CARDIFF UNIVERSITY
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UNIVERSITY OF OXFORD

COVID-19 Community Journal Club

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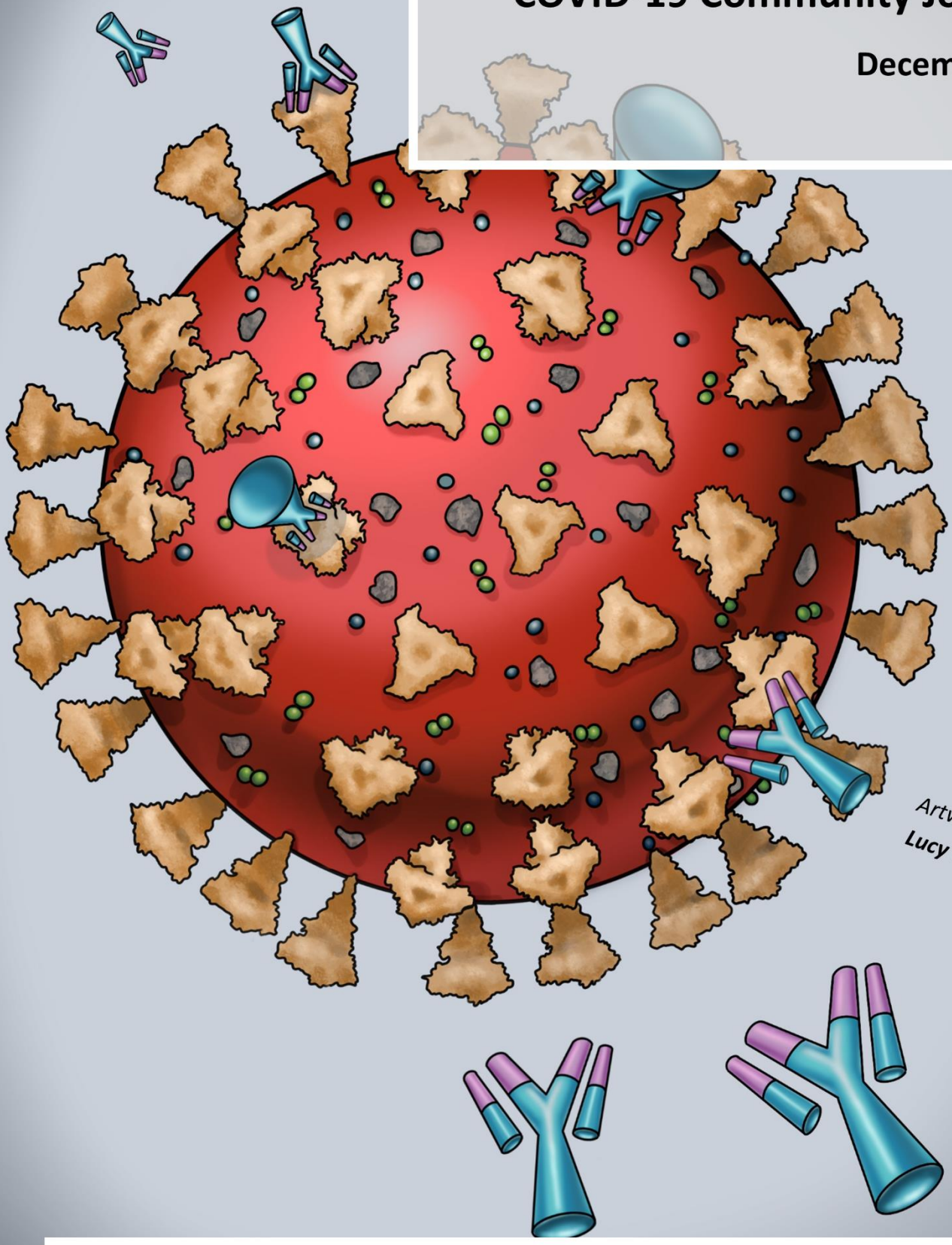

Artwork by ***Lucy Chapman***

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.

Since the first lockdown in March, the School of Medicine COVID-19 community journal club sought to help provide a balanced review of the rapidly emerging literature. When we started out, we didn't anticipate that the digests would be disseminated internationally and that we would be receiving feedback from intensivists in the US through to cancer doctors at Velindre.

Our efforts led us to collaborate with a similar initiative from Oxford University which allowed us to sustain our review output when many were allowed back into the laboratories. As a recently formed Oxford-Cardiff COVID-19 Consortium, we have been using the summarised reviews to help write a number of short communication articles to replace the current digests.

These articles, which focus on key aspects of the immune response to SARS-CoV-2, will be published by the new OUP journal *Open Immunology*, as "live reviews": allowing the authors to frequently update the information as more research findings become available.

This novel approach to reviewing literature is the first of its kind and will be pioneered by student, post-docs and ECRs from the CU SoM.

After 26 digests, 495 reviews and many beautiful arty covers (thank you to Lucy Chapman), we would like to thank everyone who has contributed to our COVID-19 journal club over the last 8 months, as well as those who read it and told us how useful it was.

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

The Oxford-Cardiff COVID-19 Literature Consortium

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SARS-CoV-2 N promotes the NLRP3 inflammasome activation to induce hyperinflammation

Pan, P. *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-101224/v1

Summary:

Pan *et al.* describe a novel mechanism for NLRP3 inflammasome activation by SARS-CoV-2 by evaluating the effect of SARS-CoV-2 proteins on the production of mature IL-1β. The authors found a direct interaction between the nucleocapsid (N) protein of SARS-CoV-2 and NLRP3 through the 260aa–340aa region of the N protein. The presence of the N protein appears to recruit NLRP3, that is otherwise disperse in the cytoplasm, and facilitates the formation of the inflammasome complex by promoting NLRP3-ASC binding. Via NLRP3 activation, the N protein induces a systemic inflammation that aggravates lung injury and shortens survival time in mouse models. The N-dependent proinflammatory activation can be prevented by NLRP3 inhibitor. This pre-print unravels how SARS-CoV-2 N protein contributes to hyperinflammation by promoting NLRP3 inflammasome, maturation of pro-inflammatory cytokine IL-1B and IL-6 production, and inducing pro-inflammatory responses in vitro and in mice.

