.org/10.1101/2020.09.01.20185447

Summary:

To determine whether antibodies produced in response to SARS-CoV-2 infection are protective, a rapid test is needed to evaluate antibody neutralisation capacity in patient plasma. Zhang *et al*. used 'Up-conversion phosphor technology-based point-of-care testing' (UPT-POCT) to quantify total antibodies, and a microneutralisation assay to assess antibody neutralising efficiency, using plasma from both recovered and PCR re-positive COVID-19 patients. UPT-POCT was found to be an effective surrogate marker for neutralising antibody (NAb) activity. This method could be employed for testing vaccine responses, screening convalescent plasma donors and assessing herd immunity.

Research Highlights:

1. Up-conversion phosphor technology-based point-of-care testing (UPT-POCT) uses the RBD of SARS-CoV-2 as the coating protein on the 'test strip' and the S1 protein as the UCP-labeling antigen. The test quantifies SARS-CoV-2-specific antibody levels using patient sera.
2. A positive correlation (correlation coefficient r= 0.9654) was found between T/C ratios reported by UPT-POCT and neutralising antibody (NAb) titres in recovered patient sera samples (519).
3. Using both re-positive patient sera and recovered patient sera, a correlation between T/C ratio and NAb titres was found, suggesting levels of neutralising antibodies can predict re-infection risk.

Impact for COVID-19 research:

- UPT-POCT could be used as a surrogate marker for neutralising antibody capacity to efficiently test vaccine responses, screen convalescent plasma donors and evaluate herd immunity etc.

Methodologies:

- Study Type: *Cohort study*

- Key Techniques: *Up-conversion phosphor technology-based point-of-care testing (UPT-POCT), microneutralisation assay.*

Limitations:

- 'Re-positive' infection patients not defined clearly.
- UPT-POCT efficacy was tested against only 1 neutralisation assay.
- UPT-POCT does not assess IgM or IgA levels, nor antibodies that recognise epitopes outside of the RBD region which could be neutralising.
- Antibody responses to SARS-CoV-2 may be short lived, and therefore, may not be an efficacious surrogate marker for re-infection.

Rare SARS-CoV-2 antibody development in cancer patients

Hempel, L. *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-71560/v1

Summary:

The authors examined 77 oncological patients (median age of 66 years) who tested positive for SARS-CoV-2 by RT-qPRC for the development of anti-SARS-CoV-2 antibodies. They suggest that only 6/77 patients showed measurable antibody levels. No correlation was detected between different types of cancers or anti-tumour treatments and the development of antibodies.

Research Highlights:

1. 6 out of 77 patients testing positive for SARS-CoV-2 by RT-qPRC developed antibodies detectable around 30 days after RT-qPCR
2. Of these 6 patients 3 developed mild symptoms, 2 experienced severe disease and 1 developed pneumonia
3. Patients who were negative for antibodies at the first test did also not develop antibodies at later time points

Impact for COVID-19 research:

- Low for clinical application
- High for the general public as a lack of antibody development means no protection after primary infection with SARS-CoV-2
- High for vaccine development, understanding why SARS-CoV-2 does not trigger antibody responses in some people will be important

Methodologies:

- Study Type: *Cohort study*

- Key Techniques: *testing for SARS-CoV-2 antibodies: Roche (Elecsys Anti-SARS-CoV-2 immunoassay)*

Limitations:

- It is unclear from the paper whether antibodies detected in the 6 patients would be protective against reinfection
- It would be interesting to know whether the 71 patients that did not develop antibodies were asymptomatic or experienced mild/severe disease
- One single test was used to detect SARS-CoV-2 antibodies
- The cohort is very specific, cancer patients have previously reported to have a both lower and incidence of COVID-19 compared to the general population depending on the study

T-cells

Systematic examination of T cell responses to SARS-CoV-2 versus influenza virus reveals distinct inflammatory profile

Law, J.C. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.27.20183319

Summary:

The T cell response to SARS-CoV-2 infection is important for clearing virus-infected cells. Why some patients with severe disease are unable to control infection is unknown. Law *et al*., 2020 furthers knowledge about the immune response to SARS-CoV-2 by characterising the *ex vivo* T cell response of 13 SARS-CoV-2 convalescent donors with a range of disease severities and compares responses to influenza virus. PBMCs collected between days 27 to 90 post-disease onset were stimulated with SARS-CoV-2 glycosylated S and recombinant N protein, peptide pools from N, M and E proteins. Outputs included cellular phenotype, cytokine production and proliferative response.

