PRIMARY IMMUNITY: Exaggerated immune phenotypes in acute inflammation to SARS-CoV-2
Immune profiling reveals ongoing neutrophil dysfunction in COVID-19 patients post-hospitalisation

Abstract

Background: Neutrophils are thought to play a pivotal role during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Increased neutrophil-to-lymphocyte ratio and heightened release of neutrophil extracellular traps (NETs) are key features of COVID-19. Further, emergency myelopoiesis potentially negatively impacts neutrophil function by promoting the release of immature and dysfunctional neutrophils from the bone marrow. This dysregulation of neutrophils during acute infection is suggested to enhance aberrant inflammatory immune responses in COVID-19 patients and could also increase the risk of bacterial co-infections. Furthermore, a high frequency of hospitalised COVID-19 patients display long-COVID symptoms including abnormal chest X-ray, increased inflammatory markers and vascular complications subsequent to SARS-CoV-2 infection. The role of neutrophils in convalescent patients with long-COVID symptoms has not been explored. Here we aimed to further investigate the role of neutrophils in contributing to prolonged inflammatory immune responses following SARS-CoV-2 infection.

Methods: Blood samples were obtained from healthy controls (HC), acute and follow-up (FU; 3-6 months) COVID-19 patients. Whole blood neutrophils or low-density neutrophils (LDN), identified by PBMC isolation, were phenotypically analysed by flow cytometry or sorted for functional assays including bacterial killing, reactive oxygen species (ROS) production, ATAC-Seq analysis and proteomics.

Results: Neutrophils of acute COVID-19 patients display significantly increased expression of degranulation and activation markers such as CD64 and CD63, but also an increased population of LDN and immature neutrophils assessed by decreased expression of CD10, CD101 and CXCR2 when compared to HC. Furthermore, compared to HC, neutrophils of acute COVID-19 patients display a decreased capability to kill bacteria and produce ROS in response to stimulation. Interestingly, even though some FU patients are comparable to HC, a subgroup of FU patients displays dysregulated neutrophils with increased total numbers, phenotypic and functional changes that maintained 3-6 months post infection.

Conclusion: Some patients maintain a dysregulated neutrophil phenotype months post recovery from SARS-CoV-2 infection. Future integration of neutrophil proteomics and ATAC-Seq data together with detailed clinical parameters may further allow us to stratify patients with dysregulated immune response to SARS-CoV-2.

Abstract Lay Summary

White blood cells, including neutrophils, are highly dysregulated in acute COVID-19 patients. Our study aims to investigate these neutrophils in convalescent Covid-19 patients and their potential role in driving long-COVID symptoms.
Abstract

The COVID-19 pandemic, caused by SARS coronavirus 2 (SARS-CoV-2), has resulted in excess morbidity and mortality as well as economic decline. To characterise the systemic host immune response to SARS-CoV-2, we performed combined single-cell transcriptome and surface proteome and T/B lymphocyte antigen receptor analyses of over 780,000 peripheral blood mononuclear cells from 130 patients with COVID-19. Our cohort, from three UK centres, spans the spectrum of disease severities ranging from asymptomatic to critical. We revealed a non-classical monocyte state that sequesters platelets and replenishes the alveolar macrophage pool; platelet activation accompanied by early priming towards megakaryopoiesis in immature haematopoietic stem/progenitor cells and expansion of megakaryocyte-primed progenitors; increased clonally expanded CD8+ effector/effector memory T cells, and proliferating CD4+ and CD8+ T cells in patients with more severe disease; and a relative decrease of IgA2 plasmablasts and plasma cells in symptomatic compared to asymptomatic patients, with an increase in IgG plasma cells with worsening disease severity that was attenuated in critical disease. We provide a template for multi-omic single cell data integration across multiple centers to rapidly build powerful resources to help combat diseases such as COVID-19.