Research Highlights:

1. SARS-CoV-2 N directly interacts with NLRP3 and both co-localise in the cytoplasm in speck structures
2. There is a N-dependent proinflammatory response with IL-1b maturation and IL-6 production
3. The N-induced NLRP3 inflammasome response relates to aggravate lung injury, accelerated death in sepsis and acute inflammation in mouse models
4. N-dependent hyperinflammation can be blocked by Ac-YVAD-cmk (NLRP3 inhibitor)

Impact for COVID-19 research:

- It unravels a novel mechanism by which SARS-CoV-2 drives hyperinflammation

Methodologies:

- Study Type: *in vitro, in vivo*
- Important cell lines/viral models used: *C57BL/6 mice, HEK293, PMA-differentiated THP-1, A549 cells*
- Key Techniques: *transduction, transfection, Co-Immunoprecipitation, ELISA, qPCR, Western Blot, His-pull-down assays, confocal microscopy and oligomerisation analysis.*

Limitations:

- The proteins were transfected into HEK293 cell line, and the levels of protein expression are not compared to the viral infection. Figure 1c shows a very big band of the N protein? That is not discussed in the text. The following wester blots are cropped.
- The levels of NLRP3 and other inflammasome components are transduced and the results of inflammasome activation might be over representative, as compared to endogenous levels.
- The fact that IL1B is dose-dependent but IL-6 production reaches a plateau is not discussed. (Fig 2j)
- The confocal images appear to be overexposed, although the quality of the images in the preprint version can be confusing.
- The authors suggest that the interaction of NLRP3 and ASC is enhanced by N protein in dose-dependent manner in HEK293T cells, and also talk about co-immunoprecipitation for N that earlier had not given this result and failed to interact with ASC. Questioning the importance of this interaction in physiological conditions.

Severe COVID-19 Is Fueled by Disrupted Gut Barrier Integrity

Giron, L.B. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.11.13.20231209

Summary:

The authors compare plasma from COVID-19 patients with different severities to healthy controls, investigating the link between SARS-CoV-2 and gut barrier integrity. They associate severe disease with tight junction permeability in the gut, microbial translocation into the blood and subsequent increased systemic inflammation.

Research Highlights:

1. Severe disease is associated with increased zonulin (drives tight junction permeability) and hospitalised patients with high plasma zonulin are more likely to die regardless of disease severity
2. Severe patients have increased plasma levels of LPS binding protein (LBP), β-glucan (marker of fungal translocation) and higher levels of soluble CD14 and myeloperoxidase
3. Higher levels of zonulin, LBP or β-glucan correlate with high levels of markers of systemic inflammation including IL-6
4. Severe disease is associated with a shift in plasma metabolites, including decreased citrulline, increased succinic acid and a higher kynurenine to tryptophan ratio which are associated with disrupted gut function and correlate with plasma IL-6 levels

5. Severe disease is associated with a shift in the plasma lipidome, marked by downregulation of glycerophospholipid and choline metabolism
6. Severe disease is associated with glycomic alterations with an apparent loss of the anti-complement activation galactosylated glycans from IgG and total plasma glycoproteins which negatively correlates with markers of translocation and inflammation

Impact for COVID-19 research:

- Moderate: the authors state that the kynurenine to trypthophan ratio alone is sufficient to distinguish hospitalised (moderate and severe) from non-hospitalised (control and mild) patients and could aid in predicting disease progression and risk of severe disease

Methodologies:

- Study Type: *In vitro*
- Key Techniques: *analysis of plasma using ELISA, Limulus Amebocyte Lysate assays and multiplex cytokine arrays; untargeted metabolomic and lipidomic analyses using mass spectrometry (MS); measurement of plasma glycomes using capillary electrophoresis and lectin microarray*

Limitations:

- The sample size (20 participants/group) is quite small and should be expanded.
- It is unclear whether gut permeability is due to effects of the gut-lung axis or direct SARS-CoV-2 infection of intestinal cells, this should be investigated.
- Also, it is unclear whether gut permeability is a result of SARS-CoV-2 infection or rather a pre-existing condition that exacerbates SARS-CoV-2 associated systemic inflammation.

Neutrophil extracellular traps induce the epithelial-mesenchymal transition: implications in post-COVID-19 fibrosis

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

Link: https://doi.org/10.1101/2020.11.09.374769

Summary:

Based on *in vitro* model and a small COVID-19 patient cohort Pandolfi *et al.* proposed, that macrophages release IL-8 and IL1β and increase NETs, causing epithelial-mesenchymal transition (EMT) and lung pathology. In lung epithelial cell line (A549) co-cultures with alveolar macrophages, neutrophils and SARS-CoV-2 virus for 48 hours. Confirmed EMT conversion, up-regulation of α-SMA (mesenchymal marker) and down-regulation of E-cadherin (epithelial marker). NETs, IL8 and IL1β (known NETosis effectors) were highest in co-cultures with alveolar macrophages and neutrophils. BAL from COVID-19 patients, showed more NETS in severe cases, positive correlation to % neutrophils and IL-8. EMT was observed in lungs of two non-survivors via IHC.

Research Highlights:

1. Cultured A549 with PMA-treated neutrophils for 24 and 48 hours, observed a fibroblast phenotype shift. Also looked at impact of isolated NETs on EMT.
2. Co-cultured A549 at air-liquid interface with alveolar macrophages, neutrophils and SARS-CoV-2 virus for 48 hours. Cells underwent EMT, up-regulated α-SMA and down-regulated E-cadherin.
3. Measured NETs via HNE-DNA complexes, IL8 and IL1β (known NETosis effectors). Highest levels in co-cultures with alveolar macrophages and neutrophils then neutrophils alone.
4. Measured NETs, IL-8 and IL-1β in BAL of mild (n=6) and severe/ICU (n=25) COVID-19 patients. More NETS in severe cases (especially non-survivors), positively correlated to compared IL-8 and neutrophil count in these samples.

Impact for COVID-19 research:

- Low- reiterating the importance of NETs in COVID-19 pathology, suggesting IL-8 and IL-1β.