Research Highlights:

1. 92% of SARS-CoV-2 convalescent patients showed lower CD4+ responses to S and N proteins than influenza A virus (PR8) and trivalent influenza vaccine and no CD8+ T cell response.
2. SARS-CoV-2-specific T cells were less multifunctional than PR8-specific T cells, particularly in severe cases.
3. High IL-10 production in response to N protein (indicates immunosuppression).
4. Granzyme B+/IFN-γ+ CD4+ and CD8+ proliferative responses to peptide pools in most patients, with CD4+ responses predominating over CD8+.
5. Peripheral T follicular helper responses to S or N strongly correlated with serum neutralization assays and RBD-specific IgA. Tfh response to SARS-CoV-2 was weaker than response to influenza virus.

Impact for COVID-19 research:

- CD4+ Th1 (pro-inflammatory) response to SARS-CoV-2 is robust and may contribute to COVID-19 pathology. Conversely, these CD4+ T cells are granzyme B+ and IFN-γ+, and may be functionally cytotoxic, thereby contributing to viral clearance via MHC class II expression on epithelial airway cells
- PBMCs from SARS-CoV-2 convalescent donors secreted high levels of IL-10 in response to N protein which may contribute to impaired antigen presentation and immunosuppression and should be considered in vaccine design

Methodologies:

- Study Type: *ex vivo*

- Important cell lines/viral models used: *Vero E6 cells*
- Key Techniques: *Development of recombinant SARS-CoV and SARS-CoV-2 spike and N protein expression in cell culture, 15-mer peptides for S, M, N and E proteins, intracellular cytokine staining, SARS-CoV-2 neutralisation assay, CFSE T cell proliferation assay, multiplex cytokine bead assay*

Limitations:

- Small sample size (n= 1 asymptomatic, 6 mild, 3 moderate and 3 severe cases)

Outcome of SARS-CoV-2 infection linked to MAIT cell activation and cytotoxicity: evidence for an IL-18 dependent mechanism

Flament, H. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.31.20185082

Summary:

Flament *et al.* evaluate MAIT cells in hospitalised patients, 51 with moderate and 51 with severe COVID-19, and paired them to age, sex and comorbidities controls. Circulating MAIT cells were dramatically reduced by COVID and the decrease correlated with the severity of the disease, with the CD8+ subset being most affected. MAIT cells showed very high levels of activation (CD69+ and CD56+), markers that suggested tissue recruitment and an increase in effector functions. MAIT alterations in activation and function preferentially associated to mortality than other T cell populations and CD69 expression was significantly correlated to fatal disease outcome. The levels of COVID-19 inflammation showed a strong correlation to the MAIT activation phenotype described, notably with IL-18. SARS-CoV-2 in vitro infected macrophages in co-culture with MAIT cells suggested a two-step process for MAIT activation, first through type I IFN pathways and later through IL-18. Altogether, this preprint shows that MAIT cells may play a pivotal role on the pathogenesis of COVID-19.

Research Highlights:

1. Profound decline of MAIT cell counts in COVID-19 patients and alteration of subset repartition.
2. Strong activation and cytotoxic phenotype of MAIT cells strongly correlated with circulating pro-inflammatory cytokines, notably IL-18
3. MAIT alterations correlated with disease severity and mortality in COVID-19 patients
4. In vitro SARS-CoV-2 infected macrophages activated MAIT cells in a cytokine-dependent manner, involving IFNα in the early phase and IL-18 in the late phase.

