2020-08-17

Welcome to Week 22 of the continuing COVID-19 saga!

Let's kick the week off with the iconic song of my teen years, Bob Dylan's Blowin' in the Wind. It seems a fitting anthem for today. Though it has been covered by a large number of artists (including Marlene Dietrich!), I'll offer up a couple of my favorites beginning with the duet version from Dylan and Joan Baez (I think this is circa 1982): https://www.youtube.com/watch?v=Io3HY7Yebjk another duet from Fort Collins, Colorado from 1976: https://www.youtube.com/watch?v=RxMQYF6EFCU Here is Bob in a 1963 TV clip when his voice was still fresh: https://www.youtube.com/watch?v=vWwgrjjIMXA

US COVID-19 STATISTICS - **Infection Rate: 1.6%; CFR: 3.1%** (IR no change; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

Bloomberg have a good article on COVID-19 vaccine manufacturing issues.

The Washington Post discusses the conundrum doctors are facing in treating COVID-19. Latina mothers have been hard hit by COVID-19.

The New York Times asks the big question, "can the area hold off a second wave of COVID-19?" New Zealand have delayed their election by four weeks because of the recent outbreak. Los Angeles look to have a very ambitious testing plan for students and workers. Here is a good article on the question of what constitutes herd immunity; as with everything else COVID-19 it is a bit complicated. Finally, here is summary of the recent research into lasting immunity, a number of papers have appeared in recent days pointing to this good news.

STAT address the Rumsfeld Paradigm about COVID-19. They also offer an interesting video on how pandemic stress alters your perception of time. Personally, I am experiencing extreme time confusion.😆

Nature have an interesting article on the new outbreak in New Zealand, noting genomics may reveal the source of the outbreak.

Thankfully, it is a slow Monday for reading papers!

MODELING

- Background: The absence of systematic surveillance for SARS-CoV-2 has curtailed accurate appraisal of transmission intensity. Our objective was to perform case detection of an entire rural community to quantify SARS-CoV-2 transmission using PCR and antibody testing. Methods: We conducted a cross-sectional survey of the prevalence and cumulative incidence of SARS-CoV-2 infection in the rural town of Bolinas, California (population 1,620), four weeks following shelter-in-place orders. Residents and county essential workers were tested between April 20th-24th, 2020. Prevalence by PCR and seroprevalence combining data from two forms of antibody testing were performed in parallel (Abbott ARCHITECT IgG to nucleocapsid protein and in-house IgG ELISA to the receptor binding domain). Results: Of 1,891 participants, 1,312 were confirmed Bolinas residents (>80% community ascertainment). Zero participants were PCR positive. Assuming 80% sensitivity, it would have been unlikely to observe these results ($p<0.05$) if there were >3 active infections in the community. Based on antibody results, estimated prevalence of

prior infection was 0.16% (95% CrI: 0.02%, 0.46%). Seroprevalence estimates using only one of the two tests would have been higher, with greater uncertainty. The positive predictive value (PPV) of a positive result on both tests was 99.11% (95% CrI: 95.75%, 99.94%), compared to PPV 44.19%-63.32% (95% CrI range 3.25%-98.64%) if only one test was utilized. Conclusions: Four weeks following shelter-in-place, active and prior SARS-CoV-2 infection in a rural Northern California community was extremely rare. In this low prevalence setting, use of two antibody tests increased the PPV and precision of seroprevalence estimates. [**note: I believe this is an update to early UCSF research on this small community by including serology testing. Clearly living in an isolated area which little incoming traffic may mean no COVID-19 infections.**] https://www.medrxiv.org/content/10.1101/2020.08.15.20175786v1

- The emergence of SARS-CoV-2 has resulted in an ongoing global pandemic with significant morbidity, mortality, and economic consequences. The susceptibility of different animal species to SARS-CoV-2 is of concern due to the potential for interspecies transmission, and the requirement for pre-clinical animal models to develop effective countermeasures. In the current study, we determined the ability of SARS-CoV-2 to (i) replicate in porcine cell lines, (ii) establish infection in domestic pigs via experimental oral/intranasal/intratracheal inoculation, and (iii) transmit to co-housed naive sentinel pigs. SARS-CoV-2 was able to replicate in two different porcine cell lines with cytopathic effects. Interestingly, none of the SARS-CoV-2-inoculated pigs showed evidence of clinical signs, viral replication or SARS-CoV-2-specific antibody responses. Moreover, none of the sentinel pigs displayed markers of SARS-CoV-2 infection. These data indicate that although different porcine cell lines are permissive to SARS-CoV-2, five-week old pigs are not susceptible to infection via oral/intranasal/intratracheal challenge. Pigs are therefore unlikely to be significant carriers of SARS-CoV-2 and are not a suitable pre-clinical animal model to study SARS-CoV-2 pathogenesis or efficacy of respective vaccines or therapeutics. [**note: here is one farm animal that may not be a reservoir for SARS-CoV-2**] https://www.biorxiv.org/content/10.1101/2020.08.15.252395v1

NEWLY REGISTERED CLINICAL TRIALS

- Amazingly, there are no new registered trials of drugs or vaccines.

CLINICAL TRIAL RESULTS

- Background: Both COVID-19 and influenza A contribute to increased mortality among the elderly and those with existing comorbidities. Changes in the underlying immune mechanisms determine patient prognosis. This study aimed to analyze the role of lymphocyte subsets in the immunopathogenesisof COVID-19 and severe influenza A, and examined the clinical significance of their alterations in the prognosis and recovery duration. Methods: By retrospectively reviewing of patients in four groups (healthy controls, severe influenza A, non-severe COVID-19 and severe COVID-19) who were admitted to Ditan hospital between 2018 to 2020, we performed flow cytometric analysis and compared the absolute counts of leukocytes, lymphocytes, and lymphocyte subsets of the patients at different time points (weeks 1-4). Results: We reviewed the patients' data of 110 healthy blood donors, 80 Non-severe COVID-19, 19 Severe COVID-19 and 43 severe influenza A. We found total lymphocytes (0.93*109/L, 0.84*109/L vs 1.78*109/L, $P < 0.0001$) and lymphocyte subsets (T cells, CD4+ and CD8+ T cell subsets) of both severe patients to be significantly lower than those of healthy donors at early

infection stages. Further, significant dynamic variations were observed at different time points (weeks 1-4). Conclusions: Our study indicates lymphopenia to be associated with disease severity and suggests the plausible role of lymphocyte subsets in disease progression, which in turn affects prognosis and recovery duration in patients with severe COVID-19 and influenza A. [**note: from China, a comparison of lymphocyte subsets in Influenza A and COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.08.15.20175455v1

DRUG DEVELOPMENT

- The emergence of SARS-CoV-2 led to pandemic spread of coronavirus disease 2019 (COVID-19), manifesting with respiratory symptoms and multi-organ dysfunction. Detailed characterization of virus-neutralizing antibodies and target epitopes is needed to understand COVID-19 pathophysiology and guide immunization strategies. Among 598 human monoclonal antibodies (mAbs) from ten COVID-19 patients, we identified 40 strongly neutralizing mAbs. The most potent mAb CV07-209 neutralized authentic SARS-CoV-2 with IC50 of 3.1 ng/ml. Crystal structures of two mAbs in complex with the SARS-CoV-2 receptor-binding domain at 2.55 and 2.70 A revealed a direct block of ACE2 attachment. Interestingly, some of the near-germline SARS-CoV-2 neutralizing mAbs reacted with mammalian self-antigens. Prophylactic and therapeutic application of CV07-209 protected hamsters from SARS-CoV-2 infection, weight loss and lung pathology. Our results show that non-self-reactive virus-neutralizing mAbs elicited during SARS-CoV-2 infection are a promising therapeutic strategy. [**note: from Germany, identification of a potent neutralizing antibody**] https://www.biorxiv.org/content/10.1101/2020.08.15.252320v1
- Virtually all SARS-CoV-2 vaccines currently in clinical testing are stored in a refrigerated or frozen state prior to use. This is a major impediment to deployment in resource-poor settings. Several use viral vectors or mRNA. In contrast to protein subunit vaccines, there is limited manufacturing expertise for these novel, nucleic acid based modalities, especially in the developing world. Neutralizing antibodies, the clearest known correlate of protection against SARS-CoV-2, are primarily directed against the Receptor Binding Domain (RBD) of the viral spike protein. We describe a monomeric, glycan engineered RBD protein fragment that is expressed at a purified yield of 200mg/L in unoptimized, mammalian cell culture and in contrast to a stabilized spike ectodomain, is tolerant of exposure to temperatures as high as 100°C when lyophilized, and upto 70°C in solution. In prime:boost guinea pig immunizations, when formulated with the MF59 like adjuvant AddaVax™, the RBD derivative elicited neutralizing antibodies with an endpoint geometric mean titer of ~415 against replicative virus, comparing favourably with several vaccine formulations currently in the clinic. These features of high yield, extreme thermotolerance and satisfactory immunogenicity suggest that such RBD subunit vaccine formulations hold great promise to combat COVID-19. [**note: here is an interesting paper from India on a thermo-tolerant fragment that may be useful as a vaccine. It may prove useful in areas where refrigeration is impractical.**] https://www.biorxiv.org/content/10.1101/2020.08.15.252437v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- SARS-CoV-2-specific antibodies, particularly those preventing viral spike receptor binding domain (RBD) interaction with host angiotensin-converting enzyme 2 (ACE2) receptor, could

offer protective immunity, and may affect clinical outcomes of COVID-19 patients. We analyzed 625 serial plasma samples from 40 hospitalized COVID-19 patients and 170 SARS-CoV-2-infected outpatients and asymptomatic individuals. Severely ill patients developed significantly higher SARS-CoV-2-specific antibody responses than outpatients and asymptomatic individuals. The development of plasma antibodies was correlated with decreases in viral RNAemia, consistent with potential humoral immune clearance of virus. Using a novel competition ELISA, we detected antibodies blocking RBD-ACE2 interactions in 68% of inpatients and 40% of outpatients tested. Cross-reactive antibodies recognizing SARS-CoV RBD were found almost exclusively in hospitalized patients. Outpatient and asymptomatic individuals' serological responses to SARS-CoV-2 decreased within 2 months, suggesting that humoral protection may be short-lived. [**note: some confounding information from Stanford on immunity. Humoral protection may be short-lived in those who have had mild disease.**]
https://www.medrxiv.org/content/10.1101/2020.08.15.20175794v1

- Understanding antibody responses to SARS-CoV-2 is indispensable for the development of containment measures to overcome the current COVID-19 pandemic. Here, we determine the ability of sera from 101 recovered healthcare workers to neutralize both authentic SARS-CoV-2 and SARS-CoV-2 pseudotyped virus and address their antibody titers against SARS-CoV-2 nucleoprotein and spike receptor-binding domain. Interestingly, the majority of individuals have low neutralization capacity and only 6% of the healthcare workers showed high neutralizing titers against both authentic SARS-CoV-2 virus and the pseudotyped virus. We found the antibody response to SARS-CoV-2 infection generates antigen-specific isotypes as well as a diverse combination of antibody isotypes, with high titers of IgG, IgM and IgA against both antigens correlating with neutralization capacity. Importantly, we found that neutralization correlated with antibody titers as quantified by ELISA. This suggests that an ELISA assay can be used to determine seroneutralization potential. *Altogether, our work provides a snapshot of the SARS-CoV-2 neutralizing antibody response in recovered healthcare workers and provides evidence that possessing multiple antibody isotypes may play an important role in SARS-CoV-2 neutralization*. [**note: from NYU, an examination of sera from recovered healthcare workers and neutralizing antibody profile.**]
https://www.biorxiv.org/content/10.1101/2020.08.15.252353v1

DIAGNOSTIC DEVELOPMENT

- Coronavirus Disease 2019 (COVID-19) is caused by severe acute respiratory coronavirus-2 (SARS-CoV-2). Fast, accurate and simple blood-based assays for quantification of anti-SARS-CoV-2 antibodies are urgently needed to identify infected individuals and keep track of the spread of disease. Methods: The study included 35 plasma samples from 22 individuals with confirmed COVID-19 by real time reverse transcriptase polymerase chain reaction and 40 non COVID-19 plasma samples. Anti-SARS-CoV-2 IgM/IgA or IgG antibodies were detected by a microfluidic quantitative immunomagnetic assay (IMA)(ViroTrack Sero COVID IgM+IgA/IgG Ab, Blusense Diagnostics, Denmark) and by enzyme-linked immunosorbent assay ((ELISA) (EuroImmun Medizinische Labordiagnostika, Germany). Results: Of the 35 plasma samples from the COVID-19 patients, 29 (82.9%) were positive for IgA/IgM or IgG by IMA and 29 samples (82.9%) were positive by ELISA. Sensitivity for only one sample per patient was 68% for IgA+IgM and 73% IgG by IMA and 73% by ELISA. For samples collected 14 days after symptom onset, the sensitivity of

both IMA and ELISA was around 90%. Specificity of the IMA reached 100% compared to 95% for ELISA IgA and 97.5% for ELISA IgG. Conclusion: IMA for COVID-19 is a rapid simple-to-use point of care test with sensitivity and specificity similar to a commercial ELISA. [**note: here are results of a new POC serology test.**]
https://www.medrxiv.org/content/10.1101/2020.08.15.20172080v1

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2020-08-18

Here is a wonderful music tribute to the great singer songwriter Townes Van Zandt. Van Zandt wrote numerous songs that have become iconic. This collection of all-star singers do justice Van Zandt's compositions: https://www.youtube.com/watch?v=Oc32Z424DCc Here is Townes Van Zandt and Nanci Griffith from 1993: https://www.youtube.com/watch?v=Kc6AsDmuD2U I did not know of Nanci Griffith until I saw these clips. She is wonderful and here she is on Letterman, singing 'From a Distance': https://www.youtube.com/watch?v=yBBi1dk-_HQ Bette Midler made this a great hit, but Griffith was the first to record it.

The New York Times has an op-ed piece from a Boston cardiologist on the increased risk of myocarditis from COVID-19. Misinformation in the age of COVID-19; fortunately, you won't find anything but the truth in this newsletter. Will fraternities and sororities lead the way to COVID-19 clusterdom? Probably. France are seeing case increases and trying to put a lid on them.

The Washington Post reports that Italy is taking measures to tamp down on increasing coronavirus cases. I still don't know if restaurants are going to make it through the pandemic, this story discusses what workers are facing. The WaPo tech columnists suggests we all start using a COVID-19 exposure app. I've written on this previously and believe it to be a very good idea. I don't think we have one for Maryland yet. Here is what it is like to be a flight attendant in this pandemic era. Well this certainly did not last long, Univ of North Carolina quickly moves to remote teaching after COVID-19 outbreaks. The bright side is it makes the campus safer for the football players. I do hope Mitch Daniels is more successful at Purdue. Parents are struggling as schools go to remote learning. I am seeing lots of requests on our neighborhood listserve for home help from two income families who are trying to figure things out. Genetic analysis of viral samples documents the suburban Baltimore/Washington March outbreak. Amazing what can be done today.