Methodologies:

- Study Type: *in vitro and cohort study*
- Key Techniques:
    - *Co-cultured A549 (lung epithelial line) at air-liquid interface with alveolar macs, neutrophils and SARS-CoV-2 found multiple factors that induce NETs*
    - *Measured IL8 and IL1ϐ by ELISA in BAL from COVID-19 patients*

Limitations:

- A549 cell line has low endogenous ACE2 expression

- *In vitro* work needs to be validated *in vivo.*

Higher expression of monocyte chemotactic protein 1 in mild COVID-19 patients might be correlated with inhibition of IFN signaling

Xi, X. *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-71401/v4

Summary:

Measured cytokines in serum and gene expression in Peripheral Blood Mononuclear Cells (PBMCs) isolated from 30 mild and 10 severe COVID-19 patients alongside 10 healthy controls. Found that Monocyte Chemotactic Protein 1 (MCP-1) was higher in serum of COVID-19 patients compared to healthy controls. In mild cases with SARS-CoV-2 *CCR2* expression was increased and *IRF3* expression reduced. Overall, it was proposed that higher MCP-1 expression may be due to inhibition of interferon (IFN) signalling.

Research Highlights:

1. MCP-1 is higher in all COVID-19 patients. IP-10 and IL-8 were higher in severe cases compared to controls.
2. Mild COVID-19 patients PBMCs had increased levels of *CCR2* and reduced *IRF3* expression compared to controls.
3. IFN-β was downregulated in mild patients compared to healthy controls.
4. No differences in *CXCR3* and *CXCR2* expression.

Impact for COVID-19 research:

- Suggest that a high MCP-1 in mild patients and low *IRF8* expression on PBMCs stratify patients by disease severity.

Methodologies:

- Study Type: *Cohort Study*
- Key Techniques: *PBMC isolation, ELISA for MCP-1, IP-10, IL-8 and IFN-β*

Limitations:

- No data shown for *IRF3* and IFN-β expression in severe cases.

T Cells

Immunodominant T-cell epitopes from the SARS-CoV-2 spike antigen reveal robust pre-existing T-cell immunity in unexposed individuals

Mahajan, S. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.11.03.367375

Summary:

A pool of 11 predicted SARS-CoV-2 immunodominant epitopes were identified using a novel TCR-binding algorithm. These peptides from both the RBD and non-RBD regions of the spike protein induced robust T-cell activation in unexposed donors, suggesting pre-existing T-cell immunity to SARS-CoV-2. These predicted epitopes gave rise to stronger T-cell responses than epitopes within overlapping peptide pools. Those pre-existing TCRs that recognised SARS-CoV-2 peptides also recognised common viral antigens such as influenza and HCMV. This suggests a pool of pre-existing SARS-CoV-2 reactive T-cells are present in many of the population who have been exposed to influenza and CMV, which may provide protection against SARS-CoV-2 infection.

Research Highlights:

1. An algorithm named 'OvcoPeptVAC' was developed to predict $CD8^+$ T-cell activating epitopes across the SARS-CoV-2 proteome. This yielded a group of eleven 15-mer peptides. PMBCs collected from 17 unexposed donors, prior to the COVID-19 pandemic, were used in a T-cell activation assay. 70% of donors had strong IFNγ responses in both $CD4^+$ and $CD8^+$ T-cells against the predicted epitopes whereas responses against SARS-CoV-2 S1 & S2 overlapping peptide pools were lower.
2. The 11 identified epitopes had low homology to SARS-CoV and common cold coronaviruses suggesting pre-existing T-cell immunity may be from TCRs recognising other viruses.
3. TCR analysis was performed on antigen-stimulated PBMCs from 2 donors. Clonal expansion of multiple CDR3s recognising HCMV, Herpes virus 5 and influenza-A peptides was seen. These CD3Rs were not amplified by the overlapping peptide pools. These TCRs were then investigated to see whether the CD3Rs were 'public' (multiple antigen specificity) or 'private' (1 antigen specific). Donor 1 had only public CD3Rs amplified, whereas donor 2 had 2 private CD3Rs.
4. Single-cell RNA sequencing was performed on PBMCs stimulated with SARS-CoV-2 peptides. A predominantly effector $CD8^+$ T-cell phenotype was found, with transition towards effector memory phenotype.
5. The 11 identified SARS-CoV-2 epitopes were tested on convalescent plasma of SARS-CoV-2 recovered patients. The algorithm predicted epitopes were recognised by COVID-19 infected patient T-cells, with a higher IFNγ response in $CD8^+$ T-cells, especially in mild-moderate patients compared to asymptomatic or severe patients.

Impact for COVID-19 research:

- Robust T-cell activating epitopes identified may have implications for vaccine design. Understanding pre-existing T-cell responses to SARS-CoV-2 may help understand herd immunity and the adaptive immune response to SARS-CoV-2.

Methodologies:

- Study Type: *In vitro*
- Key Techniques*: T-cell activation assay (flow cytometry), TCR sequencing, 10x genomics scRNA-seq.*

Limitations:

- Low sample numbers.
- Yet to find out whether these cross-reactive TCRs have any benefit in preventing SARS-CoV-2 infection and how prevalent they are in the population.

Rapid, simplified whole blood-based multiparameter assay to quantify and phenotype SARS-CoV-2 specific T cells

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

Link: https://doi.org/10.1101/2020.10.30.20223099

Summary:

The study illustrates the feasibility of a whole blood-based workflow to assess the phenotype of SARS-CoV-2 reactive T cells in exposed and unexposed individuals in a manner that is rapid (approximately 7 hours) and requires very minimal amounts of blood (approximately 400µl).

Research Highlights:

- In their 7-hour workflow, followed by cell staining, the authors were to identify SARS-CoV-2 responding CD4 T cells in all tested patients with PCR positive swabs (n=9)
- Their workflow and staining panel was also able to establish cross-reactive T cells in 40% (6/15) of individuals
- Their analysis revealed clear distinctions between individuals with symptoms versus those without, including an early differentiated memory phenotype with limited expression of IFNγ and enriched in IL2+TNFα T cells in the former, compared to a late differentiated phenotype with IL-2+IFNγ+TNFα+ T cells in the latter

Impact for COVID-19 research:

- Provides a relatively rapid workflow that requires very small amounts of blood, making it applicable for diagnostic and research purposes. The small blood volume required makes this method applicable even to pediatric cases of COVID19

Methodologies:

- Workflow: *Briefly, the workflow utilized 400µl of whole blood, incubated for 5 hours with a SARS-CoV-2 peptide pool, co-stimulatory antibodies CD28 and CD49b and Brefeldin-A; the assay concluded in a 20-minute fixation-permeabilization sequence which prepared cells for staining and analysis*

Limitations:

- Their workflow requires access to commercially available peptide pools and to multi-color flow cytometers which may not financially be available in low-resource settings.