Impact for COVID-19 research:

- Understand the immune response to SARS-CoV-2/COVID-19 and its correlation with the pathogenesis

Methodologies:

- Study Type: *Cohort study, ex vivo, in vitro.*
- Important cell lines/viral models used: *Blood from 51 moderate COVID-19 patients, 51 severe patients and 80 non-COVID patients age, sex and comorbidities matched*
- Key Techniques: *Flow cytometry,* Human Cytometric Bead Array (CBA), qPCR

Limitations:

- In figure 1 the authors show the % decrease of MAIT cells and for the CD8+ subset, it would be informative to see the other MAIT subsets. In the CD8+ subset, moderate COVID seem to have a more pronounced decrease than severe, which may be explained by other subsets.
- The authors don't comment on the fact that moderate COVID IFNγ producing cells are lower in moderate COVID than healthy controls (Fig 2E)

Note: This paper extracts very similar conclusions to another pre-print reviewed this week (see Parrot *et al*.) but has more donors and better paired with controls.

MAIT cell activation and dynamics associated with COVID-19 disease severity and outcome

Parrot, T. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.27.20182550

Summary:

Parrot *et al.* study mucosal-associated invariant T (MAIT) cells in SARS-CoV-2 infection and found profound alterations correlating to disease severity. During acute infection there was a profound and preferential lymphopenia of circulating MAIT cells but the analysis of public scRNAseq suggested its recruitment to the upper respiratory tract. Circulating MAIT cells were highly activated, with enhanced CD69 and diminished CXCR3 in both severe and mild patients, a pattern mirrored in scRNA-seq signatures of airway MAIT cells that appeared to be the main subset of airway T cells expressing IL17A. Early MAIT activation levels are linked to disease outcome and deceased patients displayed higher level of MAIT cell activation compared with patients that survived. Within 3-21 days after resolution of symptoms, MAIT cells in circulation appeared to restore CD69 levels but not CXCR3, suggesting that some phenotypical perturbations persist. Collectively, these data suggest that MAIT cells contribute to antiviral immune responses and may be involved in COVID-19 immunopathogenesis.

Research Highlights:

1. MAIT cells lymphopenia correlates with disease severity and is more pronounced than for conventional CD4 and CD8 T cells or other unconventional T cells
2. MAIT cells decline is correlated with a MAIT enrichment in the airways, possibly due to recruitment
3. Transcriptomic scRNAseq data shows MAIT cells in the airways are the prominent subset expressing IL-17
4. Higher levels of CD69 and lower levels of CXCR3 were found on patients that deceased
5. MAIT cells recover in circulation during convalescence, but some perturbation may still persist

Impact for COVID-19 research:

- Understand immunological landscape associated with SARS-CoV-2 pathogenesis, specifically on MAIT cells.

Methodologies:

- Study Type: *Cohort study, ex vivo*
- Important cell lines/viral models used: *PBMCs from SARS-CoV-2 patients and healthy donors and Publicly available scRNAseq datasets*
- Key Techniques: *flow cytometry (including* 22-parameter flow cytometry analysis and *Trucount), bioinformatics analysis (UMAP, Phenograph, PCA) and Serum proteomics*

Limitations:

- The authors compare the frequency and phenotypic characteristics of MAIT cells in acute versus convalescent patients. While cross sectional analysis may provide insight between the two phases, longitudinal samples may provide a more accurate view of alterations in the MAIT compartment considering intra-individual heterogenicity.
- There is no data on the age of the healthy controls
- The analysis of the scRNAseq data sets don't discriminate between severe and mild COVID, which would be interesting to see its correlation with MAITS in circulation
- The authors don't comment on the differences in Fig 3F/H/I between the Atlas and the Biobank CD69 expression, although there is a correlation in 3G. This might be stated in Table S3 (but not visible in the preprint)
- It would be interesting to have a more complete functional characterisation than just the expression of GrzB and Ki67.
- To investigate innate-like properties, it would be interesting to explore any alteration in their functional capacity in response to innate cytokine stimulation, given that other acute viral infections induce changes in their innate responses (Kerri G. Lal *et al*, 2020)
- Minimal evidence reported for level of serum/plasma markers associated with microbial translocation, given that MAIT cell activation could be driven by exposure to microbial products and/or microbial antigens in other viral infection (Kerri G. Lal *et al*, 2020)
- In figure 4 it would be informative to have the levels of healthy controls