The Guardian points to a single family of four as being the source of the Victoria outbreak in Australia. South Korea's outbreak seems linked to church goers.

Will there be a generation of COVID-19 parents in the same way there were Depression Era parents?

A reader asked yesterday about stem cell therapies. There are some ongoing trials for COVID-19 but as this STAT piece notes, it is difficult to tell what is scientifically sound from quack approaches.

Medscape covers a prank HCQ/azithromycin paper in an obscure journal. I have no doubt that someone has already cited this as proof of the Zombie drug's efficacy. Staying with Medscape, President Trump has a new medical advisor. Former Stanford radiologist, Scott Atlas is now working on the White House coronavirus response team. Atlas most recently has been a fellow at the Hoover Institution.

Derek Lowe on the SinoPharm Inactivated COVID-19 vaccine.

MODELING

- Frontline healthcare workers (HCW) are a high-risk population for SARS-CoV-2 infection. Here we present results from a large serosurveillance study of 10,019 asymptomatic HCW conducted during April-May 2020, in eight hospital medical centers across the state of Oregon, USA during the initial peak of the pandemic. Free and voluntary testing was performed at 14 +/- 3 day intervals, over a 4-week window at each site, utilizing a lab-developed ELISA based on the Epitope Diagnostics COVID-19 nucleocapsid IgG detection Kit. We identified 253 SARS-CoV-2 IgG seropositive individuals among 10,019 total participants, representing a cross-sectional seroprevalence of 2.53%. Subgroup analysis identified differential seropositivity by job role, ranging from 8.03% among housekeepers, odds ratio 3.17 (95% CI 1.59-5.71), to 0.00% among anesthesiologists, odds ratio 0.00 (95% CI 0-0.26), both of which were significant. Over the course of the study, 17 seroconversions (0.25%) and 101 seroreversions (1.50%) were identified. Self-reported SARS-CoV-2 swab qPCR testing, when compared with subsequent serology on study, showed only modest agreement, κ = 0.47 (95% CI 0.32-0.62). Overall*, these findings demonstrate relatively low seroprevalence and very low seroconversion rates among HCW in Oregon, USA, over a period in which aggressive social distancing measures were in place. The high rate of seroreversion observed in this cohort, and the relatively high discordance between SARS-CoV-2 serology and swab qPCR, highlight limitations of current detection methods, and stress the need for development of novel assessment methodologies to more accurately identify exposure (and/or immunity) to SARS-CoV-2 in this population*. [**note: here are the results of a large serology test of Oregon healthcare workers during the height of the pandemic**] https://www.medrxiv.org/content/10.1101/2020.08.16.20176107v1
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019, is a respiratory virus primarily transmitted from person to person through inhalation of droplets or aerosols, laden with viral particles. However, as some studies have shown, virions can remain infectious for up to 72 hours on surfaces, which can lead to transmission through contact. For this reason, a comprehensive study was conducted to determine the efficiency of protocols to recover SARS-CoV-2 from surfaces in built environments. This end-to-end (E2E) study showed that the effective combination of monitoring SARS-CoV-2 on surfaces include using an Isohelix swab as a collection tool, DNA/RNA Shield as a preservative, an automated system for RNA extraction, and reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) as the detection assay. Using this E2E approach, this study showed that, in some cases, SARS-CoV-2 viral standards were still recovered from surfaces as detected by RT-qPCR for as long as eight days even after bleach treatment. Additionally, debris associated with specific built environment surfaces appeared to negatively impact the recovery of RNA, with Amerstat inhibition as high as 90% when challenged with an inactivated viral control. *Overall, it was determined that this E2E protocol required a minimum of 1,000 viral particles per 25 cm^2 to successfully detect virus from test surfaces. When this method was employed to evaluate 368 samples collected from various built environmental surfaces, all samples tested negative, indicating that the surfaces were either void of virus or below the detection limit of the assay*. [**note: this is an interesting environmental protocol to look for virus inside buildings. Note the real-world results at the end of the abstract. This is another**

area where more data would be useful. It would be good to do representative surface testing in schools that are reopening.]
https://www.medrxiv.org/content/10.1101/2020.08.16.20172668v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check maybe tomorrow or Thursday.

CLINICAL TRIAL RESULTS

- Coronavirus disease 2019 (COVID-19) triggers distinct patterns of pneumonia progression with multiorgan disease, calling for cell- and/or tissue-type specific host injury markers. METHODS. An integrated hypothesis-free single biomarker analysis framework was performed on nasal swabs (n=484) from patients with COVID-19 in GSE152075. The origin of candidate biomarker was assessed in single-cell RNA data (GSE145926). The candidate biomarker was validated in a cross-sectional cohort (n=564) at both nucletide and protein levels. RESULTS. Phospholipase A2 group VII (PLA2G7) was identified as a candidate biomarker in COVID-19. PLA2G7 was predominantly expressed by proinflammatory macrophages in lungs emerging with progression of COVID-19. In the validation stage, PLA2G7 was found in patients with COVID-19 and pneumonia, especially in severe pneumonia, rather than patients suffered mild H1N1 influenza infection. The positive rates of PLA2G7 ranging from 29.37% to 100.00% were positively correlated with not only viral loads in patients with COVID-19 but also severity of pneumonia in non COVID-19 patients. Although Ct values of PLA2G7 in severe pneumonia was siginificantly lower than that in moderate pneumonia (P=7.2e-11), no differences were observed in moderate pneumonia with COVID-19 between severe pneumonia without COVID-19 (P=0.81). Serum protein levels of PLA2G7, also known as lipoprotein-associated phospholipase A2 (Lp-PLA2), were further found to be elevated and beyond the upper limit of normal in patients with COVID-19, especially among the re-positive patients. CONCLUSIONS. We firstly identified and validated PLA2G7, a biomarker for cardiovascular diseases (CVDs), was abnormally enhanced in COVID-19 patients at both nucletide and protein aspects. These findings provided indications into the prevalence of cardiovascular involvements seen in COVID-19 patients. PLA2G7 could be a hallmark of COVID-19 for monitoring disease progress and therapeutic response. [**note: more good work from China on a biomarker for cardiovascular disease and the linkage to COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.08.16.20175505v1

DRUG DEVELOPMENT

- Nothing new today.

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The hypothesis of attenuating SARS-CoV-2 virulence has been raised. We examined the temporal distribution of COVID-19 complications (ER visits, hospitalization, intubation/code, and/or death) among healthcare workers in one system that applied uniform screening criteria throughout the research period, and found the complication rate significantly decreased after April 15, 2020. [**note: maybe the virulence of SARS-CoV-2 is attenuating. Much more data will be needed and genomic profiles of the virus and health outcomes should inform us.**]
https://www.medrxiv.org/content/10.1101/2020.08.17.20176636v1

- SARS-CoV-2 antibody responses in children remain poorly characterized. Here, we show that pediatric patients with multisystem inflammatory syndrome in children (MIS-C) possess higher SARS-CoV-2 spike IgG titers compared to those with severe coronavirus disease 2019 (COVID-19), likely reflecting a longer time since onset of infection in MIS-C patients. [**note: from Univ of Pennsylvania, antibody responses in children with multi-inflammatory syndrome and mild and severe COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.08.17.20176552v1
- Antibody repertoire refers to the totality of the superbly diversified antibodies within an individual to cope with the vast array of possible pathogens. Despite this extreme diversity, antibodies of the same clonotype, namely public clones, have been discovered among individuals. Although some public clones could be explained by antibody convergence, public clones in naïve repertoire or virus-neutralizing clones from not infected people were also discovered. All these findings indicated that public clones might not occur by random and they might exert essential functions. However, the frequencies and functions of public clones in a population have never been studied. Here, we integrated 2,449 Rep-seq datasets from 767 donors and discovered 5.07 million public clones – ~10% of the repertoire are public in population. We found 38 therapeutic clones out of 3,390 annotated public clones including anti-PD1 clones in healthy people. Moreover, we also revealed clones neutralizing SARS-CoV-2, Ebola, and HIV-1 viruses in healthy individuals. Our result demonstrated that these clones are predisposed in the human antibody repertoire and may exert critical functions during particular immunological stimuli and consequently benefit the donors. We also implemented RAPID – a Rep-seq Analysis Platform with Integrated Databases, which may serve as a useful tool for others in the field. [**note: from China, an immunology paper that I think maybe important but don't know why**] https://www.biorxiv.org/content/10.1101/2020.08.13.249086v1
- Severe acute respiratory syndrome corona-virus 2 (SARS-CoV-2), the etiologic agent of the coronavirus disease 2019 (COVID-19), has a catastrophic effect on human health and society. Clinical findings indicated that the suppression of innate antiviral immunity, especially the type I and III interferon (IFN) production, contributes to the pathogenesis of COVID-19. However, how SARS-CoV-2 evades antiviral immunity still needs further investigations. Here, we reported that the open reading frame 9b (ORF9b) protein encoded by the SARS-CoV-2 genome inhibits the activation of type I and III IFN response by targeting multiple molecules of innate antiviral signaling pathways. SARS-CoV-2 ORF9b impaired the induction of type I and III IFNs by Sendai virus or the dsRNA mimic poly (I:C). SARS-CoV-2 ORF9b inhibits the activation of type I and III IFNs induced by the components of cytosolic dsRNA-sensing pathways of RIG-I/MDA5-MAVS signaling, including RIG-I, MDA-5, MAVS, TBK1, and IKKε rather than IRF3-5D, the active form of IRF3. SARS-CoV-2 ORF9b also suppressed the induction of type I and III IFNs by TRIF and STING, the adaptor protein of endosome RNA-sensing pathway of TLR3-TRIF signaling and the adaptor protein of cytosolic DNA-sensing pathway of cGAS-STING signaling, respectively. Mechanistically, SARS-CoV-2 ORF9b protein interacts with RIG-I, MDA-5, MAVS, TRIF, STING, TBK1, and prevents TBK1 phosphorylation, thus impeding the phosphorylation and nuclear trans-localization of IRF3 activation. Overexpression of SARS-CoV-2 ORF9b facilitates the replication of the vesicular stomatitis virus. Therefore, SARS-CoV-2 ORF9b negatively regulates antiviral immunity, thus, facilitate virus replication. This study contributes to our understanding of the molecular mechanism of how SARS-CoV-2 impaired antiviral immunity and providing an essential clue to

the pathogenesis of COVID-19. [**note: from China, further information on how SARS-CoV-2 evades antiviral immunity**] https://www.biorxiv.org/content/10.1101/2020.08.16.252973v1

DIAGNOSTIC DEVELOPMENT

- The COVID-19 pandemic continues to have an unprecedented impact on societies and economies worldwide. Despite rapid advances in diagnostic test development and scale-up, there remains an ongoing need for SARS-CoV-2 tests which are highly sensitive, specific, minimally invasive, cost-effective and scalable for broad testing and surveillance. Here we report development of a highly sensitive single molecule array (Simoa) immunoassay on the automated HD-X platform for the detection of SARS-CoV-2 Nucleocapsid protein (N-protein) in venous and capillary blood (fingerstick). In pre-pandemic and clinical sample sets, the assay has 100% specificity and 97.4% sensitivity for serum / plasma samples. The limit of detection (LoD) estimated by titration of inactivated SARS-CoV-2 virus is 0.2 pg/ml, corresponding to 0.05 Median Tissue Culture Infectious Dose (TCID50) per ml, > 2000 times more sensitive than current EUA approved antigen tests. No cross-reactivity to other common respiratory viruses, including hCoV229E, hCoVOC43, hCoVNL63, Influenza A or Influenza B, was observed. We detected elevated N-protein concentrations in symptomatic, asymptomatic, and pre-symptomatic PCR+ individuals using capillary blood from a finger-stick collection device. The Simoa SARS-CoV-2 N-protein assay has the potential to detect COVID-19 infection via antigen in blood with similar or better performance characteristics of molecular tests, while also enabling at home and point of care sample collection. [**note: this is ultra-sensitive test for the nucleocapsid protein and can work on a finger prick worth of blood.**] https://www.medrxiv.org/content/10.1101/2020.08.14.20175356v1
- Mass testing is fundamental to face the pandemic caused by the coronavirus SARS-CoV-2 discovered at the end of 2019. To this aim, it is necessary to establish reliable, fast and cheap tools to detect viral particles in biological material so to identify the people capable to spread the infection. We demonstrate that a colorimetric biosensor based on gold nanoparticle (AuNP) interaction induced by SARS-CoV-2 lends itself as an outstanding tool for detecting viral particles in nasal and throat swabs. The extinction spectrum of a colloidal solution of multiple viral-target gold nanoparticles - AuNPs functionalized with antibodies targeting three surface proteins of SARS-CoV-2 (spike, envelope and membrane) - is redshifted in few minutes when mixed to a solution containing the viral particle. The optical density of the mixed solution measured at 560 nm was compared to the threshold cycle (Ct) of a Real Time-PCR (gold standard for detecting the presence of viruses) finding that the colorimetric method is able to detect very low viral load with a detection limit approaching that of RT-PCR. Since the method is sensitive to the infecting viral particle rather than to its RNA, the achievements reported here open new perspective not only in the context of the current and possible future pandemics, but also in microbiology as the biosensor proves itself to be a powerful though simple tool for measuring the viral particle concentration. [**note: from Italy, an interesting colorimetric test for SARS-CoV-2 that approaches rt-PCR technology**] https://www.medrxiv.org/content/10.1101/2020.08.15.20175489v1

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2020-08-19

I am going to stay with Nanci Griffith for one more day. Here she is in the PBS show Austin City Limits show from 1989: https://www.youtube.com/watch?v=reZpbkE8o_M the famous Woolworth Five and Dime song is near the end of this clip. Here she is singing Kate Wolf's great song, Across the Great Divide with Emmylou Harris singing the harmony: https://www.youtube.com/watch?v=6uQNRTnsntY Finally, Nanci covers Bob Dylan's Boots of Spanish Leather: https://www.youtube.com/watch?v=k1KxthvX1Ms and for a comparison, the Dylan original: https://www.youtube.com/watch?v=iy6wryJMwVU (the full poem is in the notes of this video)

US COVID-19 STATISTICS - **Infection Rate: 1.7%; CFR: 3.1%** (IR up 0.1%; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

YIKES!!! According to my Yahoo news feed the first case of Bubonic Plague in five years was just diagnosed in California. No, the 'Black Death' is not going to return. Cases of plague are rare in the US but do occur. Good antibiotics are available!