Emily Stephenson
Newcastle University, UK

The cellular immune response to COVID-19 deciphered by single cell multi-omics across three UK centres

Abstract Lay Summary

To investigate the immune response of COVID-19, we analysed individual blood cells of 130 patients from three medical centres in the UK. We revealed several new insights to COVID-19 pathogenesis whilst providing a valuable resource, exploitable for translational studies. We also highlighted the changes to cellular components during severe disease that could be targeted for therapy.
Abstract

SARS-CoV-2-specific memory T cells will likely play a key role in long-term immune protection against COVID-19. We have systematically mapped the functional and phenotypic landscape of SARS-CoV-2-specific T cell responses in unexposed individuals, exposed family members, and individuals with acute or convalescent COVID-19. Acute-phase SARS-CoV-2-specific T cells typically display a highly activated cytotoxic phenotype that correlates with various clinical markers of disease severity, whereas convalescent-phase SARS-CoV-2-specific T cells are typically polyfunctional and display a stem-like memory phenotype. Importantly, SARS-CoV-2-specific T cells are detectable in antibody-seronegative family members and individuals with a history of asymptomatic or mild COVID-19. Our collective datasets show that SARS-CoV-2 elicits robust memory T cell responses akin to those observed after natural infection with other respiratory viruses and those observed in the context of successful vaccines, suggesting that natural exposure may prevent recurrent episodes of severe COVID-19.

T cells are essential for immune protection against viruses. The overall aim of this work is to define how T cell memory is generated and maintained in individuals who have been infected with SARS-CoV-2. Our preliminary data suggest that natural infection elicits robust T cell responses with the ability to persist over time and protect against further episodes of severe COVID-19.
IMMUNOPATHOLOGY: Mechanisms of immunopathology in severe COVID-19
Abstract

Describing the pathogenesis of SARS CoV-2 infection will be critical to elucidating why the virus elicits a wide range of symptoms and severity in patients. Post mortem tissue samples from COVID19 patients present a unique resource to study disease pathogenesis and will likely provide key insights to understanding the organ and cell type tropism of SARS CoV-2. Here, we present transcriptome-wide Digital Spatial Profiling (DSP) and integrated single cell transcriptomic analysis of infection relevant cell types in lung, upper airway, heart and kidney postmortem tissue samples from COVID19 patients. We performed multi-organ spatial characterisation of post mortem tissues from 9 COVID19 patients using the GeoMX DSP platform. We utilised the Whole Transcriptome Atlassing (WTA) on the DSP platform to measure the expression of 18,000 genes on over 300 regions of interest on FFPE tissue samples.

More, we performed cell type specific WTA profiling to assay cell types implicated in disease pathogenesis including alveolar epithelia, cardiac fibroblasts and endothelial cells across all tissues. Comparative WTA profiling revealed organ specific and shared pathological gene expression patterns in endothelial cells in COVID19. Moreover, cell2location based integration of healthy single cell RNA-Sequencing and spatial WTA data from healthy and diseased donors revealed the altered immune landscape of vasculature across multiple organs. Notably, we observed increased T-cell senescence in COVID19 in the lung parenchyma tissue, but not in blood. Taken together, our results reveal organ and cell-type specific vascular pathology in COVID19 and enable insight into the tropism of SARS CoV-2 across multiple target tissues.
Complement and complement therapies in Covid-19

Abstract

In critically ill Covid-19 patients, a hyper-inflammatory host response contributes to organ dysfunction and death. Early in the pandemic, dysregulation of the complement system was implicated as a contributor, uncontrolled activation driving inflammation and tissue damage in a vicious cycle. In these severely ill individuals, levels of complement activation products were markedly elevated in plasma, demonstrating ongoing dysregulation. Studies to date have been relatively small and focussed on severe disease with little information on complement dysregulation in mild or early disease. To address this knowledge gap, complement labs in Cardiff, Newcastle and Cambridge have developed a collaboration to undertake a comprehensive biomarker analysis of the complement system in Covid-19 in the ISARIC sample set across the three centres. We will compile data from all the assays and correlate with available clinical data and other laboratory measures to identify whether complement biomarkers can be used in early diagnosis, in prediction of disease course or outcome. We anticipate identifying a small set of complement biomarkers that, alone or in combination with other analytes, is optimal for these purposes. We will then generate a robust, rapid multiplex assay that can be adapted for use in clinical labs. We will explore whether common polymorphisms in complement proteins known to impact activity (the complotype) correlate with biomarkers or independently predict disease risk or course.