Immune Responses

Longitudinal single-cell immune profiling revealed distinct innate immune response in asymptomatic COVID-19 patients

Zhao, X-N. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.09.02.276865

Summary:

Xiang-Na Zhao *et al*. performed longitudinal single-cell RNA-seq and V(D)J sequencing of PBMCs from 3 healthy donors and 10 COVID-19 patients with asymptomatic, moderate, and severe disease. Severe disease was characterized by an increased type I interferon (IFN-I) responses, diminished T cell clonal expansion, and increased plasma B cells and BCR clonality. In contrast, moderate patients display lower expression of IFN-I related genes, robust T cell clonal expansions, and increased plasma B cells when sampled <10 days after symptom onset. The asymptomatic condition was found to be an intermediate state between moderate and healthy individuals. Asymptomatic infection was marked by a robust effector $CD4^+$ T cell clonal expansion with upregulated IFNG gene expression. Asymptomatic individuals also showed an increase in $CD56^{bri}CD16^-$ NK cells, with higher expression of cytokines/chemokines (*XCL1, XCL2,* and *IFNG*). Furthermore, these individuals display an increased proportion of a Th2-like innate immune subset expressing TNFRSF19.

Research Highlights:

1. Asymptomatic individuals lacked clonal expansion of effector CD8+ T cells but had a robust effector CD4+ T cell clonal expansion, coinciding with previously identified SARS-CoV-2-reactive CD4+ T cells in unexposed patients, suggesting pre-existing immunity may contribute to readily armed immunity.
2. Asymptomatic patients displayed distinct innate immune responses, including an increased $CD56^{bri}CD16^-$ NK subset.
3. $CD56^{bri}CD16^-$ NK subset in asymptomatic condition had upregulated cytokine-related genes including *IFNG* and *XCL2.*
4. Enrichment of less-defined subsets, Th2-like cells expressing a ciliated cell marker in asymptomatic infection.

Impact for COVID-19 research:

- Understanding immunological landscape associated with asymptomatic COVID-19 patients and highlight the importance of innate immune response towards disease progression
- For clinical setting, the study suggest that distinct NK subsets could be used in diagnosis and therapeutic interventions in COVID-19

Methodologies:

- Study Type: *in vitro analysis*
- Important cell lines/viral models used*: PBMCs from SARS-CoV-2 patients and healthy donors.*
- Key Techniques: *Droplet-based single-cell RNA sequencing, Integrating and clustering techniques for data analysis*

Limitations:

- As authors pointed, a small sample size: severe patient (n=1), moderate (n=7), and asymptomatic patients (n=2), therefore further investigation with larger cohort is needed to confirm the findings
- The analysis was performed in PBMC samples from the patients, it would be interesting to perform such analysis in samples from infected sites (i.e bronchoalveolar lavage (BAL) fluid) to fully dissect to immunological responses associated with asymptomatic in the sites of the infection.
- While the authors discussed the differential level of the cytokine-related genes in NK cells between the study groups, limited attention was given to the cytotoxic molecules, including GranzB and perforin, given the recent study linking CD56bright NK cytotoxicity and disease severity (Maucourant *et al*, 2020).

SARS-CoV-2 RNA viremia is associated with a sepsis-like host response and critical illness in COVID-19

Bermejo-Martin, J.F. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.25.20154252

Summary:

In this study Jesus Bermejo-Martin and colleagues look at how SARS-CoV-2 RNA levels in patient plasma associate with disease severity and other immunological signatures. Subject were divided in outpatients (50), hospitalised (100) and ICU patients (100). The study shows how SARS-CoV-2 RNA viremia correlates with higher levels of cytokines (CXCL10, IL-10, IL-15…), higher levels of ferritin and LDH as well as lymphopenia. Patients admitted in ICU were more likely than others to have detectable plasma SARS-CoV-2 RNA. The author points out how all these immunological response is compatible with sepsis, calling it “viral sepsis.”