The Washington Post shows how airplanes can change the seats in plane cabins. Influenza in South Africa (it's winter there) was almost eliminated. WHO are warning that young people are emerging as the main spreaders of COVID-19. I wonder if any college professors will enjoy teaching in class. There was a contentious interview with the 'My Pillow' inventor and Anderson Cooper on the use of oleandrin for COVID-19. It was the classic 'gotcha' interview and a pretty stupid one at that. Of course the extract is not a treatment in the same way tens of thousands of other compounds are not. Two points here: 1) there is an *in vitro* study that shows the extract inhibits SARS-CoV-2 growth in the standard vero cell assay, and 2) there is one clinical trial registered to study the extract. The clinical trial is just a proof of concept test and enrollment is by invitation and not randomized. I don't need to tell you how easy it is to cherry pick patients for that. I also don't particularly buy the mechanism of action for the extract as causing some type of membrane change in replicating viruses that leads to reduced infectivity. There are certainly a lot of compounds that will do that. I've seen too many weird claims for activity to put this in any other category. It would be great if there were a simple solution to coronavirus infections, but I don't see it with this compound. If you read the technical paper you begin to wonder why, it this compound has such great viricidal properties it has not been commercialized. Perhaps it just doesn't work *in vivo.* Here is how saliva-based COVID-19 tests work. Wise words from the State of Florida health commissioner about closing schools? Is Facebook having trouble deleting COVID-19 misinformation? My decision to never use Facebook is totally validated!

The University of North Carolina gets its priorities right. According to The New York Times, football will go on as scheduled despite sending students home yesterday. Chancellor Guskiewicz is a PhD in Sports Medicine. Perhaps he missed the course on infectious diseases. It is a good time to go apartment hunting in New York City. Governor DeSantis of Florida needs a different comparator for the COVID-19 infection; Osama bin Laden just doesn't work. Alabama have a large testing program in place for opening of colleges and universities. Will this frighten the virus into submission? I'm not so sure. Venezuela takes another approach, deploying the military to detain people and enforce the quarantine.

STAT asks whether COVID-19 vaccines will be safe for children and pregnant women.

The Lancet discusses the risk of COVID-19 related stroke in young individuals. Yes, the young are not immune to serious risk. The Imperial College group discusses molecular testing strategies for COVID-19 control. Finally, a report from Singapore on the Δ382 variant of SARS-CoV-2 that appears to be

associated with a milder infection. The ORF8 gene looks to be a hot spot for coronavirus mutation and we clearly need to continue genome wide looks at SARS-CoV-2 to map other deletions.

Yesterday, I provided the wrong link to the Medscpe article on the HCQ spoof. HERE is the correct link; enjoy the chuckle.

Derek Lowe on the recent immunology papers.

MODELING

- An explanation is required for the re-emergence of COVID-19 outbreaks in regions with apparent local eradication. Recent outbreaks have emerged in Vietnam, New Zealand and parts of China where there had been no cases for some months. Importation of contaminated food and food packaging is a feasible source for such outbreaks and a source of clusters within existing outbreaks. Such events can be prevented if the risk is better appreciated. [**note: I am somewhat skeptical of the risk posed by imported foods. I think there is some evidence now from New Zealand that this is not a risk. I guess more research will inform us.**] https://www.biorxiv.org/content/10.1101/2020.08.17.255166v1

NEWLY REGISTERED CLINICAL TRIALS

- Did not check today.

CLINICAL TRIAL RESULTS

- Background: From the beginning of COVID-19 pandemic, pregnant women have been considered at greater risk of severe morbidity and mortality. However, data on hospitalized pregnant women show that the symptom profile and risk factors for severe disease are similar to those among women who are not pregnant, although preterm birth, Cesarean delivery, and stillbirth may be more frequent and vertical transmission is possible. Limited data are available for the cohort of pregnant women that gave rise to these hospitalized cases, hindering our ability to quantify risk of COVID-19 sequelae for pregnant women in the community. Objective: To test the hypothesis that pregnant women in community differ in their COVID-19 symptoms profile and disease severity compared to non-pregnant women. This was assessed in two community-based cohorts of women aged 18-44 years in the United Kingdom, Sweden and the United States of America. Study design: This observational study used prospectively collected longitudinal (smartphone application interface) and cross-sectional (web-based survey) data. Participants in the discovery cohort were drawn from 400,750 UK, Sweden and US women (79 pregnant who tested positive) who self-reported symptoms and events longitudinally via their smartphone, and a replication cohort drawn from 1,344,966 USA women (162 pregnant who tested positive) cross-sectional self-reports samples from the social media active user base. The study compared frequencies of symptoms and events, including self-reported SARS-CoV-2 testing and differences between pregnant and non-pregnant women who were hospitalized and those who recovered in the community. Multivariable regression was used to investigate disease severity and comorbidity effects. Results: Pregnant and non-pregnant women positive for SARS-CoV-2 infection drawn from these community cohorts were not different with respect to COVID-19-related severity. Pregnant women were more likely to have received SARS-CoV-2 testing than non-pregnant, despite reporting fewer clinical symptoms. Pre-existing lung disease

was most closely associated with the severity of symptoms in pregnant hospitalized women. Heart and kidney diseases and diabetes were additional factors of increased risk. The most frequent symptoms among all non-hospitalized women were anosmia [63% in pregnant, 92% in non-pregnant] and headache [72%, 62%]. Cardiopulmonary symptoms, including persistent cough [80%] and chest pain [73%], were more frequent among pregnant women who were hospitalized. Gastrointestinal symptoms, including nausea and vomiting, were different among pregnant and non-pregnant women who developed severe outcomes. Conclusions: Although pregnancy is widely considered a risk factor for SARS-CoV-2 infection and outcomes, and was associated with higher propensity for testing, the profile of symptom characteristics and severity in our community-based cohorts were comparable to those observed among non-pregnant women, except for the gastrointestinal symptoms. Consistent with observations in non-pregnant populations, comorbidities such as lung disease and diabetes were associated with an increased risk of more severe SARS-CoV-2 infection during pregnancy. Pregnant women with pre-existing conditions require careful monitoring for the evolution of their symptoms during SARS-CoV-2 infection. [**note: this is a large cohort study to see if pregnancy increases the risk of COVID-19. It does not seem to which is good news but comorbidities can result in severe COVID-19 and pregnant women at risk will need to be carefully monitored.**] https://www.medrxiv.org/content/10.1101/2020.08.17.20161760v1

DRUG DEVELOPMENT

- The current pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and new outbreaks worldwide highlight the need for preventive treatments. Although angiotensin converting enzyme 2 (ACE2) is the primary receptor for SARS-CoV-2, we identified heparan sulfate proteoglycans expressed by epithelial cells, alveolar macrophages and dendritic cells as co-receptors for SARS-CoV-2. Low molecular weight heparins (LMWH) blocked SARS-CoV-2 infection of epithelial cells and alveolar macrophages, and virus dissemination by dendritic cells. Notably, potent neutralizing antibodies from COVID-19 patients interfered with SARS-CoV-2 binding to heparan sulfate proteoglycans, underscoring the importance of heparan sulfate proteoglycans as receptors and uncover that SARS-CoV-2 binding to heparan sulfates is an important mechanism for neutralization. These results have imperative implications for our understanding of SARS-CoV-2 host cell entry and reveal an important target for novel prophylactic intervention. [**note; another possible receptor for SARS-CoV-2 and possible target for drug intervention.**] https://www.biorxiv.org/content/10.1101/2020.08.18.255810v1
- The human microbiota has a close relationship with human disease and it remodels components of the glycocalyx including heparan sulfate (HS). Studies of the severe acute respiratory syndrome coronavirus (SARS-CoV-2) spike protein receptor binding domain suggest that infection requires binding to HS and angiotensin converting enzyme 2 (ACE2) in a codependent manner. Here, we show that commensal host bacterial communities can modify HS and thereby modulate SARS-CoV-2 spike protein binding and that these communities change with host age and sex. Common human-associated commensal bacteria whose genomes encode HS-modifying enzymes were identified. The prevalence of these bacteria and the expression of key microbial glycosidases in bronchoalveolar lavage fluid (BALF) was lower in adult COVID-19 patients than in healthy controls. The presence of HS-modifying bacteria decreased with age in two large survey datasets, FINRISK 2002 and American Gut, revealing one possible mechanism for the observed

increase in COVID-19 susceptibility with age. In vitro, bacterial glycosidases from unpurified culture media supernatants fully blocked SARS-CoV-2 spike binding to human H1299 protein lung adenocarcinoma cells. HS-modifying bacteria in human microbial communities may regulate viral adhesion, and loss of these commensals could predispose individuals to infection. Understanding the impact of shifts in microbial community composition and bacterial lyases on SARS-CoV-2 infection may lead to new therapeutics and diagnosis of susceptibility. [**note: another paper on the implication of heparan sulfate linkage to viral infection.**] https://www.biorxiv.org/content/10.1101/2020.08.17.238444v1

- Since the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in human breastmilk, little is known about the antiviral property of human breastmilk to SARS-CoV-2 and its related pangolin coronavirus (GX_P2V). Here we present for the first time that whey protein from human breastmilk effectively inhibited both SARS-CoV-2 and GX_P2V by blocking viral attachment, entry and even post-entry viral replication. Moreover, human whey protein inhibited infectious virus production proved by the plaque assay. We found that whey protein from different species such as cow and goat also showed anti-coronavirus properties. And commercial bovine milk also showed similar activity. Interestingly, the main antimicrobial components of breastmilk, such as Lactoferrin and IgA antibody, showed limited anti-coronavirus activity, indicating that other factors of breastmilk may play the important anti-coronavirus role. Taken together, we reported that whey protein inhibits SARS-CoV-2 and its related virus of GX_P2V. These results rule out whey protein as a direct-acting inhibitor of SARS-CoV-2 and GX_P2V infection and replication and further investigation of its molecular mechanism of action in the context of COVID-19. [**note: 'Little Miss Muffet sat on a tuffet eating her curds and whey' and did not get COVID-19. I don't know what to make of this finding.**] https://www.biorxiv.org/content/10.1101/2020.08.17.254979v1
- Drug repurposing is a rapid approach to identifying therapeutics for the treatment of emerging infectious diseases such as COVID-19. To address the urgent need for treatment options, we carried out a quantitative high-throughput screen using a SARS-CoV-2 cytopathic assay with a compound collection of 8,810 approved and investigational drugs, mechanism-based bioactive compounds, and natural products. Three hundred and nineteen compounds with anti-SARS-CoV-2 activities were identified and confirmed, including 91 approved drug and 49 investigational drugs. Among these confirmed compounds, the anti-SARS-CoV-2 activities of 230 compounds, including 38 approved drugs, have not been previously reported. Chlorprothixene, methotrimeprazine, and piperacetazine were the three most potent FDA approved drugs with anti-SARS-CoV-2 activities. These three compounds have not been previously reported to have anti-SARS-CoV-2 activities, although their antiviral activities against SARS-CoV and Ebola virus have been reported. These results demonstrate that this comprehensive data set of drug repurposing screen for SARS-CoV-2 is useful for drug repurposing efforts including design of new drug combinations for clinical trials. [**note: from NIH another drug screening effort. This a curious group of psychiatric drugs and there is no apparent mechanism proposed for antiviral activity though concentrations seem to be much better than remdesivir in this particular assay system. As I noted in the commentary on oleander extract, lots of drugs seem to be active and the old aphorism, 'many are called but few are chosen,' is apt.**] https://www.biorxiv.org/content/10.1101/2020.08.18.255877v1

- COVID-19 pandemic caused approximately 750,000 deaths and over 20 million confirmed cases of infection by SARS-CoV-2 within 8 months since the emergence of the virus. While there are no vaccines approved and considering the difficulty in meeting the large vaccination demand worldwide, the potential use of passive immunization should be considered based on existing successful therapies against many diseases. Here we demonstrate that hyperimmune globulin preparations raised in horses against the recombinant trimeric spike (S) glycoprotein of SARS-CoV-2 in the prefusion conformation provide very high ELISA titers as well as highly potent neutralizing activity against SARS-CoV-2. Five horses were subcutaneously inoculated for 6 weeks with the recombinant S protein (ectodomain, residues 1-1208). Four out of the 5 horses presented a strong immune response. Considering the average of all 5 horses, ELISA titers above 1:1,000,000 and neutralizing titers (PRNT90) reaching 1:14,604 were observed. When compared with the plasma of three convalescent COVID-19 patients, sera of immunized horses displayed approximately 140-fold higher neutralizing titers measured as PRNT90. To prevent eventual side effects caused by horse antiserum, IgG was digested with pepsin and purified by fractional salt precipitation to eliminate Fc fragments, a process that is industrially used for the production of passive immunization F(ab')2 concentrates against rabies, tetanus and snake venoms. The high neutralizing titers against SARS-CoV-2 obtained for the unprocessed sera were confirmed for the F(ab')2 fragments and were 150-fold higher than the PRNT90 neutralizing titers of plasma of three COVID-19 convalescent patients. The great advantage of using the recombinant trimeric S glycoprotein is that it is safe and provides quick adaptive immunity in horses. Our data show the perspective of using hyperimmune anti-SARS-CoV-2 F(ab')2 preparations as a passive immunization therapy in humans, similar to therapies that have been safely used for decades against rabies, tetanus and snake venoms. [**note: interesting research from Brazil on hyperimmune equine globulin preparations. It would be cool if this approach has some therapeutic usefulness.**] https://www.biorxiv.org/content/10.1101/2020.08.17.254375v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- An improved understanding of human T-cell-mediated immunity in COVID-19 is important if we are to optimize therapeutic and vaccine strategies. Experience with influenza shows that infection primes CD8+ T-cell memory to shared peptides presented by common HLA types like HLA-A2. Following re-infection, cross-reactive CD8+ T-cells enhance recovery and diminish clinical severity. Stimulating peripheral blood mononuclear cells from COVID-19 convalescent patients with overlapping peptides from SARS-CoV-2 Spike, Nucleocapsid and Membrane proteins led to the clonal expansion of SARS-CoV-2-specific CD8+ and CD4+ T-cells in vitro, with CD4+ sets being typically robust. For CD8+ T-cells taken directly ex vivo, we identified two HLA-A*02:01-restricted SARS-CoV-2 epitopes, A2/S269-277 and A2/Orf1ab3183-3191. Using peptide-HLA tetramer enrichment, direct ex vivo assessment of the A2/S269+CD8+ and A2/Orf1ab3183+CD8+ populations indicated that the more prominent A2/S269+CD8+ set was detected at comparable frequency (1.3x10-5) in acute and convalescent HLA-A*02:01+ patients. But, while the numbers were higher than those found in uninfected HLA-A*02:01+ donors (2.5x10-6), they were low when compared with frequencies for influenza-specific (A2/M158) and EBV-specific (A2/BMLF1280) (1.38x10-4) populations. Phenotypic analysis ex vivo of A2/S269+CD8+ T-cells from COVID-19 convalescents showed that A2/S269+CD8+ T-cells were predominantly negative for the CD38, HLA-DR, PD-1 and CD71 activation markers, although the

majority of total CD8+ T-cells were granzyme and/or perforin-positive. Furthermore, the bias towards naive, stem cell memory and central memory A2/S269+CD8+ T-cells rather than effector memory populations suggests that SARS-CoV2 infection may be compromising CD8+ T-cell activation. Priming with an appropriate vaccine may thus have great value for optimizing protective CD8+ T-cell immunity in COVID-19. [**note: more information on how SARS-CoV-2 impacts the immune system. This Australian paper shows that infection may compromise CE8+ T-cell activation. This is not a desirable outcome.**]
https://www.medrxiv.org/content/10.1101/2020.08.17.20176370v1