Clinical characteristics and predictive value of low CD4+T cell count in patients with moderate and severe COVID-19: A multicenter retrospective study

Wen, X. *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-46009/v2

Summary:

Previous studies with low sample sizes have shown that absolute numbers of $CD4^+$ and $CD8^+$ T cells are significantly lower in patients with severe SARS-CoV-2 disease when compared to those with moderate disease. This study has a larger cohort of 395 patients and demonstrates that a reduction of $CD8^+$ T cells indicated severity of COVID-19 and a decrease in $CD4^+$ T cells was an independent predictor of in-hospital cell death.

Research Highlights:

1. Lymphocyte and $CD8^+$ T cell count were reduced in severe patients and $CD8^+$ T cell count reflects severity of the patient's condition.
2. Patients with lower $CD4^+$ T cell counts required more oxygen inhalation, mechanical ventilation, antibiotic and glucocorticoid treatment compared to those with higher $CD4^+$ T cell counts.
3. In-hospital death rate was significantly higher in patients with lower $CD4^+$ T cell levels. These patients had a 13.659-fold increase in in-hospital death compared to patients with higher $CD4^+$T cell count

Methodologies:

- Study Type: *Retrospective study*

Limitations:

- Relatively small sample size of 395 patients
- There are no repeated blood samples

Antibodies & B Cells

Evolution of Antibody Immunity to SARS-CoV-2

Gaebler, C. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.11.03.367391

Summary:

This study presents longitudinal analysis of SARS-CoV-2 specific humoral responses in 87 COVID-19 patients at 1.3 to 6.2 months after infection. Both antibody titres against SARS-CoV-2 receptor binding domain (RBD) and antibody neutralizing capacity declined over time. However, RBD-specific memory B cells persisted up to six months after infection. These memory B cells display changes in the overall clonal composition, antibody sequence evolution clonal and are capable of generating antibodies with increased neutralizing potency and breadth. Consistent with continued evaluation of antibody responses, analysis of intestinal biopsies showed persistence of SARS-CoV-2 antigen small intestinal epithelium 3 month after infection. These data suggest that despite the declining of antibody titres, the persistence level of memory B cells which continue to evolve could provide effective humoral responses upon virus re-exposure.

Research Highlights :

1. The study performed longitudinal analysis of 87 participants with SARS-COV-2 infection for antigen binding and viral neutralization
2. Five-fold decrease in antibody neutralizing activity in plasma when measured using measured using an HIV-1 virus pseudotyped with the SARSCoV-2 spike protein.
3. The percentage of RBD-binding memory B cells increased marginally between 1.3 and 6.2 months in randomly selected individuals.
4. Consistent with the increase in IgG memory cells, the extent of somatic hypermutation for both IGH and IGL changed significantly in all selected individuals between 1.3 and 6.2 month time points.
5. The overall clonal composition of the memory B cell compartment differed at the two time points. Some of the expanded clones that were present at the earlier time point were not detectable after 6.2 months while new expanded clones appeared after 6 month of infection.
6. Memory B cells that evolved during the course of the infection express antibodies with increased neutralizing potency and breadth.
7. Examining intestinal biopsies obtained from 3 asymptomatic individuals 3 months after COVID-19 onset, showing persistence of SARS-CoV-2 using immunofluorescence, electron tomography or polymerase chain reaction in the small bowel of half of the selected participants.

Impact for COVID-19 research:

- The study suggests persistence of memory B cell responses after 6 months of infection and more interestingly these memory B cells continue to evolve.

Methodologies:

- Study Type: *ex vivo longitudinal analysis of convalescent plasma and PBMCs.*
- Key Techniques:
    - *flow cytometry live cell-based assay*
    - *SARS-CoV-2 neutralisation assay*
    - *High throughput automated serology assays*
    - *ELISA*
    - *SARS-CoV-2 pseudotyped reporter virus*
    - *Antibody sequencing, cloning and expression*
    - *Biopsies and Immunofluorescence*
    - *SARS-CoV-2 saliva PCR test*
    - *Production of monoclonal antibodies from single-sorted RBD-specific B cells*

Limitations:

- The authors note that while the study sample incorporated a longitudinal monitoring, samples collected during early phase of infection (days after infection vs 1 month) would enable to evaluate if early immune responses could influence memory B cell repertoire.
- Limitations noted by the authors include further analysis of residual SARS-CoV-2 antigens detected in the small intestine to evaluate their potential infectivity.
- sample size of the study. Therefore, future investigation in larger cohort will help in confirming the findings.
- While the study included participants with asymptomatic or mild, moderate and severe infections, minimal attention was given to the level of memory B cell responses in individual with the degree of the disease severity.
- Minimal evidence reported for clinical data and comorbidities of individuals. Therefore, it is not clear if any-existing conditions could contribute to the findings.

Prevalence of IgG Antibodies to SARS-CoV-2 in Wuhan – Implications for the Longevity of Antibodies Against SARS-CoV-2

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

Link: https://doi.org/10.1101/2020.06.13.20130252

Summary:

The authors investigate IgG antibodies from patients with SARS-CoV-2 confirmed by RT-PCR from February to April 2020 compared to healthcare workers, general workers and other patients without COVID-19 and negative RT-PCR. They found that levels of anti SARS-CoV-2 IgG antibodies do not correlate with age, gender, disease severity or comorbidities and that some patients lose IgG antibodies after 21 days post infection. They conclude that IgG antibodies to SARS-CoV-2 might not be long lasting.

