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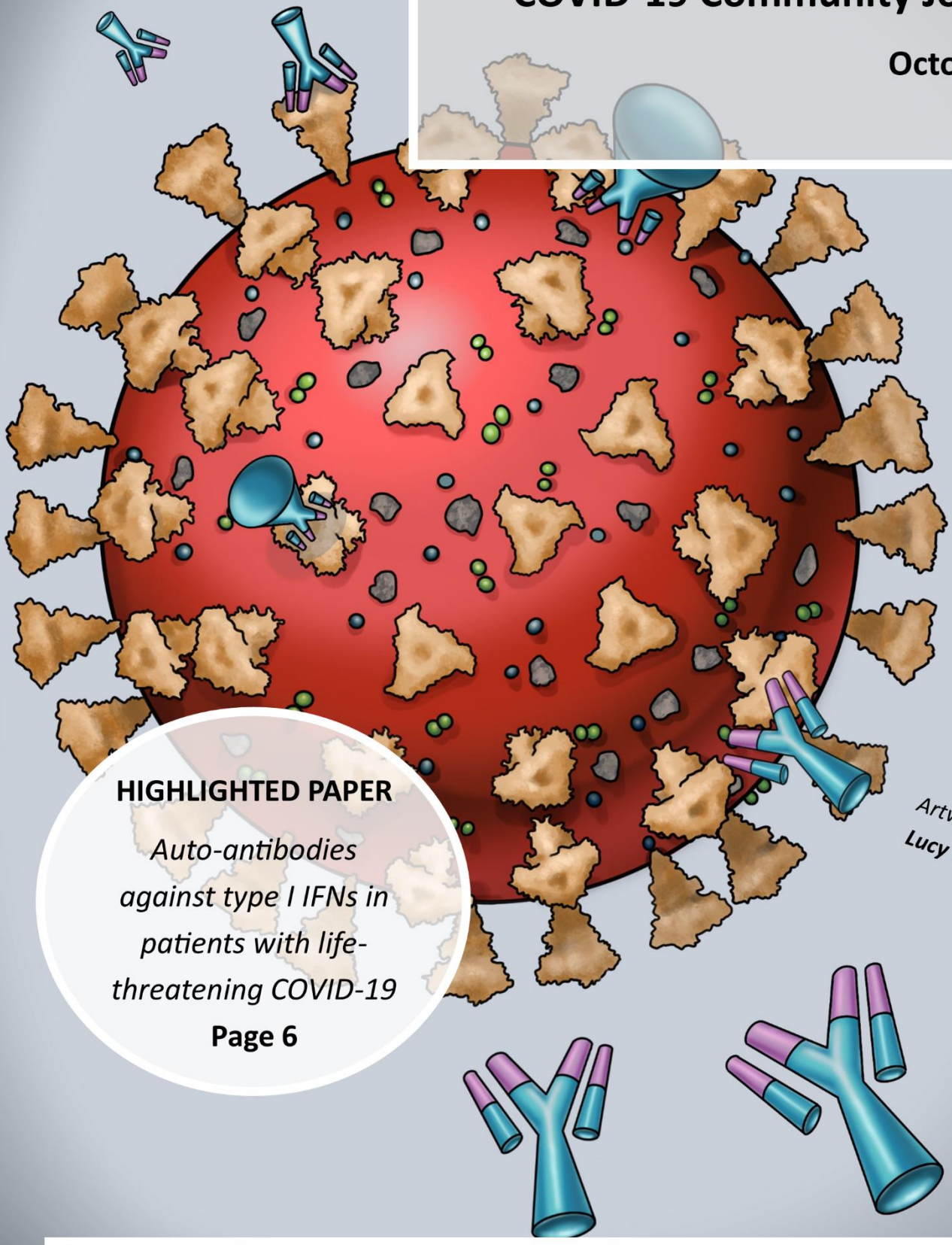

HIGHLIGHTED PAPER

Auto-antibodies against type I IFNs in patients with life-threatening COVID-19

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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.

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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.

Immune Responses and Clinical Implications

Auto-antibodies against type I IFNs in patients with life-threatening COVID-19

Bastard, P. *et al*. 2020. *Science*

Link: https://doi.org/10.1126/science.abd4585

Summary:

The authors compare the levels of neutralizing autoantibodies (auto-Abs) targeting type I interferons in asymptomatic, mild and severe COVID-19 to healthy controls. They report that no auto-Abs were found in asymptomatic and mild disease, but at least 10% of critically ill patients had auto-Abs against IFN-ω, IFN-α or both. The authors state that these antibodies are present at the onset of disease and causative of severe COVID-19 as they are able to block the ability of IFN to interfere with SARS-CoV-2 infection *in vitro.*

Research Highlights:

1. IgG auto-Abs against IFN-α2 and/or IFN-ω were detected in 13.7% of patients with life-threatening COVID-19
2. These antibodies were neutralizing *in vitro*
3. Patients with auto-Abs against IFN-α2 also had auto-Abs against all 13 IFN-α subtypes
4. Plasma from patients with neutralizing antibodies prevented the ability of IFN-α2 to block the infection of Huh7.5 cells with SARS-CoV-2 *in vitro*
5. 94% of critical COVID-19 with neutralizing auto-Abs against type I IFN were male
6. The presence of IFN targeting auto-Abs was associated with poor outcome (36.6% death)

Impact for COVID-19 research:

- Moderate: patients could be screened for auto-Abs neutralizing type I IFN to predict susceptibility to severe COVID-19 and patients that won't respond well to INF-α therapy.
- The study suggests an important role of type I IFN in combating the virus.