- Interferon-induced transmembrane proteins (IFITMs 1, 2 and 3) restrict numerous viral pathogens and are thought to prevent infection by severe acute respiratory syndrome coronaviruses (SARS-CoVs). However, most evidence comes from single-round pseudoparticle infection of cells artificially overexpressing IFITMs. Here, we confirmed that overexpression of IFITMs blocks pseudoparticle infections mediated by the Spike proteins of β-coronaviruses including pandemic SARS-CoV-2. In striking contrast, however, endogenous IFITM expression promoted genuine SARS-CoV-2 infection in human lung cells both in the presence and absence of interferon. IFITM2 was most critical for efficient entry of SARS-CoV-2 and enhanced virus production from Calu-3 cells by several orders of magnitude. IFITMs are expressed and further induced by interferons in the lung representing the primary site of SARS-CoV-2 infection as well as in other relevant tissues. Our finding that IFITMs enhance SARS-CoV-2 infection under conditions approximating the in vivo situation shows that they may promote viral invasion during COVID-19. [**note: here's a factor that may increase viral infection.**]
https://www.biorxiv.org/content/10.1101/2020.08.18.255935v1
- The SARS-CoV-2 pandemic has led to public health, economic, and social consequences that mandate urgent development of effective vaccines to contain or eradicate infection. To that end, we evaluated a novel amphiphile (AMP) vaccine adjuvant, AMP-CpG, composed of diacyl lipid-modified CpG, admixed with the SARS-CoV-2 Spike-2 receptor binding domain protein as a candidate vaccine (ELI-005) in mice. AMP immunogens are efficiently delivered to lymph nodes, where innate and adaptive immune responses are generated. Compared to alum, AMP immunization induced >25-fold higher antigen-specific T cells which produced multiple Th1 cytokines and trafficked into lung parenchyma and respiratory secretions. Antibody responses favored Th1 isotypes (IgG2bc, IgG3) and potently neutralized Spike-2-ACE2 receptor binding, with titers 265-fold higher than the natural immune response from convalescent COVID-19 patients; responses were maintained despite 10-fold dose-reduction in Spike antigen. Both cellular and humoral immune responses were preserved in aged mice. These advantages merit clinical translation to SARS-CoV-2 and other protein subunit vaccines. [**note: another new vaccine/adjuvant approach that may lead to higher antigen-specific T cells.**]
https://www.biorxiv.org/content/10.1101/2020.08.17.251728v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

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2020-08-20

Because it is my favorite song cycle and because it is Jonas Kauffmann accompanied by Helmut Deutsch, this is a must listen: https://www.youtube.com/watch?v=9M3tZE-ffxw According to the notes it was recorded on April 27 which explains the absence of an audience. This is just wonderful!!!

US COVID-19 STATISTICS - **Infection Rate: 1.7%; CFR: 3.1%** (IR up 0.1%; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

The New York Times has a nice op-ed on living with COVID-19 and risk tolerance. Here are the results of the New York City serology testing, all 1.46 million of them! Many schools don't have a full-time nurse on duty. Don't you wish you had a second passport? I sure do! Unfortunately, my ancestors were from The Russian empire, Hungary, and Romania, garden spots that I would rather not visit these days.

This indoor wrestling tournament will not end well. This Washington Post story confirms that my decision to stop watching football at all levels was the correct one. Without an office to go to, Washington power brokers are figuring out new ways of working. I must confess, it is a nice time to be retired! A Yale professor issues a stark warning to incoming students. What is wrong with this country?

The Atlantic's Ed Yong on those who have lingering illness following SARS-CoV-2 infection. Noah Feldman's 'Deep Background' podcast interviewed Univ of Colorado pediatrician Sean O'Reilly who contracted COVID-19 along with his wife over two months ago and still has lingering fatigue. O'Reilly was one of the authors of the American Academy of Pediatrics' white paper on school reopenings.

A STAT opinion piece discusses how imperfect COVID-19 tests can help control the pandemic.

Nature discusses the evidence lag over blood plasma treatment for COVID-19.

Medscape discusses the treatment of the day, oleander extract. I noted previously that we had lots of decorative oleanders growing in San Diego where I grew up. I was always admonished not to eat the flowers as they were highly poisonous. Nasturtium flowers, on the other hand, are quite tasty and can be used as a decorative addition to your summer salads. [Isn't this kind of information worth the subscription price of this newsletter?]

Things have been slow in the preprint arena over the last several days.

MODELING

- It looks like research on models is slowing way down.

NEWLY REGISTERED CLINICAL TRIALS

- This is a two-center, randomized, placebo-controlled pilot study of anti-SARS-CoV-2 equine immunoglobulin fragments F(ab')2 (INOSARS) to evaluate safety and preliminary efficacy in the treatment of hospitalized COVID-19 patients. Clinical improvement at 28 days from the start of treatment will be evaluated. [**note: I posted a paper yesterday on this approach to generating immunoglobulins and presto, a registered trial in Mexico.**] NCT04514302
- The aim of the study is to assess the safety, efficacy, and immunogenicity of AZD1222 for the prevention of COVID-19. [**note: this is the Phase 3 trial of the Oxford vaccine and AstraZeneca are running it.**] NCT04516746

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a rapidly spreading viral infection causing COVID-19 disease. There currently is no preventive or outpatient treatment therapy for Covid-19. While vaccine development for SARS-CoV-2 has been promising, there may be reduced willingness among the public to receive a vaccine developed so quickly. The intervention is metformin, a biguanide, administered in its immediate release formation, 500mg twice daily. [**note: trial is at Univ of Minnesota. There have been some observational studies suggesting a lower mortality rate of those on metformin for diabetes.**] NCT04510194

CLINICAL TRIAL RESULTS

- Sadly, no news of any kind.

DRUG DEVELOPMENT

- There is an urgent need for a safe and protective vaccine to control the global spread of SARS-CoV-2 and prevent COVID-19. Here, we report the immunogenicity and protective efficacy of a SARS-CoV-2 subunit vaccine (NVX-CoV2373) produced from the full-length SARS-CoV-2 spike (S) glycoprotein stabilized in the prefusion conformation. Cynomolgus macaques (Macaca fascicularis) immunized with NVX-CoV2373 and the saponin-based Matrix-M adjuvant induced anti-S antibody that was neutralizing and blocked binding to the human angiotensin-converting enzyme 2 (hACE2) receptor. Following intranasal and intratracheal challenge with SARS-CoV-2, immunized macaques were protected against upper and lower infection and pulmonary disease. These results support ongoing phase 1/2 clinical studies of the safety and immunogenicity of NVX-CoV2327 vaccine (NCT04368988). [**note: here is the animal data for the Novavax COVID-19 vaccine.**] https://www.biorxiv.org/content/10.1101/2020.08.18.256578v1
- Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infectious disease (COVID-19) has been threatening the world because of severe symptoms and relatively high mortality. To develop vaccines and antiviral drugs for COVID-19, an animal model of SARS-CoV-2 infection is required to evaluate the efficacy of prophylactics and therapeutics in vivo. Therefore, we examined the pathogenicity of SARS-CoV-2 in cynomolgus macaques until 28 days after virus inoculation in the present study. Cynomolgus macaques showed body temperature rises after infection and X-ray radiographic viral pneumonia was observed in one of three macaques. However, none of the macaques showed life-threatening clinical signs of disease corresponding that approximately 80% of human patients did not show a critical disease in COVID-19. A neutralizing antibody against SARS-CoV-2 and T-lymphocytes that produced interferon (IFN)-γ and interleukin (IL)-2 specifically for SARS-CoV-2 N protein were detected on day 14 in the macaque that showed viral pneumonia. On the other hand, in the other macaques, in which a neutralizing antibody was not detected, T-lymphocytes that produced IFN-γ specifically for SARS-CoV-2 N protein increased on day 7 to day 14 prior to an increase in the number of T-lymphocytes that produced IL-2. These results suggest that not only a neutralizing antibody but also cellular immunity augmented by IFN-γ has a role in the elimination of SARS-CoV-2. Thus, because of the mild clinical signs of disease and low/no antibody responses against SARS-CoV-2 in two thirds of the macaques, cynomolgus macaques are appropriate to extrapolate human responses in vaccine and drug development. [**note: another paper PETA will not like. These Japanese researchers show the utility of this animal model for SARS-CoV-2 infection.**] https://www.biorxiv.org/content/10.1101/2020.08.18.256446v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- There is an urgent need to understand the pathogenesis of the severe acute respiratory syndrome coronavirus clade 2 (SARS-CoV-2) that leads to COVID-19 and respiratory failure. Our study is to discover differentially expressed genes (DEGs) and biological signaling pathways by using a bioinformatics approach to elucidate their potential pathogenesis. The gene expression profiles of the GSE150819 datasets were originally produced using an Illumina NextSeq 500 (Homo sapiens). KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology) were utilized to identify functional categories and significant pathways. KEGG and GO results suggested that the Cytokine-cytokine receptor interaction, P53 signaling pathway, and Apoptosis are the main signaling pathways in SARS-CoV-2 infected human bronchial organoids (hBOs). Furthermore, NFKBIA, C3, and CCL20 may be key genes in SARS-CoV-2 infected hBOs. Therefore, our study provides further insights into the therapy of COVID-19. [**note: an identification of the key genes for viral infection of human bronchial organoids.B]** https://www.biorxiv.org/content/10.1101/2020.08.18.256735v1
- Disrupted antiviral immune responses are associated with severe COVID-19, the disease caused by SAR-CoV-2. Here, we show that the 73-amino-acid protein encoded by ORF9c of the viral genome contains a putative transmembrane domain, interacts with membrane proteins in multiple cellular compartments, and impairs antiviral processes in a lung epithelial cell line. Proteomic, interactome, and transcriptomic analyses, combined with bioinformatic analysis, revealed that expression of only this highly unstable small viral protein impaired interferon signaling, antigen presentation, and complement signaling, while inducing IL-6 signaling. Furthermore, we showed that interfering with ORF9c degradation by either proteasome inhibition or inhibition of the ATPase VCP blunted the effects of ORF9c. Our study indicated that ORF9c enables immune evasion and coordinates cellular changes essential for the SARS-CoV-2 life cycle. [**note: more information on the ORF9c protein and how it interferes with the immune system response. This virus has some amazing properties. Isn't evolution wonderful and sometimes bad?**] https://www.biorxiv.org/content/10.1101/2020.08.18.256776v1

DIAGNOSTIC DEVELOPMENT

- Nothing new today.

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2020-08-21

I've featured classical chamber music over the weeks but let's hear from one of the finest jazz chamber groups ever to play together. Of course it has to be the Modern Jazz Quartet that was founded in 1952 and with only one change in personnel played together for a lot of years. Here is a live concert at the North Sea Festival in The Netherlands: https://www.youtube.com/watch?v=I10wV4m4Dug

Scott Sumner is an economist whose blogs I always find interesting. He has a post on herd immunity not being a precise number. This is my opinion as well. There have been numerous models that attempt to come up with a number and I have reported on most of them. Percentage of infected needed to achieve herd immunity range from 20 to just over 70%. Part of New York City are thought to have achieved this if the value is at the lower end of the range. However, this may not be the case as it only

applies to the stochastic case of a closed system. If Queens can be isolated from the outside, herd immunity may exist. However, if the value for herd immunity is 30%, then 70% are still naïve and subject to infection. Travel in and out of Queens will subject those people to potential infection. One simple example as to how this happens are the sporadic outbreaks of measles in the US because of insufficient vaccine compliance. According to CDC, 91.5% of children receive MMR vaccination and yet outbreaks still occur. Does this mean that herd immunity for this disease is greater than 91% (quite a high number!)? No, it just means that there are variables that we still don't fully understand. As the famous literary and film quote goes, "it's complicated!"

The New York Times is reporting a new adaptive clinical trial is about to begin that pairs remdesivir and β-interferon to see if the pair improve clinical outcomes over remdesivir alone. We still need an oral antiviral that can be given in outpatient settings.

The Washington Post reports on new research showing children my play a larger role in transmission. HERE is the Journal of Pediatrics article. I totally agree with Post sports columnist, Sally Jenkins, on the sickness that is college football. The first COVID-19 cases from the Sturgis, South Dakota motorcycle rally are beginning to come in. Empty classrooms and studios is not conducive to arts education. American Airlines announces discontinuation of flights to 15 cities; I am sure other airlines will follow. The Department of Health and Human Services has barred FDA from regulating certain COVID-19 tests. The ruling applies to lab developed tests under the Clinical Laboratory Improvement Amendment (CLIA) that is administered by CMS. This has always been a gray area with some types of tests not being subject to FDA oversight but having a long history of use (genetic tests being the foremost example; the Lyme disease test I had done on a recovered tick some years ago also falls into that category). In a move to further protect their football team, North Carolina State moves to virtual classes after some severe outbreaks at several fraternity and sorority houses. Big shout out to Purdue President Mitch Daniels who is showing his mettle in suspending 36 students for violating the school's policy on social distancing and masks!!! If any university president can pull off a school opening it's Mitch! Here is an interview with Tony Fauci; I wish him well as he recovers from vocal polyp surgery. Flu shot season is coming up but for us oldies it is best to wait until September/October to get the shot.

The Lancet have a case from Brazil on an 11 year old child with multisystem inflammatory syndrome related to COVID-19 who developed cardiac failure one day after being admitted to the hospital. Post-mortem pathology showed myocarditis, likely a result of the SARS-CoV-2 infection. From the UK, pathology on nine elderly patients who died of COVID-19. Clinical data showed that the four dominant interrelated pathological processes in severe COVID-19 are diffuse alveolar damage, thrombosis, haemophagocytosis, and immune cell depletion. Additionally, we report here several novel autopsy findings including pancreatitis, pericarditis, adrenal micro-infarction, secondary disseminated mucormycosis, and brain microglial activation, which require additional investigation to understand their role in COVID-19. There is an excellent commentary on the state of COVID-19 clinical trials that is instructive to read. Money quote: "*Media reports and prepublications on* medRxiv *and* bioRxiv *represent the most frequent mechanism for data sharing, with wide public reach and usually with little detail. However, with inadequate details on the trials and only superficial scrutiny by the public and scientific decision makers, the consequences have had disastrous effects on other clinical trial funding, permissions, recruitment, and interpretation*."

Derek Lowe on the recent news about the Pfizer mRNA vaccine. I think if you have to choose this version of the vaccine, Pfizer is the way to go.

MODELING

- Nothing New

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow

CLINICAL TRIAL RESULTS

- Unfortunately nothing today.