Research Highlights:

1. The prevalence of IgG antibodies to SARS-CoV-2 was 89.8% in recovered COVID-19 patients, compared to 4% in healthcare workers, 4.6 % in general workers and 1% in other patients
2. IgG prevalence in healthcare workers and general workers increased with age
3. Among recovered COVID-19 patients, presence of anti SARS-CoV-2 IgG was not associated with disease severity, comorbidities and clinical characteristics but positively correlated with chloroquine/hydroxychloroquine and negatively with antibiotic treatment
4. 24 hospitalised COVID-19 patients lost previously detected IgG antibodies to SARS-CoV-2 after 21 days post symptom onset

Impact for COVID-19 research:

- Low: these findings are not unexpected or unprecedented

Methodologies:

- Stud Type: *Cross-sectional large cohort study*
  Key Techniques: *patients split into 4 groups: hospitalised patients with COVID-19, healthcare providers without confirmed COVID-19, general workers without confirmed COVID-19 and other patients without confirmed COVID-19. Serum was collected and tested with COVID-19 IgM/IgG antibody kits*

Limitations:

- The authors did not investigate whether IgG antibodies were neutralising and protective against SARS-CoV-2 re-infection.
- The study does not include patients with mild COVID-19 since they were admitted to a different hospital
- The last IgG test was conducted 41 days from symptom onset, it would be important to see how levels change at later timepoints

Single cell transcriptomic re-analysis of immune cells in bronchoalveolar lavage fluids reveals the correlation of B cell characteristics and disease severity of patients with SARS-CoV-2 infection

Kim, C.W. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.11.09.374272

Summary:

Bronchoalveolar lavage (BAL) fluids from patients with mild or severe COVID-19 were analysed via single cell RNA sequencing (scRNA-seq). Severe patients were found to have higher levels of activating genes for B cells along with immunoglobulin genes. Macrophages were also found to express increased levels of B cell activating factor (BAFF) in the severe cohort, with higher pro-inflammatory and Fc receptor-mediated pathway signatures. A larger population of CCR2 expressing plasma cells were also found in the severe patients. Together, these data help understand inflammatory and immune signatures in mild vs severe SARS-CoV-2 infection.

Research Highlights:

1. Using scRNA-seq analysis of BAL, the proportions of B-cells were found to be lower in the severe cohort of COVID-19 patients compared to those with mild disease. However, the actual number of B-cells was higher in the severe group. Gene set enrichment analysis (GSEA) revealed mild patients had genes for naïve or memory B-cells upregulated with higher CCR6 and CXCR3 expression. Whereas severe patients had upregulation of plasma cell and CXCR4 genes.
2. Severe patients had immunoglobulin and class-switching genes upregulated, along with CD69 and FOS in B-cells and TNFα signalling pathway genes.
3. B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) are members of the TNF superfamily involved in B-cell activation and differentiation. Gene signatures revealed severe patients were more affected by BAFF than APRIL. B-cells in the severe group had more genes upregulated that were linked to apoptosis as well as MHC-II compared to the mild group.
4. Macrophages were the most abundant cell type detected. Within the macrophage subsets, genes for IL-6, TNF, IL-1β and CCL2 were upregulated in the severe group and GSEA revealed higher inflammatory response pathways in this cohort. Fc receptor genes were expressed less in the macrophages of the mild group, whilst Fc receptor-mediated phagocytosis signalling pathways were upregulated in the severe group.
5. Severe COVID-19 patients had a higher amount of plasma cells present in BAL which express CCR2 and CCR10 highly along with upregulation of CCR2 ligands.

Impact for COVID-19 research:

- Understanding the immune landscape in severe vs mild COVID-19 infection can impact treatment of patients.

Methodologies:

- Study Type: *Cohort study.*
- Key Techniques*: single cell RNA-sequencing (scRNA-seq).*

Limitations:

- Upregulation of Fc-receptor signalling genes in macrophages of severe patients points towards antibody-dependent enhancement (ADE). However, most literature suggests ADE does not occur in SARS-CoV-2 infection.

Immunological Memory

Persistence of SARS-CoV-2 specific B- and T-cell responses in convalescent COVID-19 patients 6-8 months after the infection

Sherina, N. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.11.06.371617

Summary:

Adaptive immune response longevity to SARS-CoV-2 was investigated over 8 months using patients with mild to severe illness from Lombardy and mildly infected volunteers from Sweden. Serum antibody responses, as well as populations of memory B- and T cells were evaluated compared to historic controls. Despite a significant reduction in serum antibody levels by the end of the study, functional responses of SARS-CoV-2-specific T and memory B cells were maintained, irrespective of disease severity.

Research Highlights:

1. Samples taken within the early phases of recovery (7-28 days post symptom onset) had significantly elevated levels of both anti-S and anti-RBD IgG, IgA and IgM, when compared to historic controls.
2. Paired analysis of 27 patients found a significant decrease in serum IgA and IgM over time but no significant change in IgG. Prominent IgG responses were present in 12/15 patients who were followed up to 181-240 days post symptom onset.
3. RBD-specific producing B cells were found in 96% (25/26) of samples collected 3-8 months following symptom onset.
4. 3-8 months post symptom onset, IL-2, IFN-γ or IL-2/IFN-γ producing T cell responses were detected. The level of response (54-92%) varied with differing peptide pools.
5. Paired analysis for 5 patients found a significant increase in the number of virus-specific B and T cells in the samples taken later (median 204 days) than earlier (median 21 days), regardless of disease severity.

Impact for COVID-19 research:

- Demonstrates that there are functional B and T cells in patient's sera up to 8 months following symptom onset of SARS-CoV-2 infection. This has impact for long-term immunity studies and also the level of immunity achieved by a vaccine.

Methodologies:

- Study Type: *in vitro, cohort of patients*
- Key Techniques: *in-house ELISA to detect antibodies against RBD or spike, Human IgG SARS-CoV-2 RBD ELISPOT^{PLUS}* and Human IFN-γ/IL-2 SARS-COV-2 FluoroSpot^{PLUS} kits from Mabtech AB to analyse functional memory responses. Peptide pools were also sourced from Mabtech AB.

Limitations:

- Small group of samples, particularly sequential samples from the same patients.
- Possibly out of scope of papers aims, but showing cell phenotypes (particularly memory) would be nice.

Immunological memory to SARS-CoV-2 assessed for greater than six months after infection

Dan, J.M. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.11.15.383323

Summary:

This study assessed SARS-CoV-2 specific memory responses in a cross-sectional samples of 185 recovered COVID-19 patients at 1 and 6 months after infection. While spike IgG titers showed modest decline, RBD IgG and SARS-CoV-2 PSV neutralizing antibody titers were durable. In addition to antibodies, memory B cells specific to spike or RBD persisted for up to six months after infection. However, a decay of SARS-CoV-2-specific CD4+ T cells and CD8+ T cells were observed after 6 months of infection with a half-life of 3-5 months. Correlation analysis of different compartments of SARS-CoV-2 memory responses showed a heterogenicity of immune memory responses that wasn't attributable to gender or COVID-19 disease severity.These data suggest persistence of SARS-CoV-2 memory responses for up to six months after infection.