Methodologies:

- Study Type: *In vitro*
- Key Techniques: *analysis of plasma and serum by flow cytometry, ELISA and luciferase-based immunoprecipitation assay (LIPS), stimulation of PBMCs with IFN-α2 or IFN-ω in the presence of plasma from healthy individuals or patients with auto-Abs*

Limitations:

- More healthy participants need to be screened and followed up to see whether auto-Abs indeed exist before SARS-CoV-2 infection and drive severe disease.

Dynamic changes in serum IL-6, IL-8, and IL-10 are associated with the outcome of patients with severe COVID-19 in ICU

Li, J. *et al.* 2020. *Research Square*
Link: https://doi.org/10.21203/rs.3.rs-83336/v1

Summary:
The authors measured serum cytokines post ICU admission in 40 COVID-19 patients and retrospectively compared survivor vs non-survivor cytokine profiles, showing serum IL-6 and TNF-α higher in patients who died in ICU. IL-6 has been proposed as COVID-19 biomarkers by several studies, but antagonistic IL-6 treatments have not shown definitive success in improving COVID-19 patient outcome. IL-10 is generally considered anti-inflammatory but has been noted to be higher in sepsis patients, proposed the increase might be an attempt to moderate the immune response. This study provides useful information as to cytokine profile changes throughout COVID-19 disease.

Research Highlights:

1. Various pro- and anti-inflammatory cytokines were measured over samples taken from 40 ICU patients over 14 day (or more) period.
2. Timepoints were 1-3 days, 4-7 days, 8-13 days and 14 days onwards post ICU admission
3. Survivor and non-survivor profiles were compared retrospectively.
4. Serum IL-6, IL-10 and TNF-α was higher in severe cases where patients died.
5. IL-6, IL-8 and IL-10 were significantly associated with patient outcome despite the confounding variables e.g. age, gender, neutrophil count.

Impact for COVID-19 research:

- Could benefit disease management, looks at cytokine profile over time so could help identify high risk cases.

Methodologies:

- Study Type: *Retrospective cohort study*

Limitations:

- Only measured cytokines post-ICU admission, do not know if profiles differed before admission.
- Limited sample size, and some patients were missing particular clinical measures e.g. neutrophil counts.
- Survival was only measured during ICU, no follow up on patients who were discharged from ICU to see if they died later.

Immunometabolic Dysregulation

Metabolic stress and disease-stage specific basigin expression of peripheral blood immune cell subsets in COVID-19 patients

Siska, P.J. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.18.20194175

Summary:

Siska et al. investigated the role of immunometabolism in dysregulated immune responses in 47 SARS-CoV-2 infected patients and 16 un-infected volunteers. The level of cyclophilin A and expression of CD147 (also known as basigin, regulator of T cell activation and metabolism, and could mediate viral entry) were increased in T cells and monocytes of patients with progressed COVID-19 but resolved during recovery. T cell and monocytes from COVID-19 patient groups were characterized by different capacities to take up nutrients. While metabolic comorbidities (obesity and diabetes) affected immune cell glucose uptake capacity, increased T cell fatty acid uptake was a hallmark of COVID-19 patients with no or mild symptoms and decreased during convalescence. In addition to low nutrient uptake, T cells from progressed COVID-19 patients showed a metabolic dysregulation with increased mitochondrial mass, altered mitochondrial morphology and accumulation of ROS. Transcriptome analyses showed an increased T cell expression of ROS-related genes and basigin interaction partners and suggested a response to hypoxia by monocytes. Lastly, treatment with dexamethasone decreased T cell ROS accumulation, specifically in patients with high basigin levels. Collectively, these data suggest progressed COVID-19 is associated with a metabolic dysregulation characterized by ROS accumulation which is reversed during recovery or through pharmacologic modulation in vitro.

Research Highlights:

1. COVID-19 patients displayed altered NK and T cell frequencies, skewed T cell subset distribution and plasmablast induction.
2. The severe/critical group showed low NK cell and T cell frequencies and these changes persisted in recovering patients
3. A predominate increase in bulk and IgA+ and IgG+, but not IgM+ plasmablasts in SARS-CoV-2 infected subjects.
4. While no differences were detected in glucose uptake between COVIDD-19 patients and controls, patients with pre-existing metabolic disorders (diabetes and/or obesity) showed lower glucose uptake compared with controls. Interestingly, regardless of comorbidities, T cells from mild/asymptomatic patients showed an increase of fatty acid uptake which persist up to 4 weeks after symptom onset.
5. Basigin was strongly up-regulated on T cells from progressed COVID-19 patients and decreased during recovery. Despite increased basigin expression and high cyclophilin A plasma levels, immune cells from COVID-19 patients were likely not infected with SARS-CoV-2.