DRUG DEVELOPMENT

- Background: Severe acute respiratory syndrome coronavirus 2 (SARS−CoV−2) infections and the resulting disease, coronavirus disease 2019 (COVID−19), have spread to millions of people globally. Multiple vaccine candidates are under development, but no vaccine is currently available. Methods: Healthy adults 18−55 and 65−85 years of age were randomized in an ongoing, placebo−controlled, observer−blinded dose−escalation study to receive 2 doses at 21−day intervals of placebo or either of 2 lipid nanoparticle−formulated, nucleoside−modified RNA vaccine candidates: BNT162b1, which encodes a secreted trimerized SARS−CoV−2 receptor-binding domain, or BNT162b2, which encodes a prefusion stabilized membrane-anchored SARS−CoV−2 full-length spike. In each of 13 groups of 15 participants, 12 received vaccine and 3 received placebo. Groups were distinguished by vaccine candidate, age of participant, and vaccine dose level. Interim safety and immunogenicity data of BNT162b1 in younger adults have been reported previously from US and German trials. We now present additional safety and immunogenicity data from the US Phase 1 trial that supported selection of the vaccine candidate advanced to a pivotal Phase 2/3 safety and efficacy evaluation. Results: In both younger and older adults, the 2 vaccine candidates elicited similar dose-dependent SARS−CoV−2−neutralizing geometric mean titers (GMTs), comparable to or higher than the GMT of a panel of SARS−CoV−2 convalescent sera. BNT162b2 was associated with less systemic reactogenicity, particularly in older adults. Conclusion: These results support selection of the BNT162b2 vaccine candidate for Phase 2/3 large−scale safety and efficacy evaluation, currently underway. [**note: here is the Pfizer data for the mRNA candidate vaccine that was selected for development**] https://www.medrxiv.org/content/10.1101/2020.08.17.20176651v1
- More than one hundred vaccines against SARS-CoV-2 have been developed and some of them have entered clinical trials, but the latest results revealed that these vaccines still face great challenges. Here, we developed a novel cell-based gp96-Ig-secreting chimeric vaccine which is composed of two viral antigens, the RBD of spike protein, and a truncated nucleocapsid protein that could induce epitope-specific cytotoxic T lymphocytes but low antibody response. Syrian hamsters immunized with the cell-based vaccine produced high level of SARS-CoV-2 specific NAbs and specific T cell immunity which could eliminate RBD-truncated N-expressing cells, without the induction of antibody against N protein and other observed toxicity. This study provides a proof of concept for clinical testing of this safe, effective and cost-effective vaccine against SARS-CoV2 infection. [**note: another vaccine candidate from China. This is an**

interesting chimeric vaccine that they say is cost-effective. I don't know enough about the cell line they are using to know whether it is really that cost effective as they are not manufacturing at scale. It will be interesting to see if this ever goes into a trial given the stiff competition in the vaccine arena.]
https://www.biorxiv.org/content/10.1101/2020.08.19.258244v1

- Neutralizing antibodies (Abs) have been considered as promising therapeutics for the prevention and treatment of pathogens. After the outbreak of COVID-19, potent neutralizing Abs to SARS-CoV-2 were promptly developed, and a few of those neutralizing Abs are being tested in clinical studies. However, there were few methodologies detailly reported on how to rapidly and efficiently generate neutralizing Abs of interest. Here, we present a strategically optimized method for precisive screening of neutralizing monoclonal antibodies (mAbs), which enabled us to identify SARS-CoV-2 receptor-binding domain (RBD) specific Abs within 4 days, followed by another 2 days for neutralization activity evaluation. By applying the screening system, we obtained 198 Abs against the RBD of SARS-CoV-2. Excitingly, we found that approximately 50% (96/198) of them were candidate neutralizing Abs in a preliminary screening of SARS-CoV-2 pseudovirus and 20 of these 96 neutralizing Abs were confirmed with high potency. Furthermore, 2 mAbs with the highest neutralizing potency were identified to block authentic SARS-CoV-2 with the half-maximal inhibitory concentration (IC50) at concentrations of 9.88 ng/ml and 11.13 ng/ml. In this report, we demonstrated that the optimized neutralizing Abs screening system is useful for the rapid and efficient discovery of potent neutralizing Abs against SARS-CoV-2. Our study provides a methodology for the generation of preventive and therapeutic antibody drugs for emerging infectious diseases. [**note: a new way to rapidly screen for neutralizing antibodies from China.**]
https://www.biorxiv.org/content/10.1101/2020.08.19.253369v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Detailed knowledge about the dynamics of SARS-CoV-2 infection is important for unraveling the viral and host factors that contribute to COVID-19 pathogenesis. Old-World nonhuman primates recapitulate mild-moderate COVID-19 cases, thereby serving as important pathogenesis models. We compared African green monkeys inoculated with SARS-CoV-2 or inactivated virus to study the dynamics of virus replication throughout the respiratory tract. RNA sequencing of single cells from the lungs and mediastinal lymph nodes allowed a high-resolution analysis of virus replication and host responses over time. Viral replication was mainly localized to the lower respiratory tract, with evidence of replication in the pneumocytes. Macrophages were found to play a role in initiating a pro-inflammatory state in the lungs, while also interacting with infected pneumocytes. Our dataset provides a detailed view of changes in host and virus replication dynamics over the course of mild COVID-19 and serves as a valuable resource to identify therapeutic targets. [**note: virus dynamics in the African green monkey**]
https://www.biorxiv.org/content/10.1101/2020.08.20.258087v1
- The current SARS-CoV-2 pandemic is accompanied by high morbidity and mortality rates, and there is a compelling need for effective vaccines and therapeutic agents to lessen the severity of COVID-19 disease. Appropriate animal models are essential for testing of vaccines and therapeutics and for mechanistic studies of infection and the host response. The Spike (S) protein of SARS-COV-2 has a high affinity for the human ACE2 receptor, which is expressed on

multiple cell types including alveolar epithelial and vascular endothelial cells. Wild-type mice are not susceptible to developing coronavirus-mediated diseases. Accordingly, several human (h)ACE2 transgenic mouse models have been developed for coronavirus research. However, these mice have failed to closely mimic important aspects of the human immunopathological responses to SARS-CoV-2. We report herein that DRAGA (HLA-A2.HLA-DR4.Rag1KO.IL-2R. gammac KO.NOD) mice infused with human hematopoietic stem cells from cord blood reconstitute a fully functional human immune system, as well as engraft human epithelial and endothelial cells, sustain SARS-CoV-2 infection, and develop severe COVID-19-like symptoms. In pilot experiments, infected mice developed parenchymal and epithelial lung infiltrations with granzyme B+ and perforin+ CD8+ T cells and alveolar CD61+ microthrombi, mimicking human immunopathological responses to SARS-CoV-2. We propose the DRAGA mouse as a novel pre-clinical tool for studying COVID-19 immunopathology and human immune responses to SARS-CoV-2, including events leading to the cytokine storm and coagulopathies, as well as for testing of candidate vaccines and therapeutics. [**note: another animal model, this time a genetically modified mouse.**] https://www.biorxiv.org/content/10.1101/2020.08.19.251249v1

- Understanding and eliciting protective immune responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an urgent priority. To facilitate these objectives, we have profiled the repertoire of human leukocyte antigen class II (HLA-II)-bound peptides presented by HLA-DR diverse monocyte-derived dendritic cells pulsed with SARS-CoV-2 spike (S) protein. We identify 209 unique HLA-II-bound peptide sequences, many forming nested sets, which map to sites throughout S including glycosylated regions. Comparison of the glycosylation profile of the S protein to that of the HLA-II-bound S peptides revealed substantial trimming of glycan residues on the latter, likely introduced during antigen processing. Our data also highlight the receptor-binding motif in S1 as a HLA-DR-binding peptide-rich region. Results from this study have application in vaccine design, and will aid analysis of CD4+ T cell responses in infected individuals and vaccine recipients. [**note: more on the Spike protein and antigen processing.**] https://www.biorxiv.org/content/10.1101/2020.08.19.255901v1
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China at the end of 2019, and became pandemic. The zoonotic virus most likely originated from bats, but definite intermediate hosts have not yet been identified. Raccoon dogs (Nyctereutes procyonoides) are kept for fur production, in particular in China, and were suspected as potential intermediate host for both SARS-CoV6 and SARS-CoV2. Here we demonstrate susceptibility of raccoon dogs for SARS-CoV-2 infection after intranasal inoculation and transmission to direct contact animals. Rapid, high level virus shedding, in combination with minor clinical signs and pathohistological changes, seroconversion and absence of viral adaptation highlight the role of raccoon dogs as a potential intermediate host. The results are highly relevant for control strategies and emphasize the risk that raccoon dogs may represent a potential SARS-CoV-2 reservoir. Our results support the establishment of adequate surveillance and risk mitigation strategies for kept and wild raccoon dogs. [**note: here is an animal that is new to me!! The raccoon dog may be a host to SARS-CoV-2 virus in China.]** https://www.biorxiv.org/content/10.1101/2020.08.19.256800v1

DIAGNOSTIC DEVELOPMENT

- The coronavirus disease 2019 (COVID-19) pandemic caused by Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) is in urgent need of therapeutic options. High-throughput

screening (HTS) offers the research field an opportunity to rapidly identify such compounds. In this work, we have developed a homogeneous cell-based HTS system using AlphaLISA detection technology for the SARS-CoV-2 nucleocapsid protein (NP). Our assay measures both recombinant NP and endogenous NP from viral lysates and tissue culture supernatants (TCS) in a sandwich-based format using two monoclonal antibodies against the NP analyte. Viral NP was detected and quantified in both tissue culture supernatants and cell lysates, with large differences observed between 24 hours and 48 hours of infection. We simulated the viral infection by spiking in recombinant NP into 384-well plates with live Vero-E6 cells and were able to detect the NP with high sensitivity and a large dynamic range. Anti-viral agents that inhibit either viral cell entry or replication will decrease the AlphaLISA NP signal. Thus, this assay can be used for high-throughput screening of small molecules and biologics in the fight against the COVID-19 pandemic. [**note: a new approach to detecting the nucleocapsid protein. This is probably more of a research tool.**]
https://www.biorxiv.org/content/10.1101/2020.08.20.258129v1

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2020-08-22

Gershwin and dance, a good coupling indeed. No, we are not going with Fred and Ginger though they are fantastic in every movie they did. This time the dance is ballet, and the choreographer is George Balanchine: https://www.youtube.com/watch?v=VM_91kZVQ1o&t=8s Yes, I know you all want to see Fred and Ginger. Here they are in the closing number 'Shall We Dance': https://www.youtube.com/watch?v=VS9rCo-sA9Q

From my Yahoo news feed, here is a very good summary of the vaccine landscape from one of the academic developers. I agree that we need multiple vaccines and we also need to track performance of each one.

As this Washington Post story shows, we can all learn from Rhode Island. The have established protocols and opened childcare facilities. Student newspapers at some colleges argue that administrators ae partly to blame for the recent outbreaks on campus. This sports column really sends the wrong message.

The New York Times has an interesting story on the development of a new algorithm for pooled SARS-CoV-2 testing developed by a group of Israeli scientists. They were able to achieve an eight-fold increase in testing efficiency and a similar reduction in cost. A link to the full paper is HERE and I would note that the senior author is a professor at the same campus of the Open University where my sister is a professor of Biblical Studies!!! Antibody tests may not be as helpful as we once thought.

Lots of papers to review today!!!! There is disappointing news on the use of convalescent serum from Brown University. In an elderly cohort, mortality was not reduced but time to discharge was. The authors note that it is a small study and further trials need to be done.

MODELING

- Wastewater-based epidemiology (WBE) has emerged as an effective environmental surveillance tool in monitoring fecal-oral pathogen infections within a community. Congruently, SARS-CoV-2

virus, the etiologic agent of COVID-19, has been demonstrated to infect the gastrointestinal tissues, and be shed in feces. In the present study, SARS-CoV-2 RNA was concentrated from wastewater, sludge, surface water, ground water, and soil samples of municipal and hospital wastewater systems and related environment in Wuhan during the COVID-19 middle and low risk periods, and the viral RNA copies quantified using RT-qPCR. From the findings of this study, during the middle risk period, one influent sample and three secondary treatment effluents collected from Waste Water Treatment Plant 2 (WWTP2), as well as two influent samples from wastewater system of Hospital 2 were SARS-CoV-2 RNA positive. One sludge sample collected from Hospital 4; which was obtained during low risk period, was positive for SARS-CoV-2 RNA. These study findings demonstrate the significance of WBE in continuous surveilling and monitoring of SARS-CoV-2 at the community level, even when the COVID19 prevalence is low. Therefore, the application of WBE is principally useful in tracking the level of infections in communities and the risk assessment of the secondary environment. [**note: Wuhan wastewater surveillance information.**]
https://www.medrxiv.org/content/10.1101/2020.08.19.20172924v1

- A fundamental problem dealing with the Covid-19 pandemic has been to estimate the rate of infection, since so many cases are asymptomatic and contagious just for a few weeks. For example, in the US, estimate the proportion P(t) = N/330 where N is the US total who have ever been infected (in millions)at time t (months, t =0 being March 20). This is important for decisions on social restrictions, and allocation of medical resources, etc. However, the demand for extensive testing has not produced good estimates. In the US, the CDC has used the blood supply to sample for anti-bodies. Anti-bodies do not tell the whole picture, according to the Karolinska Instituet , many post infection cases show T-cell immunity, but no anti-bodies. We introduce a method based on a difference-differential equation (dde) for P(t). We emphasize that this is just for the present, with no prediction on how the pandemic will evolve. The dde uses only x=x(s), which is the number/million testing positive, and y=y(s), the number/million who have been tested for all time $0 < s < t$ (months), with no assumptions on the dynamics of the pandemic. However, we need two parameters. First, R , the ratio of asymptomatic to symptomatic infected cases. Second, T , the period of active infection when the virus can be detected. Both are random variables with distribution which can be estimated. For fixed R, we prove uniform bounds $(1+ R)\, x/(y +1) < P(t) < (1+ R)\, x(t)$, are best possible, with range depending on T . One advantage of our theory is being able to estimate P for many regions and countries where x and y is the only information available. [**note: this is an interesting model from a Univ of Maryland mathematician. I like the paper because he spends time going over the origin of the equation and the various assumption. Very clearly written but like everything else COVID-19 related, it may or may not be correct.**]
https://www.medrxiv.org/content/10.1101/2020.08.17.20176602v1
- The evolution of coronavirus disease (COVID-19) into a pandemic has severely hampered the usage of public transit systems. In a post-COVID-19 world, we may see an increased reliance on autonomous cars and personal rapid transit (PRT) systems, with inherent physical distancing, over buses, trains, and aircraft for intracity, intercity, and interstate travel. However, air travel would continue to be the dominant mode of intercontinental transportation for humans. In this study, we perform a comprehensive computational analysis of typical intercontinental aircraft ventilation systems to determine the seat where environmental factors are most conducive to

human comfort with regards to air quality, protection from orally or nasally released pollutants such as CO2 and coronavirus, and thermal comfort levels. Air velocity, temperature, and air pollutant concentration emitted from the nose/mouth of fellow travelers are considered for both Boeing and Airbus planes. In each plane, first class, business class, and economy class sections were analyzed. We present conclusions as to which is the optimum seat in each section of each plane and provide the data of the environmental conditions to support our inferences. The findings may be used by the general public to decide which seat to occupy for their next intercontinental flight. Alternatively, the commercial airliners can use such a model to plan the occupancy of the aircraft on long-duration intercontinental flights (viz., Airbus A380 and Boeing B747). [**note: planning an airline trip? This paper may help you select the right seat!]**
https://www.medrxiv.org/content/10.1101/2020.08.17.20176909v1