Research Highlights:

1. The study performed cross-sectional and longitudinal analysis of 185 participants with SARS-CoV-2 infection to assess three compartments of memory responses (B cells, CD4 T cells and CD8 T cells).
2. SARS-CoV-2 spike IgG titers were nearly stable greater than 6 months after infection, when assessing all COVID19 subjects by cross-sectional analysis.
3. The frequency of SARS-CoV-2 memory B cells increased several months post-infection suggesting that development of B cell memory to SARS-CoV-2 appeared to be robust and likely long-lasting.
4. Analysis T cell memory responses showed a greater percentage of individuals with detectable circulating SARS-CoV-2 memory CD8+ T cells observed at 1 month post infection (61%, (30/49 individuals) than more than 6 months after infection (50%, (9/18 individuals)) showing that SARS-CoV-2 memory CD8+ T cells declined over time.
5. Phenotypic analysis showed that the majority of SARS-CoV-2-specific memory CD8+ T cells were TEMRA with small subsets of TCM and TEM.
6. Circulating SARS-CoV-2 memory CD4+ T cell responses were robust with approximately one third of COVID-19 cases at 1 month PSO had > 1.0% SARS-CoV-2-

specific CD4+ T cells. However, SARS-CoV-2 memory CD4+ T cells declined over the 6 month time frame of this study.

Impact for COVID-19 research:

- The study suggests persistence of SARS-CoV-2 memory responses after 6 months of infection.

Methodologies:

- Study Type: *ex vivo cross-sectional longitudinal analysis of convalescent plasma and PBMCs.*
- Key Tec*hniques:*
  - *flow cytometry live cell-based assay*
  - *SARS-CoV-2 neutralisation assay*
  - *SARS-CoV-2 ELISA*
  - *SARS-CoV-2 specific memory B cells detection assay*
  - *Activation induced markers (AIM) T cell assay*

Limitations:

- Limitation noted by the authors include population representativeness; the sampling was geographically restricted, most of the subjects were from California or New York, USA.
- The authors note that while the study sample incorporated a longitudinal monitoring, samples collected during early phase of infection (days after infection vs 1 month) would enable to evaluate if early immune responses could influence SARS-CoV-2 memory B and T cell repertoire.
- While the study included longitudinal analysis of 2 time-points in some individuals, limitations noted by the authors include further analysis of three or more time-points to assess long-term kinetics of SARS-CoV-2 memory responses.
- While the study included participants with asymptomatic or mild, moderate and severe infections, minimal attention was given to the level of memory responses in individual with the degree of the disease severity.

Immune memory in mild COVID-19 patients and unexposed donors from India reveals persistent T cell responses after SARS-CoV-2 infection

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

Link: https://doi.org/10.1101/2020.11.16.20232967

Summary:

Immunological memory and cross-reactivity to SARS-CoV-2 has great influence in vaccine research. Ansari *et al*. examined two arms of adaptive immunity – $CD4^+$ T cells and B cells in an Indian population who had either recovered from mild COVID-19 or were unexposed (pre-pandemic). Immunity was detected up to 5 months post diagnosis but more interestingly, the authors identified a high magnitude of cross-reactive $CD4^+$ T cells to non-spike epitopes in 70% of unexposed individuals. Despite a high incidence, COVID-19 fatality in India is low: future work will be needed to determine where this cross-reactivity is responsible.

Research Highlights:

1. Samples collected 2-5 months post diagnosis showed high IgG titers against spike and nucleoprotein, which demonstrated neutralizing capacity.
2. 15/42 unexposed donors had IgG titres against SARS-CoV-2 nucleoprotein. Reactivity against the nucleoproteins of common cold strains HCoV-OC43 and HCoV-NL63 were seen in both donors and patients. A significant increase with recovered patients was only seen with HCoV-OC43.
3. All recovered COVID-19 patients had a high frequency of activation induced marker (AIM+) (OX40+CD137+) T cells in response to a HLA Class II predicted spike megapool.
4. An almost similar magnitude of response to a megapool of non-spike domain peptides was seen in both groups. 22/32 of unexposed individuals showed a significant increase in response compared to DMSO control.
5. Memory B cell responses to spike and nucleoprotein were found $\geq$ 4 months of recovery from mild disease. A 5-8 fold increase in response between spike and nucleoprotein was seen with IgG and IgA, but not IgM.

Impact for COVID-19 research:

- Provides further evidence regarding long-term immunity following a mildly severe SARS-CoV-2 infection.
- Demonstrates cross-reactivity in unexposed donors between common cold strain HCoV-OC43 and SARS-CoV-2 nucleoprotein. The authors highlight this as an area of potential further research, to assess the impact on this finding.

Methodologies:

- Study Type: *in vitro, ex vivo*
- Key Techniques: *IgG ELISAs against Spike and Nucleoprotein, Activation induced cell marker flow cytometry for $CD4^+$ T cells using peptide megapools, SARS-CoV-2 specific B cell ELISPOT*

Limitations:

- Relatively few sample numbers – only include one population so cross-reactivity may differ for different populations/demographics/disease severity.
- Would've been to see CD8 populations and functional ELISPOTs for the T-cell data.

Genetic Variants and Disease

A functional genomics approach to understand host genetic regulation of COVID-19 severity

Kuijpers, Y. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.11.10.20229203

Summary:

Yunus, K et al explore the physiological significance of the host genetic variants that influence susceptibility to severe COVID-19. By characterizing individuals with high risk employing the detailed clinical, immunological and multi-omics dataof the Human Functional Genomics Projects (HFGP). Although other similar studies have been published, this study compiles and analyses the data obtained in two Dutch cohorts of healthy volunteers from the 500functional genomics (500FG) cohort and the 300 Bacillus Calmetter-Guerin (300BCG) cohort to demonstrate that genetic variability explains an important component of the increased susceptibility to severe COVID-19 and its association with defective innate immune responses, endothelial function dysregulation is influenced by polymorphisms in sex chromosomes. Thus, the importance of this study in the development of novel treatment and prevention strategies for severe COVID-19

Research Highlights:

1. Covid-19 loci are enriched for expression in immune organs, chemokine signaling pathways and enhancer region
2. 3p21.31 loci are associated with lower production of monocyte-derived cytokines
3. Von Willebrand Factor (VWF) and lymphocytes are strongly colocalized with ABO loci
4. Men have a higher genetic severity risk of COVID-19 than woman

Impact for COVID-19 research:

- Considering their results this might suppose a benefit to clinicians as it shows who might be have a higher risk for severe COVID-19 although a genetic analysis shouldbe done to identify those individuals.