6. T cells from progressed COVID-19 patients showed a metabolic dysregulation with increased mitochondrial mass, altered mitochondrial morphology and accumulation of ROS. Transcriptome analyses showed an increased T cell expression of ROS-related genes and basigin interaction partners and suggested a response to hypoxia by monocytes.
7. T cell receptor stimulation induced ROS accumulation, accompanied by basigin up-regulation in T cells from COVID-19 patients and controls. Treatment with dexamethasone decreased T cell ROS accumulation specifically in T cells from patients with high basal basigin levels.
8. The progressed COVID-19 is associated with increased T cell basigin expression, and a metabolic dysregulation hallmarked by ROS accumulation that is reversed during recovery or through pharmacologic modulation in vitro.

Impact for COVID-19 research:

- Understand contribution of metabolic reprogramming in SARS-CoV-2 infection and its impact in immune dysfunction.
- The study argues in favor of using agents that reduce oxidative stress in COVID-19 therapy.

Methodologies:

- Study Type: *in vitro*
- Important cell lines/viral models used: *SARS-CoV-2-infected patients*
- Key Techniques:
    1. *Flow cytometry analysis of metabolic and mitochondrial parameters.*
    2. *Assessment of mitochondrial morphology was performed by Electron microscopical analysis*
    3. *RNA-seq analysis*
    4. *Enzyme-linked immunosorbent assay (ELISA) to measure plasma level of Cyclophilin A*

Limitations:

- The study showed glucose uptake by measurement of 2NDBG. The glucose transporter (Glut) was not measured in the study.
- The sample size is relatively small, particularly for asymptomatic/mild group (n=7) and moderate group (n=5). Therefore, future studies could confirm the findings in larger cohort.
- The study measure glucose uptake and mitochondrial mass by flow cytometric analysis. The data could be strengthened by measuring metabolic profile of immune cells from COVID-19 patients (glycolytic rate of Oxidative phosphorylation) by Seahorse technology.

T-cells

T cell anergy in COVID-19 reflects virus persistence and poor outcomes

Renner, K. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.21.20198671

Summary:

Hoang *et* al. applied novel approaches to analyse T cell reactivity in 55 COVID-19 patients. T cell activation was measured not only by cytokine production but also by downstream impacts on various cell types. The authors showed that these assays are more sensitive and consistent than classical readouts with PBMCs. Ventilated patients, particularly patients with fatal outcomes, showed lower responses of basophils, pDCs, monocytes and neutrophils to T cell activation and reduced T cell-derived cytokines (IL-3, GM-CSF and IFN-gamma), suggesting an impairment in T cell reactivity in these patients. Gender-specific differences in T cell-induced downstream effects on monocytes were observed. T cell anergy was associated with disease severity and prolonged viral replication. However, impairment in T cell reactivity was reversible in critically ill patients after recovery. Finally, a score was developed based on CD123-upregulation on monocytes, CD11b-upregulation on neutrophils and basophil count to predict fatal outcomes and identify patients who might benefit from strategies to overcome T cell anergy.

Research Highlights:

1. Impaired T cell reactivity (or T cell anergy) is a hallmark of COVID-19 patients and correlates with disease severity
2. IL-2 partially reversed T cell anergy
3. Whole blood is superior to PBMCs to quantify T cell anergy in COVID-19 patients
4. T cell anergy is reversible in critically ill COVID-19 patients after recovery
5. Basophil counts were markedly reduced in ventilated COVID-19 patients that subsequently died on the ICU
6. The numbers of pDCs were decreased while the total count of neutrophils was increased in ventilated COVID-19 patients
7. A combination of three parameters: CD123-upregulation on monocytes, CD11b-upregulation on neutrophils and basophil numbers could predict fatal outcome of patients on mechanical ventilation

Impact for COVID-19 research:

- Developing a sensitive approach to measure T cell reactivity in COVID-19 patients.
- Risk/prognosis estimation
- Potential target for therapy for critically ill patients

Methodologies:

- Study Type: *Clinical immune monitoring*
- Important cell lines/viral models used: *SARS-CoV-2-infected patients*
- Key Techniques:
    1. *T cell activation assays in whole blood with anti-CD3 antibodies.*
    2. *Flow cytometry*
    3. *ELISA*
    4. *Bioinformatic analysis for predictive score for death in ventilated COVID-19 patients.*

Limitations:

- Changes in protein expression on different subsets (including basophils, pDCs, monocytes and neutrophils) were presented as % of control. While the differences can be shown in %, the fold change could be a better representation of the data.
- T cell activation in PBMC was performed by stimulating T cells with anti-CD3 for 24 hours. It is not clear whether the same effect will be seen in a shorter stimulation (6h).
- No statistical tests were performed in longitudinal analysis of changes of monocytes and neutrophils, making it hard to predict the significance of the data.
- The cohort in the study included hospitalised COVID-19 patients. While perhaps outside the scope of the study, it would be interesting to compare T cell anergy in patients with less severe outcomes (i.e asymptomatic patients).
- T cell activation was measured by TCR stimulation (CD3). Minimal evidence presented for SARS-CoV-2 specific T cell responses.