Research Highlights:

1. SARS-CoV-2 RNA positivity in patient plasma is independently associated with some plasma cytokines (G-CSF, IL-10, CXCL10, IL1-ra, CCL-2, IL-15)
2. SARS-CoV-2 RNA positivity in patient plasma is independently associated with ferritin and LDH

3. SARS-CoV-2 RNA positivity in patient plasma is independently associated with lymphopenia
4. SARS-CoV-2 RNA in patient plasma is independently associated with critical illness
5. Severe COVID-19 patients display a sepsis-like phenotype

Impact for COVID-19 research:

- Most of the data in this article were already known. It has been established that severe COVID-19 patients have elevated pro-inflammatory cytokines, LDH and ferritin as well as lymphopenia
- The study suggests IL-15 level as possible clinical signature for critical illness

Methodologies:

- Study Type: *patient cohort*
- Key Techniques: *cytokine measurement, viral RNA detection, IgG detection SARS-CoV-2*

Limitations:

- The main limitation of the study is its failure to address viral levels anywhere but in the plasma. The study suggests that plasma viral RNA could be somewhat deleterious for COVID-19 outcome, but it could simply reflect overall viral load. All the phenotypic characteristics that they correlate with plasma SARS-Cov-2 RNA are already known to be associated with severe COVIS-19
- As they point out presence of live virus in peripheral blood is not confirmed
- The study suggests that worst COVID-19 outcome is driven by TLR stimulation by viral RNA in the periphery, but there are no direct experiments to prove that.

Genomic and Transcriptomic Analysis

Genome-wide bioinformatic analyses predict key host and viral factors in SARS-CoV-2 pathogenesis

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

Link: https://doi.org/10.1101/2020.07.28.225581

Summary:

Ferrarini *et al*. aimed to characterize the host response triggered specifically by SARS-CoV-2 infection. They addressed this using a publicly available RNA-seq dataset of human lung cells infected with SARS-CoV-2 and other respiratory viruses. Based on identification of SARS-CoV-2 specific gene, isoform, and pathway responses, they predicted putative interactions between the viral RNA genome and human RNA-binding proteins (RBP). Four viral sequence variants were identified to potentially play a role in disease severity. The host factors identified could serve as potential novel drug target.

Research Highlights:

1. Analysis of host-pathogen interaction on a cellular level which specifically contribute to SARS-CoV-2 replication and pathogenesis
2. Identification of RBP binding sites, which interact with SARS-CoV-2 in a conserved and specific way
3. SARS-CoV-2 virus protein translation could be EIF4B-dependent
4. Creation of publicly available workflow for mechanistic analysis on emerging pathogens

Impact for COVID-19 research:

- Interaction of SARS-CoV-2 with human cells on a molecular level helps understanding the specific mechanism of pathology, creating novel drug targets/prophylactics
- The findings need to be validated in vitro/in vivo as it's an in silico study

Methodologies:

- Study Type: *in silico*
- Important cell lines/viral models used: *NHBE, A549, Calu-3, original SARS-CoV-2, influenza A virus, respiratory syncytial virus and human parainfluenza virus 3 (dataset from Blanco-Melo et al., Cell, 2020)*
- Key Techniques: *bioinformatics*

Limitations:

- Host interaction analysis based on cell line data not always applicable to in vivo scenario; also doesn't differentiate between different severities of COVID-19

- As stated in the discussion, there might be a bias regarding collection time of viral genomes in the pandemic

Immune transcriptomes of highly exposed SARS-CoV-2 asymptomatic seropositive versus seronegative individuals from the Ischgl community

Lee, H.K. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.01.20185884

Summary:

The authors investigate immune cell transcriptomes from 43 asymptomatic seropositive and 52 highly exposed seronegative individuals to investigate immune responses in asymptomatic COVID-19. They also compare four mildly symptomatic seropositive cases to four highly exposed seronegative participants from the same family to validate their approach. The authors do not detect any differences in chemokine and cytokine expression between seronegative and asymptomatic seropositive cases and only little change in PBMC gene expression.