Methodologies:

- Study Type: *cohort study.*
- Important cell lines/viral models used: *500FG cohort study which consist of 451 healthy individuals of European ancestry with genotype measurement. 300BCG consist of 313 healthy Europeans that participated in a BCG vaccination study.*
- Key Techniques: *Cohorts were phased using Eagle v2.4 with the European population of HRC 1.1 2016 hg 2019 reference panel. For the immune parameter quantitative trait locus (QTL) profile they used an R package MatrixeQTL, for the colocalization analysis they used the 'coloc' package. For the functional analysis of genomic loci they used the*

FUMA pipeline to identified significant independent SNPs as variants. All the gene-sets were obtained from MsigDB, WikiPathways, and GWAS-catalog reported gene-sets.

Limitations:

- As their data comes from two different cohort studies (300BCG and 500FG) the stimuli used in measuring cytokine production are different. Thus it is not possible to replicate their findings
- As they mention in their discussion young adults are overrepresented which might lead to a biased conclusion considering that COVID-19 affects mostly elder people
- Both, 500FG and 300BCG consist in healthy cohorts studies it would be good to count with a COVID-19 patients' cohort

Virology

ORF3a mediated-incomplete autophagy facilitates SARS-CoV-2 replication

Qu, Y. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.11.12.380709

Summary:

The study by Qu et al investigates how SARS-CoV-2 affects autophagy. Specifically, the authors find that autophagy flux is disrupted following infection, a function they attribute to ORF3a. Through a set of immunoprecipitation experiments, Qu et al show that ORF3a binds to UVRAG, which is involved in autophagosome maturation, disrupting the binding to Beclin-1. However, the SARS-CoV ORF3a, which is similar, does not carry out these functions. Lastly, the authors illustrate that depletion of the autophagy-related protein ATG3 hampers SARS-CoV-2 replication. The autophagy pathway emerges as a double-edged sword in SARS-CoV-2 infection, with initial steps being beneficial for viral replication.

Research Highlights:

1. The SARS-CoV-2 protein ORF3a blocks autophagy flux
2. ORF3a interacts with UVRAG, which is involved in autophagosome maturation
3. The similar SARS-CoV ORF3a does not bind UVRAG
4. Atg3 knockout cells block SARS-CoV-2 replication

Impact for COVID-19 research:

- This article suggests that autophagy flux is disrupted following SARS-CoV-2 infection, while depleting central autophagy mediators reduces viral replication. Autophagy induction has previously been shown to limit SARS-CoV-2 spread *in vitro* (Gassen et al, BioRxiv, 2020). It is possible that the virus uses autophagosomes as a niche, while avoiding degradation in the lysosome. Therefore, drugs that target autophagy downstream of the UVRAG-Beclin-1 interaction might be relevant therapeutic targets for COVID-19.

Methodologies:

- Study Type: *in vitro*
- Important cell lines/viral models used: *SARS-CoV-2 strain SH01 (GenBank: MT121215.1); HeLa-hACE2, HEK293T, MEF, A549, Vero-E6, Calu-3*.
- Key Techniques: *LC3 shift assay (GFP-RFP) microscopy, co-IP, LC3-I/LC3-II conversion Western blot*

Limitations:

- Western blot quantification should be provided since LC3-I and LC3-II both increase in Figure 1A after infection
- Secondary structure prediction of SARS-CoV versus SARS-CoV-2 ORF3a would be useful to visualize changes in amino acids between the two.
- In figure S2D, I think the authors mean IP: Flag-Atg14 (Flag-Beclin-1 written).

Therapeutics and PPE

Intranasal fusion inhibitory lipopeptide prevents direct contact SARS-CoV-2 transmission in ferrets

de Vries, R.D. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.11.04.361154

Summary:

Here they show the potency of a dimeric form of a SARS-CoV-2 S-specific lipopeptide as an inhibitor, preventing this way viral entry in ferrets. This lipopeptide corresponds to the highly conserved heptad repeat (HR) domain at the C-terminus of the S protein. With this study they demonstrate the efficacy of SARS-CoV-2 S-derived HRC-peptide as a candidate antiviral that can be administered by inhalation or intranasal spray on the contrary to the majority of compounds which are injected. Not only that but also its other benefits such as its cost, long shelf life and storage.

Research Highlights:

1. [SARS-CoV-2-HRC-peg4]2-chol peptide inhibits SARS-CoV-2 entry
2. [SARSHRC-PEG4]2-chol prevents SARS-CoV-2 transmission in vivo
3. [SARSHRC-PEG4]2-chol-treated animals do not seroconvert

Impact for COVID-19 research:

- As it shown in this study this peptide could become a possible candidate to prevent COVID-19 infection as it demonstrates a highly efficacy inhibiting SARS-CoV-2 transmission in ferrets

Methodologies:

- Study Type: *in vivo.*
- Important cell lines/viral models used: *SARS-CoV-2 virus seronegative female ferrets*
- Key Techniques*: β-Gal complementation-based assay, cell toxicity assay using MTT cell proliferation assay, virus neutralization of ferret sera when incubated with 100TCID50 of virus*

Limitations:

- Ferrets although they have been considered a good animal model to study SARS-CoV-2 their clinical signs are limited.
- The peptide treatments were given after 3 or 4 DPI and it doesn't show for how long ferrets are protected against Covid-19

De novo design of potent and resilient hACE2 decoys to neutralize SARS-CoV-2

Linsky, T.W. *et al*. 2020. *Science*

Link: https://doi.org/10.1126/science.abe0075

Summary:

There is a need to develop therapeutics that will withstand mutational escape from single-strand RNA viruses such as SARS-CoV-2. The authors have here reported on the discovery of *de novo* hACE2 decoys from a computational protein design strategy. Decoys were designed to fully preserve the binding interface to prevent mutational escape. CTC-445 was found to be the best of 35,000 generated computational hACE2 decoys and was later modified by directed evolution to create CTC-445.2: a thermostable molecule with nanomolar affinity and high specificity to RBD. CTC-445.2 can potently neutralise SARS-CoV-2 and provides prophylactic protection in a hamster model.