Longitudinal high-throughput TCR repertoire profiling reveals the dynamics of T cell memory formation after mild COVID-19 infection

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

Link: https://doi.org/10.1101/2020.05.18.100545

Summary:

Minervina *et al.* characterise the dynamics and specificity of the T cell repertoire for two mild COVID patients through high-throughput TCR sequencing. Three different T cell dynamics are identified: TCR clonotypes constant through all timepoints, clonotypes that contract after day 15 of symptoms onset (contracting subset); and clonotypes with an unexpected late clonal expansion peaking at day 37 (expanding subset). Both CD4 and CD8 T cells participate actively in the response and are found in central (CM) and effector memory (EM) subsets, with a bias for CD4 to CM and CD8 to EMRA. Pre-COVID samples from the same patients show pre-existing cross-reactive T cell responses associated with the contracting subset. These pre-

infection clones appear to switch from CM to EM phenotype. Overall, this preprint shows two waves of clonal expansion driven by different TCRs reactive to SARS-CoV-2.

Research Highlights:

1. Identification of different memory T cell dynamics formed after mild COVID
2. Identification of pre-existing SARS-CoV-2 reactive TCRs
3. Phenotyping of the T cell memory response for mild COVID
4. Characterisation of TCRs reactive to SARS-CoV-2
5. Description of Public TCR sequence motifs of SARS-CoV-2 reactive clones.

Impact for COVID-19 research:

- The study identifies an expected clonal expansion and an unexpected later clonal expansion for T cells.

Methodologies:

- Study Type: *cohort study, TCR sequencing.*
- Important cell lines/viral models used: *cells from 2 mild COVID patients*
- Key Techniques: *ELISA, FACS, 5'-RACE TCR sequencing, bioinformatics analysis*

Limitations:

- Analysis limited to two patients with mild COVID, it would have been interesting to have some more samples and to compare it to severe or asymptomatic.
- After the matching and identification of the epitopes with the MIRA dataset, there is no functional assays to prove the predictions.

Antibodies

Comparative evaluation of six immunoassays for the detection of antibodies against SARS-CoV-2

Perez-Garcia, F. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.08.20190488

Summary:

In this study Perez-Garcia *et al.* evaluates for the first time six different commercial immunoassay (Alltest, One Step, Seroflash, Dia.Pro, COV2T, Elecsys). Demonstrating how reliable they are for COVID-19 diagnosis. Although this study doesn't show any new data, as it's a comparison it gives an idea of how sensitivity and specific these immunoassays are and which of those is the best option to consider for COVID-19 testing.

Research Highlights:

1. One Step, Dia.Pro, Elecsys and COV2T achieved the best diagnostic performance results
2. All the immunoassays evaluated showed a specificity of 100% and sensitivities over 97% from 14 days after onset of the symptoms

Impact for COVID-19 research:

- The data shown in this study do not alter our view of the disease but it might be useful to decide which type of test is the best candidate and which are their limitations

Methodologies:

- Study Type*: Retrospective study*
- Important cell lines/viral models used: *80 serum samples from patients with positive PCR for SARS-CoV-2*
- Key Techniques: Immunoassays used: *Three LFAs (Alltest, One Step and SeroFlash), one ELISA (Dia.Pro) and two CLIAs (Elecsys and COV2T)*

Limitations:

- This study was conducted in a single institution. It would be good to count with the participation of other institution to reinforce their findings and increase the number of participants.
- They only considered 6 different type of test. Although the number of test available is high it would be worth it to consider more tests to compare with.
- The participants were positive for COVID-19 and confirmed by PCR, all of them symptomatic. It might be something to consider to test those asymptomatic too or a follow up test.

A natural mutation between SARS-CoV-2 and SARS-CoV determines neutralization by a cross-reactive antibody

Wu, N.C. *et al*. 2020. *bioRxiv*
Link: https://doi.org/10.1101/2020.09.21.305441

Summary:
Better understanding of antibody cross-reactivity will benefit therapeutic and vaccine designs. Wu *et* al. used the highly neutralizing monoclonal antibody CR3022, which was isolated from a recovered SARS patient, to probe the antigenic variation between SARS-CoV and SARS-CoV-2. CR3022 recognises a 28-residue epitope which is largely conserved between the strains, but has a much greater affinity to SARS-CoV and cannot neutralise SARS-CoV-2. P384A mutation of SARS-CoV-2 increases CR3022's binding affinity and promotes neutralisation, with similar potency to that of SARS-CoV. Further analysis demonstrated that there is considerable flexibility in the binding of CR3022 and SARS-CoV RBD.