Numerous small studies and a handful of larger trials of anti-complement drugs in Covid-19, almost all in severe disease, have been reported; most have targeted the terminal pathway and results have been mixed, implying that targeting early complement activation events may be necessary to ameliorate inflammatory pathology in this disease. It is anticipated that a better understanding of precisely how and when complement is activated in COVID-19, and the capacity to stratify based on complement dysregulation, will enable better clinical trials of anti-complement drugs by targeting the right pathway at the right time in the relevant individual.

Abstract Lay Summary

Uncontrolled inflammation is a major cause of severe disease, organ damage and abnormal clotting in Covid-19. Complement is a highly inflammatory system in the blood that defends against infection; over-activation of complement has been shown in severe Covid-19 and drugs that inhibit complement suggested as potential therapies. Here we describe a study in progress to show when and how complement is activated in Covid-19 and identify opportunities for treatment with complement blocking drugs.
PROTECTIVE IMMUNITY: Protective immune responses against SARS-CoV-2
Abstract

The SARS-CoV-2 Immunity & Reinfection Evaluation (SIREN) study is a large multicentre prospective cohort study of NHS healthcare workers in the UK. Participants attend regular SARS-CoV-2 PCR and antibody testing (every 2–4 weeks) and complete questionnaires every 2 weeks on symptoms and exposures. We have undertaken two major analyses to date investigating rates of SARS-CoV-2 reinfection and COVID-19 vaccine coverage and the short-term effectiveness of the BNT162b2 mRNA vaccine against infection.

Between June 18 2020 and January 11 2021, we detected 155 reinfections in the baseline positive cohort of 8278 participants and 1704 new PCR positive infections in the negative cohort of 17383 participants. The incidence density was 7.6 reinfections per 100 000 person-days in the positive cohort, compared with 57.3 primary infections per 100 000 person-days in the negative cohort, between June, 2020, and January, 2021. The adjusted IRR was 0.159 for all reinfections (95% CI 0.13–0.19) compared with PCR-confirmed primary infections. The median interval between primary infection and reinfection was more than 200 days. A previous history of SARS-CoV-2 infection was associated with an 84% lower risk of infection and 93% lower risk of symptomatic infection, with median protective effect observed 7 months following primary infection.

Between 7 December 2020 and 5 February 2021 there were 977 new infections in the unvaccinated cohort, an incidence density of 14 infections per 10 000 person-days; the vaccinated cohort had 71 new infections 21 days or more after their first dose (incidence density of eight infections per 10 000 person-days) and nine infections 7 days after the second dose (incidence density four infections per 10 000 person-days). A single dose of BNT162b2 vaccine showed vaccine effectiveness of 70% (95% CI 55–85) 21 days after first dose and 85% (74–96) 7 days after two doses in the study population.

Abstract Lay Summary

The SIREN study investigates immunity following both SARS-CoV-2 infection and COVID-19 infection by following-up over 44,000 healthcare workers in 135 hospitals across the UK for 12 months with regular antibody and PCR testing. To date we have shown that for most people, immunity following infection protects against reinfection for an average of 7 months, reducing the risk by 84% of having a subsequent infection and 93% of having a symptomatic infection. We have also shown that the BNT162b2 mRNA vaccine is effective in reducing both symptomatic and asymptomatic infection, with a 70% reduction in infection after one dose and 85% reduction after two doses.
Abstract

In 2019, COVID-19, a disease caused by the novel coronavirus – SARS-CoV-2 emerged causing a global health crisis with over 120 million people infected and 2.7 million deaths. Understanding the immune response against SARS-CoV-2 is pertinent for better disease management and diagnostics, rational and optimal design of vaccines and developing safer public health policies.