- The relationship between specific humidity and influenza/SARS-CoV-2 in the Netherlands is evaluated over time and at regional level. Design: Parametric and non-parametric correlation coefficients are calculated to quantify the relationship between humidity and influenza, using five years of weekly data. Bayesian spatio-temporal models-with a Poisson and a Gaussian likelihood-are estimated to find the relationship between regional humidity and the daily cases of SARS-CoV-2 in the municipalities and provinces of the Netherlands. Results: An inverse (negative) relationship is observed between specific humidity and the incidence of influenza between 2015 and 2019. The space-time analysis indicates that an increase of specific humidity of one gram of water vapor per kilogram of air (1 g/kg) is related to a reduction of approximately 5% in the risk of COVID-19 infections. Conclusion: The increase in humidity during the outbreak of the SARS-CoV-2 in the Netherlands helped to reduce the risk of regional COVID-19 infections. Public policies that promote higher levels of specific humidification-above 6 g/Kg-can lead to significant reductions in the spread of respiratory viruses, such as influenza and SARS-CoV-2. [**note: is this a case of correlation not being causation? Low humidity typically occurs during winter months when influenza is prevalent. There is also reduced sunlight and maybe that is more of a factor than humidity. If increased humidity moderates SARS-CoV-2 infection, why is Brazil so hard hit?**]
https://www.medrxiv.org/content/10.1101/2020.08.18.20177444v1

NEWLY REGISTERED CLINICAL TRIALS

- This is a first-in-human (FIH), Phase 1, single-center, randomized, double-blind, placebo-controlled, single ascending dose study to evaluate the safety, tolerability, PK and immunogenicity of AK119, a humanized monoclonal antibody targeting the CD73. The study will consist of 4 cohorts of healthy subjects. Eight subjects will be enrolled per cohort, randomized in a 3:1 ratio to receive a single dose of either the active drug AK119 (N=6) or matching placebo (N=2). Approximately 32 subjects (24 receiving active drug and 8 receiving placebo) will participate in this study. [**note: this is a trial in New Zealand. It's not clear what the mechanism is.**] NCT04516564

- The purpose of this study is to measure the effect of the Shingrix vaccine on your immune system and whether that has any effect on the body's ability to fight off other infections such as COVID-19. We hypothesize that: H1: Shingrix vaccination will elevate acute and trained immunity & H2: For 6 months following the first injection, increased levels of acute and trained immunity is associated with less disease, including fewer hospitalizations and deaths associated

with flu, pneumonia, and COVID-19. [**note: having gotten my Shingrix shots last year, I certainly hope this trial works!!!**] NCT04523246

- This study aims to evaluate the safety and immunogenicity of the preventative vaccine, AdimrSC-2f, in healthy volunteers aged from 20 to 60 years old. [**note: this is a baculovirus derived vaccine developed by Adimmune Corp and is being administered in Taiwan.**] NCT04522089

CLINICAL TRIAL RESULTS

- Early clinical reports have suggested that the prevalence of thrombotic complications in the pathogenesis of COVID-19 may be as high as 30% in intensive care unit (ICU)-admitted patients and could be a major factor contributing to mortality. However, mechanisms underlying COVID-19-associated thrombo-coagulopathy, and its impact on patient morbidity and mortality, are still poorly understood. Methods: We performed a comprehensive analysis of coagulation and thromboinflammatory factors in plasma from COVID-19 patients with varying degrees of disease severity. Furthermore, we assessed the functional impact of these factors on clot formation and clot lysis. Results: Across all COVID-19 disease severities (mild, moderate and severe) we observed a significant increase (6-fold) in the concentration of ultra-large von Willebrand factor (UL-VWF) multimers compared to healthy controls. This is likely the result of an interleukin (IL)-6 driven imbalance of VWF and the regulatory protease ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motifs, member 13). Upregulation of this key pro-coagulant pathway may also be influenced by the observed increase (~6-fold) in plasma α-defensins, a consequence of increased numbers of neutrophils and neutrophil activation. Markers of endothelial, platelet and leukocyte activation were accompanied by increased plasma concentrations of Factor XIII (FXIII) and plasminogen activator inhibitor (PAI)-1. In patients with high FXIII we observed alteration of the fibrin network structure in in vitro assays of clot formation, which coupled with increased PAI-1, prolonged the time to clot lysis by the t-PA/plasmin fibrinolytic pathway by 52% across all COVID-19 patients (n=23). Conclusions: We show that an imbalance in the VWF/ADAMTS13 axis causing increased VWF reactivity may contribute to the formation of platelet-rich thrombi in the pulmonary vasculature of COVID-19 patients. Through immune and inflammatory responses, COVID-19 also alters the balance of factors involved in fibrin generation and fibrinolysis which accounts for the persistent fibrin deposition previously observed in post-mortem lung tissue. [**note: more on the coagulation accompanying COVID-19**] https://www.medrxiv.org/content/10.1101/2020.08.18.20159608v1
- Growing clinical evidence has implicated complement as a pivotal driver of COVID-19 immunopathology. Deregulated complement activation may fuel thrombotic microangiopathy and NET-driven immunothrombosis, thereby exacerbating cytokine-driven hyper-inflammation and multi-organ failure. Complement therapeutics have gained traction as candidate drugs for countering the detrimental consequences of SARS-CoV-2 infection. Whether blockade of terminal complement effectors (C5, C5a, or C5aR1) can elicit similar outcomes to upstream intervention at the level of C3 remains debated. Here we have compared the clinical efficacy of the C5-targeting mAb eculizumab with that of the compstatin-based C3-targeted drug candidate AMY-101 in small independent cohorts of severe, mainly non-intubated COVID-19 patients. Our exploratory study indicates that therapeutic complement inhibition abrogates COVID-19 hyper-inflammation. Both C3 and C5 inhibitors elicit a robust anti-inflammatory response, reflected by

a steep decline in CRP and IL-6 levels, associated with marked lung function improvement and resolution of SARS-CoV-2-associated ARDS. C3 inhibition afforded broader therapeutic control in COVID19 patients by attenuating both C3a and sC5b-9 generation and preventing FB consumption. This broader inhibitory profile of anti-C3 treatment was associated with a more robust decline of neutrophil counts, a greater decline of median LDH levels and more prominent lymphocyte recovery within the first 7 days of treatment. These early clinical results offer important insight into the differential mechanistic basis and underlying biology of C3 and C5 inhibition in COVID-19. They point to a broader pathogenic involvement of C3-mediated pathways and set the stage for larger prospective trials that will benchmark these complement-targeting agents in COVID-19. [**note: the role of Complement C3 vs C5 inhibition in severe COVID-19.]** https://www.medrxiv.org/content/10.1101/2020.08.17.20174474v1

- The efficacy of convalescent plasma (CP) for the treatment of COVID-19 remains unclear. Methods A matched cohort analysis of hospitalized patients with severe COVID-19. The impact of CP treatment on all cause in-hospital mortality was evaluated using univariate and multivariate Cox proportional-hazards models, and the impact of CP treatment on the time to hospital discharge was assessed using a stratified log-rank analysis. Results 64 patients who received CP a median of 7 days after symptom onset were compared to a matched control group of 177 patients. Overall in-hospital mortality was 14.9%. There was no significant difference in the risk of in-hospital mortality between the two groups (adjusted hazard ratio [aHR] 0.93, 95% confidence interval [CI] 0.39 − 2.20). There was also no significant difference in the overall rate of hospital discharge (rate ratio [RR} 1.28, 95% CI 0.91 − 1.81), but a subgroup analysis of patients 65-years-old or greater who received CP demonstrated a significantly increased hospital discharge rate among these patients (RR 1.86, 95% CI 1.03 − 3.36). There was a greater than expected frequency of transfusion reactions in the CP group (2.8% reaction rate observed per unit transfused). Conclusions The use of CP in this study was a safe treatment for COVID-19. There was no overall significant reduction of in-hospital mortality or increased rate of hospital discharge associated with the use of CP in this study, although there was a signal for improved outcomes among the elderly. Further adequately powered randomized studies should target this subgroup when assessing the efficacy CP treatment. [**note: this is a huge bummer. In this study, convalescent plasma seems not to be effective at reducing mortality. Date to discharge was reduced which was a good thing. We need to see further data here and also for MaB treatment.**] https://www.medrxiv.org/content/10.1101/2020.08.18.20177402v1
- Children with SARS-CoV-2 infection typically have mild symptoms that do not require medical attention, leaving a gap in our understanding of the spectrum of illnesses that the virus causes in children. METHODS: We conducted a prospective cohort study of children and adolescents (<21 years of age) with a SARS-CoV-2-infected close contact. We collected nasopharyngeal or nasal swabs at enrollment and tested for SARS-CoV-2 using a real-time PCR assay. RESULTS: Of 382 children, 293 (77%) were SARS-CoV-2-infected. SARS-CoV-2-infected children were more likely to be Hispanic (p<0.0001), less likely to have asthma (p=0.005), and more likely to have an infected sibling contact (p=0.001) than uninfected children. Children ages 6-13 years were frequently asymptomatic (39%) and had respiratory symptoms less often than younger children (29% vs. 48%; p=0.01) or adolescents (29% vs. 60%; p<0.0001). Compared to children ages 6-13 years, adolescents more frequently reported influenza-like (61% vs. 39%; p<0.0001), gastrointestinal (27% vs. 9%; p=0.002), and sensory symptoms (42% vs. 9%; p<0.0001), and had

more prolonged illnesses [median (IQR) duration: 7 (4, 12) vs. 4 (3, 8) days; p=0.01]. Despite the age-related variability in symptoms, we found no differences in nasopharyngeal viral load by age or between symptomatic and asymptomatic children. CONCLUSIONS: Hispanic ethnicity and an infected sibling close contact are associated with increased SARS-CoV-2 infection risk among children, while asthma is associated with decreased risk. Age-related differences in the clinical manifestations of SARS-CoV-2 infection must be considered when evaluating children for COVID-19 and in developing screening strategies for schools and childcare settings. [**note: this Duke study looks at the rate of SARS-CoV-2 infection in children. It's consistent with previous studies. We still don't know the rate of transmission of asymptomatic infected small children.**] https://www.medrxiv.org/content/10.1101/2020.08.18.20166835v1

- The heterogeneous disease course of COVID-19 is unpredictable, ranging from mild self-limiting symptoms to cytokine storms, acute respiratory distress syndrome (ARDS), multi-organ failure and death. Identification of high-risk cases will enable appropriate intervention and escalation. This study investigates the routine laboratory tests and cytokines implicated in COVID-19 for their potential application as biomarkers of disease severity, respiratory failure and need of higher-level care. From analysis of 203 samples, CRP, IL-6, IL-10 and LDH were most strongly correlated with the WHO ordinal scale of illness severity, the fraction of inspired oxygen delivery, radiological evidence of ARDS and level of respiratory support ($p \leq 0.001$). IL-6 levels of >3.27pg/ml provide a sensitivity of 0.87 and specificity of 0.64 for a requirement of ventilation, and a CRP of >37mg/L of 0.91 and 0.66. Reliable stratification of high-risk cases has significant implications on patient triage, resource management and potentially the initiation of novel therapies in severe patients. [**note: more on the biomarkers for severe COVID-19 from the UK.**] https://www.medrxiv.org/content/10.1101/2020.08.18.20168807v1
- A decrease in blood cell counts, especially lymphocytes and eosinophils, has been described in patients with severe SARS-CoV-2 (COVID-19), but there is no knowledge of the potential role of their recovery in these patients prognosis. This article aims to analyse the effect of blood cell depletion and blood cell recovery on mortality due to COVID-19. Design: This work is a multicentre, retrospective, cohort study of 9,644 hospitalised patients with confirmed COVID-19 from the Spanish Society of Internal Medicine SEMI-COVID-19 Registry. Setting: This study examined patients hospitalised in 147 hospitals throughout Spain. Participants: This work analysed 9,644 patients (57.12% male) out of a cohort of 12,826 patients over 18 years of age hospitalised with COVID-19 in Spain included in the SEMI-COVID-19 Registry as of 29 May 2020. Main outcome measures: The main outcome measure of this work is the effect of blood cell depletion and blood cell recovery on mortality due to COVID-19. Univariate analysis was performed to determine possible predictors of death and then multivariate analysis was carried out to control for potential confounders. Results: An increase in the eosinophil count on the seventh day of hospitalisation was associated with a better prognosis, including lower mortality rates (5.2% vs 22.6% in non-recoverers, OR 0.234 [95% CI, 0.154 to 0.354]) and lower complication rates, especially regarding to development of acute respiratory distress syndrome (8% vs 20.1%, p=0.000) and ICU admission (5.4% vs 10.8%, p=0.000). Lymphocyte recovery was found to have no effect on prognosis. Treatment with inhaled or systemic glucocorticoids was not found to be a confounding factor. Conclusion: Eosinophil recovery in patients with COVID-19 is a reliable marker of a good prognosis that is independent of prior treatment. This finding could be used to guide discharge decisions. [**note: this is a nice cohort study from Spain looking**

at the rebound in eosinophil counts in COVID-19 patients. It may be a good marker for gauging recovery.] https://www.medrxiv.org/content/10.1101/2020.08.18.20172874v1