Research Highlights:

1. Of the 4/28 residues within CR3022's epitope that are not conserved between SARS-CoV and SARS-CoV-2, only mutation at residue 384 (P -> A) increased the antibody's affinity for SARS-CoV-2 spike protein (K_D = 1.4 nM) from WT (K_D = 68 nM).
2. P384A mutation promoted CR3022-mediated neutralisation of SARS-CoV-2 pseudovirus (IC_{50} – 3.2 µg/ml) which was similar to SARS-CoV (IC_{50} – 5.2 µg/ml).
3. Neutralisation of P384A mutant was similar at both IgG and Fab formulation.
4. Amino acid substitution at 384 alters the number of hydrogen bonds formed between the RBD and V_H S96 of CR3022 (A = 3, P = 1). This may impact the differences in CR3022 binding to SARS-CoV and SARS-CoV-2 RBD.
5. Structural analysis revealed that 3 CR3022 Fabs could simultaneously bind to one SARS-CoV S protein, whilst all three RBDs were in the "up" conformation. In addition, CR3022 could make quaternary contacts with the N-terminal domain during interaction.

Impact for COVID-19 research:

- Understanding antibody-epitope interactions, and the possibility of cross reactivity between antibodies, will better aid designs of therapeutics and vaccine candidates.

Methodologies:

- Study Type: *in vitro, in silico*
- Important cell lines/viral models used: *SARS-CoV and SARS-CoV-2 pseudovirus*
- Key Techniques: *crystallography, negative-strain electron microscopy, cryo-EM, biolayer interferometry for binding assays, neutralisation assay*

Limitations:

- Suggest differences in binding affinity could be caused by less hydrogen bonds between CR3022 and RBD at residue 384. This mutation is discussed in isolation and

does not consider the impact the differences at other non-conserved residues (372, 420, 519) would have on structural interaction.
- No statistics for both binding affinity and neutralisation assays.
- Figure 1 – results are representative of only two replicates.

A potent SARS-CoV-2 neutralizing human monoclonal antibody that reduces viral burden and disease severity in Syrian hamsters

Fagre, A.C. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.09.25.313601

Summary:

A panel of human monoclonal antibodies (mAbs) against the SARS-CoV-2 receptor binding domain (RBD) were identified using a yeast display library. These mAbs were able to neutralize the virus *in vitro* and the lead candidate was able to reduce viral load in Syrian hamsters under SARS-CoV-2 challenge, as well as improve lung pathology and reduce monocyte infiltration. This compound therefore has potential as a therapy to be used for COVID-19 patients.

Research Highlights:

1. A human mAb library displayed on yeast was used to identify a panel of mAbs against the SARS-CoV-2 RBD region. A series of assays such as MACS and FACS using recombinant SARS-CoV-2 RBD and competition ELISA with ACE2 were used to identify the most potent mAb candidates.
2. The most potent mAb; AvGn-B, efficiently blocked RBD-ACE2 binding with an IC_{50} value of 2.2 nM. *In vitro*, AvGn-B also showed 100% cell death protection against SARS-CoV-2.
3. AvGn-B was administered to Syrian hamsters on day 2 of SARS-CoV-2 infection. There was a significant reduction in lung viral load compared to the untreated group. However, there was no significant difference between lung viral load in the mice treated with AvGn-B and the isotype antibody control (IgG).
4. Pulmonary pathology was decreased in the AvGn-B treated group, as well as a reduction in inflammatory cell infiltrates in the pulmonary parenchyma of the AvGn-B high dose group.
5. The untreated group and IgG isotype control group had higher macrophage infiltration within the lungs seen by immunofluorescence, which was reduced dose-dependently by AvGn-B treatment.

Impact for COVID-19 research:

- If this mAb is successful, it could be used as a treatment for COVID-19 patients; there are currently very limited treatment options for those with severe COVID-19.

Methodologies:

- Study Type: *In vitro* & *in vivo*.
- Important cell lines/viral models used: Vero E6 cells.
- Key Techniques: *In vitro neutralisation assay, competition ELISA (SARS-CoV-2 RBD, ACE2), lung histopathology, immunofluorescence.*

Limitations:

- No improvement of animal weight loss with AvGn-B treatment.
- No difference in viral load in the animals treated with AvGn-B compared to those treated with the isotype control.

A potent synthetic nanobody targets RBD and protects mice from SARS-CoV-2 infection

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

Link: https://doi.org/10.21203/rs.3.rs-75540/v1

Summary:

In this study Li *et al*. demonstrate for first time that nanobodies can protect mice from live SARS-CoV-2. The most potent construct developed suggested an antagonistic mechanism to block the ACE2-RBD interaction. Not only they test their efficacy but they analyze the mechanism of binding by crystallography. In summary, they develop an efficient *in vitro* platform to generate neutralizing sybodies with a high affinity, neutralization activity and *in vivo* stability making them good candidates to treat COVID-19.