Research Highlights:

1. CTC-445.2 demonstrated favourable affinity for SARS-CoV-2 RBD (K_D ~ 21.0 nM) and could compete with hACE2 for binding (IC_{50} ~ 10.4 nM) despite sharing little amino acids with the receptor. CTC-445.2 is thermodynamically hyperstable and its potency could be increased with increased avidity.
2. CTC-445.2 can simulatensously bind all three RBDs of the trimeric S protein in an "up" and "partially-down" conformation.
3. A strong correlation between binding to hACE2 and CTC-445.2 decoy (R^2 = 0.84) following site saturation mutagenesis of the SARS-CoV-2 RBD binding interface demonstrates an intrinsic resilience of the decoy to RBD mutational escape. Some mutations which reduce binding to decoy but not hACE2 were found, so escape is still theoretically possible.
4. hACE2 decoys fully neutralised SARS-CoV-2 two *in vitro* systems. Effect was greater when decoys were present throughout infection compared to pre- and post- infection only.
5. Single intranasal prophylactic dose of CTC-455.2d provided Syrian hamsters with 100% protection from lethal SARS-CoV-2 challenge ($5x10^5$ PFUs). Treatment group demonstrated little respiratory distress and modest weight loss compared to control.

Impact for COVID-19 research:

- This study details the discovery and efficacy of a novel potential therapeutic for SARS-CoV-2. Intrinsic resilience to mutational escape of the virus gives this decoy superiority over some potential monoclonal antibody candidates.

Methodologies:

- Study Type: *In silico, in vitro, in vivo*
- Important cell lines/viral models used: *Vero E6 and Calu-3 cells for in vitro neutralisation,*

- Key Techniques: *de novo protein design including a plethora of techniques, ELISA competition assays, biolyaer interferometry, Cryo-EM, VSV-luc pseudovirus neutralisation, viral infection assays, prophylactic dosing to hamsters*

Limitations:

- No evidence of statistical analysis limits weight of findings from *in vivo* work.

Rapidly self-sterilizing PPE capable of 99% SARS-CoV-2 deactivation in 30 seconds

Zinn, A.A. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.11.16.384040

Summary:

The authors have produced a self-sterilizing copper configuration, named ActiveCopper (aCu) that is capable of killing SARS-CoV-2 and other microbes in seconds – much faster than any other copper configuration. This paper demonstrates the testing carried out on this new material to illustrate that it could offer a defence against most microbes and should be used for protective equipment such as masks.

Research Highlights:

1. A cellulose/polyester fabric blend (commonly used to make surgical masks) and a rayon/polyester blend were tested by incubating the aCu formulations with Salmonella enterica bacteriophage and counting the plaques. 100% kill rates occurred between 30 seconds and 3 minutes.
2. Sustained antimicrobial efficacy was tested according to EPA protocol and showed no degradation after 14 days.
3. After 2 years of seasonal exposure, the copper did not change its morphology and was able to kill 100% of phages within 60 seconds.
4. Samples challenged with human influenza A (H1N1 and H3N2) and feline calicivirus VR-782 showed concentrations of all viruses had reduced by >99% within 2 hours.
5. The aCu-coated fabrics were shown to be safe for human use and proximity to the respiratory system by conducting shedding tests under simulated breathing conditions.

Impact for COVID-19 research:

- *Efficacy of aCU makes it a promising candidate for the development of self-cleaning PPE*

Neuropathology

COVID-19-associated olfactory dysfunction reveals SARS-CoV-2 neuroinvasion and persistence in the olfactory system

Melo, G.D. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.11.18.388819

Summary:

The authors examined the olfactory systems of patients with acute loss of smell (anosmia), detecting viral replication in the olfactory epithelium and sensory neurons associated with local inflammation. They found that SARS-CoV-2 RNA and antigens can persist locally in olfactory sensory neurons several months after general symptom resolution, associated with prolonged olfactory function loss and high levels of IL6 and myeloid cell infiltration.
They further used a hamster model to show that SARS-CoV-2 invades the CNS via the retrograde olfactory pathway.

Research Highlights:

1. All 5 patients with anosmia investigated had detectable SARS-CoV-2 RNA and high numbers of Iba1+ cells in olfactory mucosa samples which were not observed in controls
2. SARS-CoV-2 infected cells in the patients included mature sensory neurons, sustentacular cells and myeloid cells
3. In SARS-CoV-2 infected hamsters, viral RNA and SARS-CoV-2 nucleoprotein were detected in Iba1+ cells and uncharacterised cells in the olfactory bulb
4. SARS-CoV-2 infection in hamsters was associated with loss of ciliation in olfactory neuroepithelium, budding of viral particles from deciliated cells and SARS-CoV-2 nucleoprotein antigen detection in sensory neurons axons reaching the olfactory bulb, along with upregulations in Il6, Cxcl10, IfnbI and Il1b
5. Of 4 patients with persistent anosmia (110-196 days post first symptom) none had detectable SARS-CoV-2 RNA in nasopharyngeal samples, but all had detectable SARS-CoV-2 RNA in olfactory mucosa cytological samples, associated with abundant Iba1+ cells and upregulation of IL6 gene expression in 3/4 patients similar to levels in acute COVID-19

Impact for COVID-19 research:

- Moderate: the fact that prolonged anosmia is associated with SARS-CoV-2 persistence in the olfactory system could be important for patient management. Further, the paper suggests an important way in which SARS-CoV-2 could enter the CNS causing neuroinflammation.

Methodologies:

- Study Type: *In vitro, in vivo*

- Key Techniques: *nasal mucosa brush sampling, quantitative PCR and immunofluorescence; intranasal inoculation of Syrian golden hamsters with SARS-CoV-2, behavioural testing, scanning electron microscopy*

Limitations:

- The sample size (5 participants/group) is too small and needs significant expansion.
- Immunofluorescence should be improved, Figure 1 showing co-staining of mature olfactory neurons and viral nucleoprotein is unconvincing