DRUG DEVELOPMENT

- It becomes more and more obvious that deregulation of host metabolism play an important role in SARS-CoV-2 pathogenesis with implication for increased risk of severe course of COVID-19. Furthermore, it is expected that COVID-19 patients recovered from severe disease may experience long-term metabolic disorders. Thereby understanding the consequences of SARS-CoV-2 infection on host metabolism can facilitate efforts for effective treatment option. We have previously shown that SARS-CoV-2-infected cells undergo a shift towards glycolysis and that 2-deoxy-D-glucose (2DG) inhibits SARS-CoV-2 replication. Here, we show that also pentose phosphate pathway (PPP) is remarkably deregulated. Since PPP supplies ribonucleotides for SARS-CoV-2 replication, this could represent an attractive target for an intervention. On that account, we employed the transketolase inhibitor benfooxythiamine and showed dose-dependent inhibition of SARS-CoV-2 in non-toxic concentrations. Importantly, the antiviral efficacy of benfooxythiamine was further increased in combination with 2DG. [**note: I don't know much about the compound they tested other than the link about the structure. There is a European patent HERE. Unlike a lot of papers I have been reading, these researchers at least have a plausible mechanism for inhibition!.**]
https://www.biorxiv.org/content/10.1101/2020.08.19.257022v1
- COVID-19 (coronavirus disease 2019) is a pandemic caused by SARS-CoV-2 (severe acute respiratory syndrome-coronavirus 2) infection affecting millions of persons around the world. There is an urgent unmet need to provide an easy-to-produce, affordable medicine to prevent transmission and provide early treatment for this disease. The nasal cavity and the rhinopharynx are the sites of initial replication of SARS-CoV-2. Therefore, a nasal spray may be a suitable dosage form for this purpose. The main objective of our study was to test the antiviral action of three candidate nasal spray formulations against SARS-CoV-2. We have found that iota-carrageenan in concentrations as low as 6 mcg/ mL inhibits SARS-CoV-2 infection in Vero cell cultures. The concentrations found to be active in vitro against SARS-CoV-2 may be easily achieved by the application of nasal sprays already marketed in several countries. Xylitol at a concentration of 5 % m/V has proved to be viricidal on its own and the association with iota-carrageenan may be beneficial, as well. [**note: I'm beginning to wonder if this *in vitro* test is worth anything. Lots of compounds show up as inhibitory without a plausible mechanism of action. I imagine that if they tested the fine Procter & Gamble dish detergent, Dawn™, it would be viricidal as well. Does this mean that we all should do twice daily nasal rinses with this product? Probably not (disclosure: as a P&G shareholder I would benefit greatly were this to be shown as a viable COVID-19 preventive treatment).**]
https://www.biorxiv.org/content/10.1101/2020.08.19.225854v1
- An effective response to the ongoing coronavirus disease (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) will require a range of complementary preventive modalities. The current studies were conducted to evaluate the in vitro SARS-CoV-2 antiviral activity of astodrimer sodium, a dendrimer with broad spectrum antimicrobial activity, including against enveloped viruses in in vitro and in vivo models, that is marketed for antiviral and antibacterial applications. We report that astodrimer sodium inhibits

replication of SARS-CoV-2 in Vero E6 cells when added to cells 1-hour prior to or 1-hour post infection, with 50% effective concentrations reducing virus-induced cytopathic effect (EC50) ranging from 0.090 to 0.742 µM (0.002 to 0.012 mg/mL). The selectivity index (SI) in these assays was as high as 2197. Astodrimer sodium was also effective in a virucidal evaluation when mixed with virus for 1 hour prior to infection of cells (EC50 1.83 µM [0.030 mg/mL]). Results from a time of addition study, which showed infectious virus was below the lower limit of detection at all time points tested, were consistent with the compound inhibiting early virus entry steps. The data were similar for all investigations and were consistent with the potent antiviral activity of astodrimer sodium being due to inhibition of virus-host cell interactions, as previously demonstrated for other viruses. Further studies will confirm if astodrimer sodium binds to SARS-CoV-2 spike protein and physically blocks initial association of the virus with heparan sulfate proteoglycans on the host cell. Given the in vitro effectiveness and significantly high SI, astodrimer sodium warrants further investigation for potential as a nasally administered or inhaled antiviral agent for SARS-CoV-2 prevention and treatment applications. [**note: yet another compound with *in vitro* activity! This is a topical microbicide developed by Starpharma who are the authors of this paper.**
https://www.biorxiv.org/content/10.1101/2020.08.20.260190v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Adaptive Biotechnologies has built an immune medicine platform based on the sequencing of immune receptors (immunoglobulins, B-cell receptors [BCRs] and T-cell receptors [TCRs]) with myriad applications in health and disease. This broad platform technology can be used to assess the diversity of the cellular adaptive immune system and track disease-associated TCRs and BCRs during the course of infection. The SARS-CoV-2 virus is spreading rapidly throughout the world, causing significant morbidity and mortality. Researchers, governments, and biotechnology companies are mobilizing to develop and distribute diagnostic and therapeutic alternatives to try to curb this global pandemic. Methods: In collaboration with our partners LabCorp/Covance, Adaptive Biotechnologies has opened the ImmuneRACE study to prospectively collect samples from individuals who have been infected with SARS-CoV-2, who have recovered from SARS-CoV-2 infection, or who have been exposed to someone infected with SARS-CoV-2. Discussion: We believe that the information contained within the genetics of the adaptive immune response to SARS-CoV-2 can improve our understanding of the immunobiology of this devasting virus and may inform efforts to improve current diagnostic and therapeutic approaches. To facilitate scientific and clinical advancement in the fight against COVID-19, the TCR sequence data resulting from the primary aims of this study will be made publicly available to scientists and researchers across the globe, an effort made possible through a collaboration with Microsoft. Trial registration: ImmuneRACE is registered with the US National Institutes of Health and can be accessed at ClinicalTrials.gov (NCT04494893). [**note: this looks like it will be a useful tool in tracking immune responses.**]
https://www.medrxiv.org/content/10.1101/2020.08.17.20175158v2
- Monitoring the levels of SARS-CoV-2 specific antibodies such as IgG, M and A in COVID-19 patient is an alternative method for diagnosing SARS-CoV-2 infection and an simple way to monitor immune responses in convalescent patients and after vaccination. Here, we assessed the levels of SARS-CoV-2 RBD specific antibodies in twenty-seven COVID-19 convalescent

patients over 28-99 days after hospital discharge. Almost all patient who had severe or moderate COVID-19 symptoms and a high-level of IgG during the hospitalization showed a significant reduction at revisit. The remaining patients who had a low-level IgG during hospitalization stayed low at revisit. As expected, IgM levels in almost all convalescent patients reduced significantly or stayed low at revisit. The RBD-specific IgA levels were also reduced significantly at revisit. We also attempted to estimate decline rates of virus-specific antibodies using a previously established exponential decay model of antibody kinetics after infection. The predicted days when convalescent patients' RBD-specific IgG reaches to an undetectable level are approximately 273 days after hospital discharge, while the predicted decay times are 150 days and 108 days for IgM and IgA, respectively. This investigation and report will aid current and future studies to develope SARS-CoV-2 vaccines that are potent and long-lasting. [**note: length of antibody decline following SARS-CoV-2 infection. The real question is whether there is any immune memory at the point where antibody levels are not measurable.**]
https://www.medrxiv.org/content/10.1101/2020.08.17.20175950v1

- SARS-CoV-2 recently emerged as a human pathogen and is the causative agent of the COVID-19 pandemic. A molecular framework of how the virus manipulates host cellular machinery to facilitate infection remains unclear. Here, we focus on SARS-CoV-2 NSP1, which is proposed to be a virulence factor that inhibits protein synthesis by directly binding the human ribosome. Using extract-based and reconstitution experiments, we demonstrate that NSP1 inhibits translation initiation on model human and SARS-CoV-2 mRNAs. NSP1 also specifically binds to the small (40S) ribosomal subunit, which is required for translation inhibition. Using single-molecule fluorescence assays to monitor NSP1-40S subunit binding in real time, we demonstrate that eukaryotic translation initiation factors (eIFs) modulate the interaction: NSP1 rapidly and stably associates with most ribosomal pre-initiation complexes in the absence of mRNA, with particular enhancement and inhibition by eIF1 and eIF3j, respectively. Using model mRNAs and an inter-ribosomal-subunit FRET signal, we elucidate that NSP1 competes with RNA segments downstream of the start codon to bind the 40S subunit and that the protein is unable to associate rapidly with 80S ribosomes assembled on an mRNA. Collectively, our findings support a model where NSP1 associates with the open head conformation of the 40S subunit to inhibit an early step of translation, by preventing accommodation of mRNA within the entry channel. [**note: another viral factor that interacts with protein synthesis.]**
https://www.biorxiv.org/content/10.1101/2020.08.20.259770v1
- SARS-CoV-2 has infected millions of people and is on a trajectory to kill more than one million globally. Virus entry depends on the receptor-binding domain (RBD) of the spike protein. Although previous studies demonstrated anti-spike and -RBD antibodies as essential for protection and convalescent plasma as a promising therapeutic option, little is known about the immunoglobulin (Ig) isotypes capable of blocking virus entry. Here, we studied spike- and RBD-specific Ig isotypes in plasma/sera from two acutely infected and 29 convalescent individuals. Spike- and RBD-specific IgM, IgG1, and IgA1 antibodies were produced by all or nearly all subjects at varying levels and detected at 7-8 days post-disease onset. IgG2, IgG3, IgG4, and IgA2 were also present but at much lower levels. All samples also displayed neutralizing activity. IgM, IgG, and IgA were capable of mediating neutralization, but neutralization titers correlated better with binding levels of IgM and IgA1 than IgG. [**note: more good work from the Mt. Sinai group on the antibody profiles from COVID-19 patients. IgM and IgA binding seem to be more**

important than IgG. I wonder what profiles of vaccinated individuals look like.]
https://www.medrxiv.org/content/10.1101/2020.08.18.20177303v1

DIAGNOSTIC DEVELOPMENT

- No news today.

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2020-08-23

You cannot ever go wrong with Bach as this clip from MASH shows: https://www.youtube.com/watch?v=0zWbpZPsrfc For this reflection Sunday, here is the concerto for violin in a minor featuring Julia Fischer and the Baltic Sea Youth Philharmonic (it's always good when young musicians can play with accomplished artists): https://www.youtube.com/watch?v=sDyDHvZKl8g and as an added treat here is the concerto for 3 violins in D major: https://www.youtube.com/watch?v=WDkDbvtQaSE

US COVID-19 STATISTICS - **Infection Rate: 1.7%; CFR: 3.1%** (IR unchanged; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

The New York Times notes how difficult it is controlling student behavior on college campuses. Outdoor dining is all the rage in NYC but how long can it last and will restaurants survive? Life in China is returning to normal.

The Washington Post covers the outbreak of COVID-19 in the Midwest. President Trump is now taking on the FDA, accusing them of impeding enrollment in clinical trials for political reasons. I'll restrain myself and only say these types of comments are baseless. Remember motels and roadside inns? They seem to be making a comeback. Don't let your brain become numb to COVID-19 risks!

STAT have a piece on the President's tweets about the FDA Deep State phenomenon. Here is a nice piece by veteran FDA watcher Ed Silverman on the same topic.

MODELING

- BCG have heterogeneous immunity to certain pathogens other than Mycobacterium tuberculosis effect. At early times during COVID-19 pandemic heterogeneous immunity towards (SARS-CoV-2), was hypothesized and statistical correlation between of BCG vaccination practices and COVID-19 mortality variances among countries was statistically proved . These studies was criticized because of low evidence of such studies and possible confounding factors. For that reason this study was designed to look for impact of duration of cessation of BCG programs on Covid-19 mortality looking for the hypotheses by different design and looking forward to support previous studies. Methods: Total number of studied group is 14 countries which has stopped BCG vaccination programs. Through applying stem-leaf plot for exploring data screening behavior concerning Covid-19 Mortality for obsolescence duration of cessation of mass BCG vaccination programs, as well as (nonlinear regression of compound model) for predicted shape behavior for that group. Results: Slope value shows highly significant effectiveness of obsolescence of cessation of mass BCG vaccination programs on Covid -19 mortality at P-value<0.000. Obsolescence of duration of cessation of mass BCG vaccination

programs has strongly negatively associated with Covid-19 mortality in countries which stopped BCG vaccination programs. Conclusion: The longer the cessation duration of BCG programs, the higher the Covid-19 mortality is, and vice versa. [**note: Carl Bergstrom, co-author of 'Calling Bullshit: The Art of Scepticism in a Data-Driven World' would figure out the flaws in this study within a minute (it took me three).**]
https://www.medrxiv.org/content/10.1101/2020.08.20.20178889v1

- The COVID-19 pandemic has sparked an intense debate about the factors underlying the dynamics of the outbreak. Mitigating virus spread could benefit from reliable predictive models that inform effective social and healthcare strategies. Crucially, the predictive validity of these models depends upon incorporating behavioral and social responses to infection that underwrite ongoing social and healthcare strategies. Formally, the problem at hand is not unlike the one faced in neuroscience when modelling brain dynamics in terms of the activity of a neural network: the recent COVID19 pandemic develops in epicenters (e.g. cities or regions) and diffuses through transmission channels (e.g., population fluxes). Indeed, the analytic framework known as "Dynamic Causal Modeling" (DCM) has recently been applied to the COVID-19 pandemic, shedding new light on the mechanisms and latent factors driving its evolution. The DCM approach rests on a time-series generative model that provides - through Bayesian model inversion and inference - estimates of the factors underlying the progression of the pandemic. We have applied DCM to data from northern Italian regions, which were the first areas in Europe to contend with the COVID-19 outbreak. We used official data on the number of daily confirmed cases, recovered cases, deaths and performed tests. The model - parameterized using data from the first months of the pandemic phase - was able to accurately predict its subsequent evolution (including social mobility, as assessed through GPS monitoring, and seroprevalence, as assessed through serologic testing) and revealed the potential factors underlying regional heterogeneity. Importantly, the model predicts that a second wave could arise due to a loss of effective immunity after about 7 months. This second wave was predicted to be substantially worse if outbreaks are not promptly isolated and contained. In short, dynamic causal modelling appears to be a reliable tool to shape and predict the spread of the COVID-19, and to identify the containment and control strategies that could efficiently counteract its second wave, until effective vaccines become available. [**note: it is still unclear whether immunity is totally lost following infection and recovery from SARS-CoV-2 infection. We are still in the midst of the Rumsfeld Paradigm and models cannot fully take this into account.**] https://www.medrxiv.org/content/10.1101/2020.08.20.20178798v1
- Background: Calls are increasing for widespread SARS-CoV-2 infection testing of people from populations with a very low prevalence of infection. We quantified the impact of less than perfect diagnostic test accuracy on populations, and on individuals, in low prevalence settings, focusing on false positives and the role of confirmatory testing. Methods: We developed a simple, interactive tool to assess the impact of different combinations of test sensitivity, specificity and infection prevalence in a notional population of 100,000. We derived numbers of true positives, true negatives, false positives and false negatives, positive predictive value (PPV, the percentage of test positives that are true positives) and overall test accuracy for three testing strategies: (1) single test for all; (2) add repeat testing in test positives; (3) add further repeat testing in those with discrepant results. We also assessed the impact on test results for individuals having one, two or three tests under these three strategies. Results: With sensitivity

of 80%, infection prevalence of 1 in 2,000, and specificity 99.9% on all tests, PPV in the tested population of 100,000 will be only 29% with one test, increasing to >99.5% (100% when rounded to the nearest %) with repeat testing in strategies 2 or 3. More realistically, if specificity is 95% for the first and 99.9% for subsequent tests, single test PPV will be only 1%, increasing to 86% with repeat testing in strategy 2, or 79% with strategy 3 (albeit with 6 fewer false negatives than strategy 2). In the whole population, or in particular individuals, PPV increases as infection becomes more common in the population but falls to unacceptably low levels with lower test specificity. Conclusion: *To avoid multiple unnecessary restrictions on whole populations, and in particular individuals, from widespread population testing for SARS-CoV-2, the crucial roles of extremely high test specificity and of confirmatory testing must be fully appreciated and incorporated into policy decisions.* [**note: this is from the UK and looks at various testing scenarios when imperfect diagnostic tests are used for surveillance. I found it useful reading.**] https://www.medrxiv.org/content/10.1101/2020.08.19.20178137v1