Research Highlights:

1. SR4, MR17 and MR3 neutralize SARS-CoV-2 by competitively blocking the ACE2-RBD binding
2. LR1, LR5 and MR3 bind to RBD.
3. The biparatopic LR5-MR3 sybodies were more potent than either sybodies alone despite the length of GS-linker.
4. The MR3-MR3 showed optimal neutralization activity with the longest GS-linker and similar activity to inhibit pseudotypes harboring the original SARS-CoV-2 S or the D614G mutant. Turning to be the most potent divalent sybody.

5. The addition of ABD to the MR3-MR3 gave the sybody the capacity to bind human albumin while retaining its ability to bind RBD and extend its in vivo stability, displaying neutralization activity up to 24hrs p.i

Impact for COVID-19 research:

- This study does not alter out view of the disease but it demonstrates the efficacy and stability in vivo of a sybodies. What they propose here is a new alternative to antibodies to treat Covid-19. As it is demonstrated, the MR3-MR3-ABD sybodie should be considered as a candidate and therefore should be considered in clinical trial.

Methodologies:

- Study Type: *in vitro and in vivo.*
- Important cell lines/viral models used: *Escherichia coli MC1061 was used to express all the sybodies except the MR3-MR3-ABD which was expressed in Pichia Pastoris GS115 and SMD1168H.*
- Key Techniques: *SARS-CoV-2 S-RBD binders were selected by perming one round of ribosome display using three high-diversity libraries and three rounds of phage display using the RBD as the bait under increasingly stringent conditions. This was followed by an ELISA and screened by fluorescence-detector size exclusion chromatography (FSEC) assay. Other techniques are bio-layer interferometry assay for binding kinetics; Crystallization and subsequent data collection at beamline BL19U1 structure determination for which they used 2Fo-Fc maps in Coot and refined using Phenix.*

Limitations:

- In the in vivo study only one single doses of 25mg/kg is administered. Might be worth to use different doses of the sybodies.

SARS-CoV-2 Reinfection

Reinfection with SARS-CoV-2 and Failure of Humoral Immunity: a case report.

Goldman, J.D. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.22.20192443

Summary:

Goldman *et al.* define a new SARS-CoV-2 reinfection case by whole viral genome sequencing and correlate the lack of protection to a weak humoral immune response. This preprint describes a case-study from a patient that was found re-infected 140 days (March and July) after the first SARS-CoV-2 PCR positive test but found less severely ill in re-infection. Whole genome sequencing of the viruses indicated that first and second infection were not due to prolonged viral shedding but to 2 genetically distinct virus. Antibody responses were evaluated only after re-infection and poor responses were found against spike and RBD (receptor binding domain) with a rapid decay. Overall, this pre-print describes a rare case of re-infection and correlates it with a failure in humoral immune responses.

Research Highlights:

1. Description of a re-infection SARS-CoV-2 case after 140 days of the first infection
2. Whole genome sequence of the virus shows 2 genetically distinct virus, last with D614G mutation.
3. Poor anti-spike and anti-RBD antibody responses were found in the patient after re-infection
4. No evidence of antibodies blocking RBD-ACE2 binding

Impact for COVID-19 research:

- It describes a case of re-infection and correlates it with profound defects in humoral immune responses. Helps to understand the immune correlates for humoral immunity that might prevent re-infection.

Methodologies:

- Study Type: *Patient-case study*
- Key Techniques: *whole genome sequencing,*

Limitations:

- The authors attribute re-infection to a poor humoral response from the first infection but there is no proof to that, other than a general weak humoral immunity for this patient after re-infection.
- The authors link the re-infection to the fact that first generated immune responses can't protect from re-infection of the now more circulating virus harbouring the D614G mutation. However, most studies show that antibodies against the Spike are

able to neutralize WT and D614G viruses and therefore re-infection may be ascribed in this case to malfunctioning of the humoral immunity.

Asymptomatic reinfection in two healthcare workers from India with genetically distinct SARS-CoV-2

Gupta, V. *et al*. 2020. *Clinical Infectious Diseases*

Link: https://doi.org/10.1093/cid/ciaa1451

Summary:

This paper looks at two case studies of genetically distinct SARS-CoV-2 reinfections – the first time that reinfections are reported in asymptomatic patients – raising questions about the immunity obtained following infection. It is possible that many SARS-CoV-2 reinfections are undetected indicating a requirement for strict surveillance in healthcare systems.