Research Highlights:

1. 16 hallmark gene sets are expressed at higher levels in mild cases compared to seronegative ones. 5 of them are involved in immune regulation, while 11 are not directly linked to the immune response.
2. Very little change (11 induced, 7 down-regulated) is detected in PBMC gene expression between seropositive asymptomatic and seronegative participants (data stated to be in supplements which cannot be accessed)
3. No differences are observed in chemokine and cytokine levels of seropositive asymptomatic and seronegative patients.

Impact for COVID-19 research:

- Very little clinical impact. It's interesting to see that asymptomatic COVID-19 does not seem to trigger an immune response. Would be interesting to investigate gene expression differences in non-immune cells.
- It would be good to see the gene expression data for asymptomatic and seronegative participants and to compare affected genes to mild and severe cases of COVID-19.

Methodologies:

- Study Type: *In silico*
- Key Techniques: *RNA sequencing from PBMCs, cytokine profiling from plasma*

Limitations:

- The patient sample is very specific, all participants are from a small rural region in Austria with very low predefined risk factors for COVID-19.
- Supplementary figures are not accessible, would be especially interesting to see data on PBMC gene expression for seronegative and seropositive asymptomatic participants.

Vaccines

Prime-boost protein subunit vaccines against SARS-CoV-2 are highly immunogenic in mice and macaques

Tan, H.X. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.09.01.278630

Summary:

In this preprint, the authors present their data comparing the efficacy and immunogenicity of both homologous and heterologous prime-boost vaccination of mice and macaques with either a viral spike (S) or a RBD subunit vaccine. S vaccines elicited superior immunogenicity in mice and comparable responses to RBD vaccination in macaques. Study highlights the potential of using recombinant spike protein as a vaccine candidate and highlights key differences in vaccine responses between mice and macaques.

Research Highlights

1. In mice, serum antibody titres, and GC B Cell and TFH cell responses are markedly improved with a single immunization of S rather than RBD
2. Homologous (RBD-RBD or S-S) or heterologous (RBD-S or S-RBD) prime boost vaccination of mice, elicited poor immunogenic responses if S was not included in the vaccination strategy.
3. Homologous (RBD-RBD or S-S) and heterologous (S-RBD) vaccination of macaques, elicited comparable immunogenic responses with all vaccine strategies utilized.
4. A comparison of serum and neutralizing Ab titres revealed that responses to vaccination in mice far exceed those observed in convalescent Covid patients*see limitation below.

Impact for COVID-19 research:

- Provides evidence that the immunological response to vaccination can be improved by including the spike protein alongside the RBD.
- Demonstrates the differences in the immunological response between mouse and non-human primates to the same vaccine-strategy, with particular reference to the differing VH gene usage and how this impacts on the generation of B cell responses post vaccination.

Methodologies:

- Study Type: *In vivo (mouse and NHP).*
- Important cell lines/viral models used: *Vaccine study*
- Key Techniques: *Flow cytometric analysis of S and RBD-specific B cells and TFH cells, BCR sequencing, ACE2-RBD inhibition ELISA.*

Limitations:

- As with all macaques studies the numbers are small, however, it is difficult to draw firm conclusions with a N of 2. Furthermore, the presentation of data in Figure 3 is particularly difficult to interpret for some of the panels.
- There are differences between the vaccine approaches used in the mice and macaques and this is not discussed by the authors. A vaccination dose-response, at least in mice should have been considered.
- In the macaque model, the RBD-S prime-boost vaccine has not been studied and the authors provide no explanation as to why.
- The patients recruited for the convalescent and B Cell studies, are mainly mild infections, but do include some severe individuals, would have been useful to highlight the severe patients in Figure 4.