- Dental procedures often produce splatter and aerosol which have potential to spread pathogens such as SARS-CoV-2. Mixed guidance exists on the aerosol generating potential of orthodontic procedures. The aim of this study was to evaluate aerosol and/or splatter contamination during an orthodontic debonding procedure. Material and Methods: Fluorescein dye was introduced into the oral cavity of a mannequin. Orthodontic debonding was carried out in triplicate with filter papers placed in the immediate environment. Composite bonding cement was removed using a slow-speed handpiece with dental suction. A positive control condition included a high-speed air-turbine crown preparation. Samples were analysed using digital image analysis and spectrofluorometric analysis. Results: Contamination across the 8-metre experimental rig was 3% of the positive control on spectrofluorometric analysis and 0% on image analysis. There was contamination of the operator, assistant, and mannequin, representing 8%, 25%, and 28% of the positive control spectrofluorometric measurements, respectively. Discussion: *Orthodontic debonding produces splatter within the immediate locality of the patient. Widespread aerosol generation was not observed. Conclusions: Orthodontic debonding procedures are low risk for aerosol generation, but localised splatter is likely. This highlights the importance of personal protective equipment for the operator, assistant, and patient*. [**note: this is a useful real world simulation of orthodontic procedures.**] https://www.medrxiv.org/content/10.1101/2020.08.19.20178319v1
- Ferrets (Mustela putorius furo) are mustelids of special relevance to laboratory studies of respiratory viruses and have been shown to be susceptible to SARS-CoV-2 infection and onward transmission. Here, we report the results of a natural experiment where 29 ferrets in one home had prolonged, direct contact and constant environmental exposure to two humans with symptomatic COVID-19. We observed no evidence of SARS-CoV-2 transmission into the household ferret population via RT-PCR and ELISA. To better understand this discrepancy in experimental and natural infection in ferrets, we compared SARS-CoV-2 sequences from natural and experimental mustelid infections and identified two surface glycoprotein mutations associated with mustelids. While there is evidence that ACE2 provides a weak host barrier, one mutation only seen in ferrets is located in the novel S1/S2 cleavage site and is computationally predicted to decrease furin activity. *These data suggest that host factors interacting with the novel S1/S2 cleavage site are a barrier in ferret SARS-CoV-2 susceptibility and that domestic ferrets are at low risk of natural infection*. [**note: I don't know anyone keeping pet ferrets but**

for those who do, this paper might be important.]
https://www.biorxiv.org/content/10.1101/2020.08.21.254995v1

NEWLY REGISTERED CLINICAL TRIALS

- It's Sunday, and I've taken the day off.

CLINICAL TRIAL RESULTS

- Hundreds of thousands of deaths have already been recorded for patients with the severe acute respiratory syndrome coronavirus (SARS-CoV-2; aka COVID-19). Understanding whether there is a relationship between comorbidities and COVID-19 positivity will not only impact clinical decisions, it will also allow an understanding of how better to define the long-term complications in the groups at risk. In turn informing national policy on who may benefit from more stringent social distancing and shielding strategies. Furthermore, understanding the associations between medications and certain outcomes may also further our understanding of indicators of vulnerability in people with COVID-19 and co-morbidities. Methods: Electronic healthcare records (EHR) from two London hospitals were analysed between 1st January and 27th May 2020. 5294 patients presented to the hospitals in whom COVID status was formally assessed; 1253 were positive for COVID-19 and 4041 were negative. This dataset was analysed to identify associations between comorbidities and medications, separately and two outcomes: (1) presentation with a COVID-19 positive diagnosis, and (2) inpatient death following COVID-19 positive diagnosis. Medications were analysed in different time windows of prescription to differentiate between short-term and long-term medications. All analyses were done with controls (without co-morbidity) matched for age, sex, and number of admissions, and a robustness approach was conducted to only accept results that consistently appear when the analysis is repeated with different proportions of the data. Results: We observed higher COVID-19 positive presentation for patients with hypertension (1.7 [1.3-2.1]) and diabetes (1.6 [1.2-2.1]). We observed higher inpatient COVID-19 mortality for patients with hypertension (odds ratio 2.7 [95% CI 1.9-3.9]), diabetes (2.2 [1.4-3.5]), congestive heart failure (3.1 [1.5-6.4]), and renal disease (2.6 [1.4-5.1]). *We also observed an association with reduced COVID-19 mortality for diabetic patients for whom anticoagulants (0.11 [0.03-0.50]), lipid-regulating drugs (0.15 [0.04-0.58]), penicillins (0.20 [0.06-0.63]), or biguanides (0.19 [0.05-0.70]) were administered within 21 days after their positive COVID-19 test with no evidence that they were on them before, and for hypertensive patients for whom anticoagulants (0.08 [0.02-0.35]), antiplatelet drugs (0.10 [0.02-0.59]), lipid-regulating drugs (0.15 [0.05-0.46]), penicillins (0.14 [0.05-0.45]), or angiotensin-converting enzyme inhibitors (ARBs) (0.06 [0.01-0.53]) were administered within 21 days post-COVID-19-positive testing with no evidence that they were on them before. Moreover, long-term antidiabetic drugs were associated with reduced COVID-19 mortality in diabetic patients (0.26 [0.10-0.67]).* Conclusions: We provided real-world evidence for observed associations between COVID-19 outcomes and a number of comorbidities and medications. These results require further investigation and replication in other data sets. [**note: this is an observational study from the UK on just over 5000 patients. Clearly we need much more observational data to understand whether these drug effects on reduced mortality hold.**
https://www.medrxiv.org/content/10.1101/2020.08.20.20174169v1

- Rationale: Chronic obstructive pulmonary disease (COPD) is a risk factor for severe COVID-19. Inhaled corticosteroids (ICS) are commonly prescribed for the prevention of acute exacerbations in people with COPD, but their use is associated with increased risk of respiratory infections. The effects of ICS on SARS-CoV-2 susceptibility or COVID-19 severity are currently unknown. Objectives: To determine the effects of ICS treatment on the bronchial epithelial cell expression of key SARS-CoV-2-related genes in volunteers with COPD. Methods: We performed a randomized, open-label, parallel treatment trial of 12 weeks treatment with ICS in combination with long-acting beta-agonist (formoterol/budesonide 12/400 µg twice daily or salmeterol/fluticasone propionate 25/250 µg twice daily), or treatment with LABA only (formoterol 12 µg twice daily), in volunteers with mild to very severe COPD. We obtained bronchial epithelial cell samples via bronchoscopy before and after treatment, and determined transcriptome-wide gene expression by RNA sequencing. Main Results: 63 volunteers were randomized to receive treatment. Compared to formoterol alone, formoterol/budesonide treatment decreased the expression of the SARS-CoV-2 receptor gene *ACE2* and the host cell protease gene *ADAM17*. These genes were highly co-expressed with innate immune response genes, particularly those of the type I interferon and anti-viral response pathways, which also tended to decrease following ICS treatment. Conclusions: This is the first randomized controlled trial to show that ICS affect the expression of key SARS-CoV-2-related genes in COPD. Their relation to important anti-viral response genes may have critical implications for SARS-CoV-2 susceptibility or COVID-19 severity in this vulnerable population. [**note: this is a useful trial looking at the effect of inhaled corticosteroids in COPD patients. SARS-CoV-2 related gene expression is down regulated. One might expect the same result in asthmatics who are treated with the same drug. Maybe this is the prophylactic treatment we should all be on!**] https://www.medrxiv.org/content/10.1101/2020.08.19.20178368v1
- Background The COVID-19 pandemic, caused by the coronavirus SARS-CoV-2, is rapidly spreading worldwide. There is limited information about prognostic markers that could help clinicians to identify COVID-19 patients with a poor prognosis. Serum levels of the immune activation marker neopterin has shown to be of prognostic value in patients with SARS. The aim of this study was to investigate whether serum neopterin is associated with the severity of COVID-19. Methods We included 34 patients with confirmed COVID-19 between March 3 and March 30, 2020. Fifteen patients had mild disease and did not require hospitalization, whereas 19 patients developed severe COVID-19 requiring intensive care. Concentrations of serum neopterin, tryptophan, and kynurenine were measured at and repeatedly after inclusion. Results We found a more than two-fold higher mean concentration of neopterin in severely ill patients (mean value 42.0 nmol/L (SD 18.2)) compared to patients with mild symptoms (16.9 nmol/L (SD 11.0)). All of the severe cases had elevated neopterin concentrations (>9.1 nmol/L) at the initial sampling with values ranging from 17.2 to 86.7 nmol/L. In comparison, 10 of 15 patients with mild disease had neopterin levels above 9.1 nmol/L, with concentrations in the range from 4.9 to 31.6 nmol/L. Neopterin levels gradually decreased during the course of COVID-19, but severe cases maintained elevated levels for a longer period. Moreover, lower levels of tryptophan and higher levels of kynurenine, indicating an increased tryptophan catabolism, were seen in the group with severe cases. Conclusions In conclusion, we found that serum neopterin levels are associated with the severity of COVID-19. Our findings suggest that neopterin could be used as a prognostic marker, but further studies are needed to elucidate

how it can be used in clinical praxis. [**note: another possible marker for severe COVID-19**] https://www.medrxiv.org/content/10.1101/2020.08.19.20178178v1

- A growing body of evidence shows that poor vitamin D status has been associated with an increased susceptibility to viral and bacterial respiratory infections. In this study, we aimed to examine the association between vitamin D and COVID-19 risk and outcomes, and to explore potential causal effects. We used logistic regression to identify associations between different vitamin D variables (25-hydroxyvitamin D concentration (25-OHD), ambient UVB and genetically-predicted 25-OHD concentrations) and COVID-19 (risk of infection, hospitalisation and death) in 495,780 participants from UK Biobank. We subsequently performed a Mendelian Randomisation (MR) study to test if there was any causal effect. In total, 1,746 COVID-19 cases and 399 COVID-19 deaths occurred between March and June 2020. We found significant inverse associations between COVID-19 infection and 25-OHD in univariable models, but these associations were non-significant after adjustment for confounders. Ambient UVB was strongly and inversely associated with hospitalization and death. Although the main MR analysis showed that genetically-predicted vitamin D levels were not causally associated with COVID-19 risk, MR sensitivity analysis using weighted mode method indicated a potential causal effect (OR=0.72, 95% CI:0.53-0.98; P=0.041). In conclusion, our study found suggestive evidence of association between vitamin D and the risk or severity of COVID-19 but further studies are needed. [**note: more observational data on Vitamin D. There are clinical trials on going that may provide the definitive answer. In the meantime, both Mrs. G and I are taking 1000 IU of Vitamin D daily (this does not constitute medical advice as neither of us are MDs).**] https://www.medrxiv.org/content/10.1101/2020.08.18.20177691v1

DRUG DEVELOPMENT

- An efficacious and affordable vaccine is urgently needed. The Val308-Gly548 of Spike protein of SARS-CoV-2 linked with Gln830-Glu843 of Tetanus toxoid (TT peptide) (designated as S1-4) and without TT peptide (designated as S1-5), and prokaryotic expression, chromatography purification and the rational renaturation of the protein were performed. The antigenicity and immunogenicity of S1-4 protein was evaluated by Western Blotting (WB) in vitro and immune responses in mice, respectively. The protective efficiency of it was measured by virus neutralization test in Vero E6 cells with SARS-CoV-2. S1-4 protein was prepared to high homogeneity and purity by prokaryotic expression and chromatography purification. Adjuvanted with Alum, S1-4 protein stimulated a strong antibody response in immunized mice and caused a major Th2-type cellular immunity compared with S1-5 protein. Furthermore, the immunized sera could protect the Vero E6 cells from SARS-CoV-2 infection with neutralization antibody GMT 256. The candidate subunit vaccine molecule could stimulate strong humoral and Th1 and Th2-type cellular immune response in mice, giving us solid evidence that S1-4 protein could be a promising subunit vaccine candidate. [**note: yet another vaccine prototype from China. This one is a recombinant viral protein with a T helper epitope as a built-in adjuvant. This one has the advantage of being produced in an E. coli production system. So many vaccines, too little time for development. I'm sure some very good candidates will never be trialed.**] https://www.biorxiv.org/content/10.1101/2020.08.21.262188v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Background: Patients infected with SARS-CoV-2 exhibit a highly variable clinical course, varying from barely discernible signs of disease, to moderate flu-like symptoms and, occasionally, with life-threatening pneumonia and/or cytokine storm. The relationship between the nasopharyngeal virus load, IgA and IgG antibodies to both the S1-RBD-protein and the N-protein as well the neutralizing activity (NAbs) against SARS-CoV-2 in the blood of moderately afflicted COVID-19 patients has not been investigated longitudinally so far. Methods: Several new serological methods to examine these parameters were developed and validated for the longitudinal investigation in three patients of a family which underwent a mild course of COVID-19. Findings: *We observed that the virus load had almost completely disappeared after about four weeks, whereas serum antibodies showed a contrasting course. IgA levels to S1-RBD-protein and, to a lesser extent, to the N-protein, peaked about three weeks after clinical disease onset but declined soon thereafter. IgG levels rose continuously, reaching a plateau approximately six weeks after disease onset. NAbs in serum reached a peak about four weeks after disease onset but dropped to a lower level about six weeks later*. Interpretation: Our data establishes associations of virus neutralization and a serological immune response foremost against Sars-CoV-2 S1-RDB-protein in a longitudinal manner. [**note: this is a viral and immunological profile of a very small group of patients who had mild COVID-19.**]
  https://www.medrxiv.org/content/10.1101/2020.08.20.20174912v1
- Natural infection of SARS-CoV-2 in humans leads to the development of a strong neutralizing antibody response, however the immunodominant targets of the polyclonal neutralizing antibody response are still unknown. Here, we functionally define the role SARS-CoV-2 spike plays as a target of the human neutralizing antibody response. In this study, we identify the spike protein subunits that contain antigenic determinants and examine the neutralization capacity of polyclonal sera from a cohort of patients that tested qRT-PCR-positive for SARS-CoV-2. Using an ELISA format, we assessed binding of human sera to spike subunit 1 (S1), spike subunit 2 (S2) and the receptor binding domain (RBD) of spike. To functionally identify the key target of neutralizing antibody, we depleted sera of subunit-specific antibodies to determine the contribution of these individual subunits to the antigen-specific neutralizing antibody response. We show that epitopes within RBD are the target of a majority of the neutralizing antibodies in the human polyclonal antibody response. These data provide critical information for vaccine development and development of sensitive and specific serological testing. [**note: an analysis of polyclonal neutralizing antibodies and their binding sites.**]
  https://www.biorxiv.org/content/10.1101/2020.08.21.261727v1

DIAGNOSTIC DEVELOPMENT

- Nothing today, though there is a paper up in the Modeling section on the use of imperfect diagnostic tests.