Research Highlights:

1. Two healthcare workers who were asymptomatic were reinfected with genetically distinct SARS-CoV-2
2. Both patients were reinfected within 4 months
3. Seven variants each for the two patients mapped to predicted immune epitopes
4. Both individuals had a higher viral load upon reinfection
5. Genetic variant 22882T>G (S:N440K) was found in patient 2 reinfection - possibly confers to resistance to neutralizing antibodies

Impact for COVID-19 research:

- Highlights the need for surveillance of SARS-CoV-2 reinfections, especially in the healthcare setting

Methodologies:

- Study Type: *Case-study*
- Key Techniques: *Sequencing-ready libraries were prepared using capture-based (TWIST Biosciences) as well as amplicon-based (COVIDSeq, Illumina) approaches*

Limitations:

- Only two patients were investigated

Virology

KIM-1/TIM-1 is a Receptor for SARS-CoV-2 in Lung and Kidney

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

Link: https://doi.org/10.1101/2020.09.16.20190694

Summary:

This study highlights how uptake of SARS-Cov-2 virosomes into human alveolar basal epithelial cells and pig kidney lines was significantly reduced upon blockade of KIM-1. Equally, the uptake of SARS-CoV-2 virosomes increased upon upregulation of KIM-1 on human tubuloids and KIM-1 was shown to bind to SARS-CoV-2 spike protein ectodomain in-vitro. Overall, these observations suggest that KIM-1 may be a receptor for SARS-CoV-2 in both the lung and kidney.

Research Highlights:

1. KIM-1 was shown to be expressed in the autopsy lung samples of 10/11 patients who had died from SARS-CoV-2 infection; equally, 3 live biopsies of infected patients and 14/30 post-mortem biopsies showed KIM-1 expression
2. Uptake of fluorescent SARS-CoV-2 virosomes into A549 human alveolar basal epithelial cells was significantly reduced with anti-KIM-1 IgG or with TW-37, an inhibitor of KIM-1 mediated endocytosis; comparable observations were made using mouse primary lung epithelial cells
3. SARS-CoV-2 virosomes were uptaken into pig kidney lines expressing human KIM-1, an effect that did not occur in controls without KIM-1 expression and that was shown to be independent of ACE2 expression
4. KIM-1 expressing cells of human three dimensional tubuloids endocytosed SARS-CoV-2 virosomes, and effect that was enhanced when KIM-1 expression was increased
5. Finally, KIM-1 was shown to bind to the SARS-CoV-2 spike protein ectodomain and GST-RBD *in-vitro*, with an EC50 of 19nM and 10nM respectively.

Impact for COVID-19 research:

- Implies that KIM-1 facilitated endocytosis of SARS-CoV-2 may contribute to the development of acute kidney injury, a major complication of SARS-CoV-2 infection, associated with higher morbidity and mortality of the virus. Given this, targeted treatment directed at KIM-1 may be therapeutic/prophylactic for acute kidney injury occurring secondary to SARS-CoV-2 infection

Methodologies:

- Study Type: *In-vitro*
- Key Techniques: *SARS-CoV-2 fluorescent virosomes composed of liposomes conjugate to spike ectodomain; internalization assay of virosomes; human renal tubuloids as a three-dimensional in-vitro model of kidneys*

Limitations:

- No assessment of KIM-1 expression of healthy volunteers was assessed, likely due to the difficult of obtaining kidney biopsies from healthy volunteers. Despite clearly shown *in-vitro*, assessing the contribution of KIM-1 to acute kidney injury using an *in-vivo* model would have strengthened their claims. Likewise, it would have been interesting to see if blocking KIM-1, either via antibody block or with TW-37, reduced uptake of virosomes into tubuloids.

Age is associated with increased expression of pattern recognition receptor genes and ACE2, the receptor for SARS-CoV-2: implications for the epidemiology of COVID-19 disease

Bickler, S.W. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.06.15.134403

Summary:

Bickler *et al*. look at a human dermal fibroblast dataset of genome wide RNA-seq profiles and compare gene expression levels between children (under 10) and the elderly (over 80) to flag any significant differences in key expression. The older cohort were linked to an increased expression of pattern recognition receptor (PRRS), ACE2 and four additional genes known to interact with SAR-C0V-2 proteins. PRR expression has the potential to predict risk of severe disease in patients.

Research Highlights:

1. TLR3, TLR4 and IHIF1 were differentially expressed between the older and younger groups (log2fc>1.0).
2. TLR4 was significantly increased in the elderly compare to the young (log2fc>2.6)
3. Nucleotide-binding oligomerization domain-containing protein (NOD1) and Cyclic GMP-AMP synthase showed a negative correlation with age.
4. ACE-2 expression increased with age and correlated with expression of 19 PRR genes, although variable in the over 80's.
5. ADAM9, FBLN5, FAM8A1 and CLIP4 which bind to SARS-CoV-2 proteins ORF8, M and Nsp13 increased with age.

Impact for COVID-19 research:

- PRRs play a key role in disease progression, therefore expression profiles of key PRRS could be a potential indicator of patients' risk of severe outcomes for SARS-CoV-2.

Methodologies:

- Study Type: *cohort study*
- Key Techniques: *Limma-Voom to identify differentially expressed genes*
  Cytoscape – identify protein-protein interactions

Limitations:

- Overall, relatively small numbers in each cohort
- No health profiles of subjects made available that may influence expression levels of PRRs.
- Minority groups poorly represented.
- No in vitro/vivo experiments performed to strengthen findings.