IFN-α Therapy

Engineered interferon alpha effectively improves clinical outcomes of COVID-19 patients

Li, C. *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-65224/v1

Summary:

The study by Li *et al*. investigates the use of recombinant super compound interferon (rSIFN-co) compared to IFN-α treatment in COVID-19 patients in a single-blind, randomized trial. Specifically, the study enrolled 94 moderate-to-severe COVID-19 patients, who were also being treated with anti-viral agents. The authors found that the time to clinical improvement for the rSIFN-co group was faster (11.5 days) compared to the IFN-α group (14 days), as well as the rate of radiological improvement and negative test (for viral RNA) conversion. This study accentuates the potential for the use of rSIFN-co for the clinical management of COVID-19 patients.

Research Highlights:

1. Treating moderate and severe COVID-19 patients with rSIFN-co compared to IFN-α resulted in faster clinical improvement
2. The rate of improvement in radiological tests was accelerated for patients on rSIFN-co treatment compared to IFN-α
3. Patients treated with rSIFN-co had a negative test faster

Impact for COVID-19 research:

- This research is interesting in light of a study showing that severe COVID-19 patients have low IFN-α levels (Hadjadj, Science, 2020). Together, these results highlight the dampened type I interferon response in COVID-19 patients with severe disease, and that therapeutics reversing this might benefit patients. This investigation could aid disease management, although it would be important to assess the effects of using rSIFN-co in comparison to a placebo prior to extensive administration.

Methodologies:

- Study Type: *Clinical trial*
- Key Techniques: *SARS-CoV-2 qRT-PCR, chest CT scans*

Limitations:

- It would be important to include a control group (not receiving rSIFN-co or IFN-α) before extensive administration proceeds.
- Different concentrations of rSIFN-co (12 million IU) and IFN-α (5 million IU) were used in the study, casting some doubts about the clinical superiority of rSIFN-co, since the effects could be attributed to higher dosage.

Cardiology

SARS-CoV-2 infects human pluripotent stem cell-derived cardiomyocytes, impairing electrical and mechanical function

Marchiano, S. *et al*. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.30.274464

Summary:

Cardiovascular pathologies are commonly observed following SARS-CoV-2 infections. The underlying mechanisms of cardiopathologies however remain elusive. Marchiano et al. use hPSC systems to show that cardiomyocytes express the viral entry receptor ACE2 and can be readily infected by SARS-CoV-2. Further the authors demonstrate that infected hPSC-cardiomyocytes exhibit impair electrophysiological and contractile features. This study could provide further insight in the cardiological effects of SARS-CoV-2 infections and help with design of treatments for long-term effects of COVID-19.

Research Highlights:

1. SARS-CoV-2 can infect and replicated in hPSC-derived cardiomyocytes.
2. In the absence of cytopathic effects, SARS-CoV-2 can change electrophysiological cardiomyocyte properties (reduced beating rate, increased duration of field potential (QT interval proxy)).
3. In a three-dimensional engineered heart tissue, SARS-CoV-2 impairs contractile properties as measured by twitch forces.

Impact for COVID-19 research:

- Cardiovascular pathologies following (even mild) SARS-CoV-2 infection are often observed. The preprint uses advanced in vitro models to get a mechanistical understanding of the pathology induced by SARS-CoV-2. The overall findings are in line with previous studies. Together these studies shed an interesting light on SARS-CoV-2-induced cardiopathologies.

Methodologies:

- Study Type: *in vitro*
- Important cell lines/viral models used: *hPSC system used from different donors, SARS-CoV-2/ USA-WA1/2020*
- Key Techniques: *scRNAseq, contractility analysis with 3D-engineered heart tissue, multi-electrode assay to measure electrophysiology*

Limitations:

- Electrophysiological properties a most strongly impacted by high MOI (question of physiological relevance), low MOI of 0.1 shows only very modest impact on electrophysiological properties.

- As the authors outline, hPSC are known for being functionally immature.
- Especially in light of the absence of cytopathic effects, it remains unclear in how far stress responses (for instance induced through innate sensing mechanism, rather than infection) can impact on heart physiology and contractility. Similar observations have been made by Pérez-Bermejo et al., who has suggested that other soluble factors may have an impact on the cytoskeleton of iPSC-derived cardiomyocytes.
- Using controls with inflammatory mediators and their impact on cardiac physiology would promote the authors conclusion of direct effects of the virus on heart function.