Vaccine Development

Pathogenicity, immunogenicity, and protective ability of an attenuated SARS-CoV-2 variant with a deletion at the S1/S2 junction of the spike protein

Wang, P. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.24.264192

Summary:

Ca-DelMut is an attenuated SARS-CoV-2 vaccine candidate. Hamsters vaccinated with Ca-DelMut were fully protected from live challenge with two different SARS-CoV-2 strains. Immunization reduced viral titers to undetectable levels in the lungs, induced RBD specific neutralizing antibodies, abrogated weight loss and reduced lung pathology. Infection with Ca-DelMut did not induce the high levels of proinflammatory cytokines (INF-g, CCR4, IL-10, IL-21, TNF-a) seen in WT virus infections. Notably, there was no activation of IL-6, which has been recognized as a biomarker for COVID-19 disease severity.

Research Highlights:

1. Researchers adapted a variant of SARS-CoV-2 with a deletion in the S1/S2 junction of the spike protein, 'Ca-DelMut'
2. Ca-DelMut replicates efficiently in vitro
3. Ca-DelMut does not induce elevated levels of proinflammatory cytokines or body weight loss in a hamster model
4. Ca-DelMut did trigger a neutralizing antibody response in hamsters
5. Ca-DelMut immunized hamsters challenged with WT SARS-CoV-2 showed no body weight loss and had no signs of viral replication in the respiratory tract

Impact for COVID-19 research:

- Develop a vaccine for SARS-CoV-2/COVID19

Methodologies:

- Study Type: *in vitro, in vivo (hamster)*
- Important cell lines/viral models used:
    - *Cell lines: Vero E6, Calu-3*
    - *Viral models: SARS-CoV-2 WT virus HK-13 and HK-95 for viral challenge, SARS-CoV-2 WT virus HK-001a for neutralization assay, SARS-CoV-2 Cal-DelMut as candidate vaccine*
- Key Techniques: *Anti-spike RBD IgG ELISA, intranasal vaccination of hamsters, histopathological assessment of hamster lung and nasal tissue, plaque assay, neutralization assay of sera from hamsters, qRT-PCR for cytokine profiling*

Limitations:

- N=3 for each sub-group, which is too small to confer statistical significance to the results.
- No mention of blinding e.g. for the histological analysis.
- No description of replicates for ELISA data. Would be good to see raw data with CVs calculated in the Supplementary Figures.
- Done in hamster model, rather than a non-human primate model. This may not translate well to humans.
- Uses replication in Vero E6 cells to claim that Ca-DelMut replicates more efficiently in vitro than WT virus, but Vero E6 is a limited model and the Calu-3 data is more equivocal.
- No statistical comparison included for IL-6 and IL-13 in Figure S3.
- Likely outside scope of study, but no long-term data on antibody titers or protective efficacy.
- The paper does not discuss potential safety issues with using an attenuated live virus as a vaccine, and potential to revert to a more pathogenic form in vivo.

Prophylactic intranasal administration of a TLR2 agonist reduces upper respiratory tract viral shedding in a SARS-CoV-2 challenge ferret model

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

Link: https://doi.org/10.1101/2020.09.25.309914

Summary:

In this study Pamela Proud and colleagues investigate the prophylactic action of TLR2 agonist INNA-051 against SARS-CoV-2 when administered intranasally in ferrets. Groups of 6 ferrets were treated i.n. with 3 different doses of INNA-051 or PBS 4 and 1 day before infection. Levels of SARS-CoV-2 RNA where measured in nasal and throat swabs up to day 12-14 post-infection and in the lungs at day 3 and 12-14. Treatment with TLR2 agonists does not cause changes in temperature or weight in the ferrets, suggesting lack of excessive inflammation. Any of the treatment conditions significantly reduces the viral load in the swabs (from day 5 post infection), as well as in the lungs at day 3.

Research Highlights:

1. Prophylactic treatment with INNA-051 in ferret does not cause adverse effects (weight loss, fever)
2. Prophylactic treatment with INNA-051 reduces upper respiratory tract viremia in ferret after SARS-CoV-2 infection.

Impact for COVID-19 research:

- While a vaccine is still preferable for its long-term effect a prophylactic treatment against SARS-CoV-2 could potentially be useful for situation of elevated risk of community transmission. The possible applications are nonetheless limited

Methodologies:

- Study Type: *animal model*
- Important cell lines/viral models used*: SARS-CoV-2 Victoria/01/2020*
- Key Techniques: *RT-qPCR*

Limitations:

- While the authors do not declare any conflict of interest the study has been founders and partially designed by Ena Respiratory, the company producing INNA-051
- The study only address inflammation caused by the INNA-051 treatment by temperature and weight loss, there could be other side effects less systemic
- The study fail to determine if it is possible to use this prophylactic approach repeatedly on the same subject or it this could cause sensitization. This is a major point to clarify for real word applications.
- It would be interesting to see if the treatment works if applied right after exposure.
- The TLR2 agonists has only been studied in animal models, interactions with human immune system or with other pathogen frequently present in the population is unknown
- Ferrets only develop mild COVID-19 symptoms, the efficacy against severe infections is unknown