Using highly sensitive immunological assays in a cohort of COVID-19 convalescent HCWs from Oxford University Hospital NHS Foundation Trust, we characterised the SARS-CoV-2-specific immune response following natural infection. We found strong ex vivo IFNγ ELISpot (S1/S2 = 34/75; structural and accessory = 65/103) and proliferative responses to M (CD4+ 69/107; CD8+ 50/107), NP (CD4+ 63/107; CD8+ 56/107) and ORF3 (CD4+ 26/107; CD8+ 24/107) in PCR-confirmed SARS-CoV-2 infected volunteers that were rarely detected in uninfected volunteers. By contrast, >90% of convalescent or unexposed people show proliferative responses to spike subunits S1/S2, indicating pre-existing cross-reactive T cell populations. Interestingly, our assays show that highly exposed but antibody seronegative HCWs with recent COVID-19-compatible illness show T cell response patterns characteristic of prior infection. To address longevity of the SARS-CoV-2-specific immune response, we followed a subgroup of HCWs across 6 months to determine the durability of their SARS-CoV-2-specific T and B cell immune response. We found waning but detectable SARS-CoV-2-specific memory T cells with proliferative potential, presence of neutralising antibodies as well as anti-Spike and anti-NP total IgG present 6 months post-infection.

Taken together, our study has shown that detection of SARS-CoV-2-specific immune responses are critically dependent on assay and antigen selection. SARS-CoV-2-specific T cells and humoral responses wane over time but memory responses are readily detectable up to 6 months after initial infection in most people. The durability of T and humoral responses months after infection has implications for subsequent SARS-CoV-2 re-exposure and vaccine responses.

Abstract Lay Summary

In 2019, COVID-19, a disease caused by a newly discovered virus – SARS-CoV-2 emerged. To date, there have been 120 million infections with 2.7 million deaths globally. Understanding the immune response against SARS-CoV-2 is necessary for better disease management and diagnostics, vaccine design as well as developing safer public health policies.

Using highly sensitive immunological assays, we found that strong T cell responses were made in a cohort of HCWs who recovered from COVID-19. The T cell responses in these convalescent patients were targeted towards specific viral proteins from SARS-CoV-2. Using these assays, we also show that highly exposed but antibody seronegative HCWs with recent COVID-19-compatible illness show T cell response patterns characteristic of prior infection. The durability of the immune response against SARS-CoV-2 is also an important question. We address this by following a subgroup of HCWs across 6 months. We found that although the magnitude of the immune response waned over time, it was still detectable and functional 6 months post-infection.

To summarise, our study has shown that detection of SARS-CoV-2-specific immune responses are critically dependent on the assay used for screening and the viral proteins used as antigens. SARS-CoV-2-specific T cells and antibodies wane over time but are readily detectable up to 6 months after initial infection in most people. These has implications for subsequent SARS-CoV-2 re-exposure and vaccine responses.
Abstract

Antibody testing to establish viral immunity is well established for a number of viruses including rubella in pregnancy and CMV in transplant. Testing aids decision making around risk of infection and need for booster vaccination. For SARS-CoV-2 key questions remain around what is a normal antibody response post natural infection or vaccination and can this level be used as a surrogate for protection in the short and long term. To help answer these questions the COCO health care worker study was set up, in the spring of 2020 to measure spike antibody levels and relate this to clinical and ethno demographic data. Key findings from this seroprevalence study included establishing the occupational risk of being a HCW and that not just patient facing roles were at high risk. Asymptomatic seroconversion occurred in 17% of individuals and this was associated with lower antibody levels than those that had suffered a symptomatic illness. Older age, non white ethnicity and higher body mass index were also associated with a higher antibody response. Two thirds of individuals had detectable antibodies at 6 months and were associated with a 79% reduction in risk of reinfection and persistent neutralizing antibodies in the laboratory. Vaccination was associated with detectable antibodies reliably from 14 days with more rapid and larger magnitude responses in those that had previous suffered from COVID-19 infection. With the role out of community asymptomatic PCR testing, the need for antibody testing for seroprevalence studies reduces but there remains an individual and public health need to understand long lived immunity following natural infection and vaccination to guide booster vaccination and cross reactivity to variants of concern.

Alex Richter
University of Birmingham, UK

Abstract Lay Summary

Antibody testing is used to determine rates and risk factors for COVID-19 infection in a large health care worker cohort. The size and longevity of the antibody response is also examined following natural infection and vaccination.
PROTECTIVE IMMUNITY: Immune responses to COVID in the national ISARIC consortium
Abstract

The inflammatory response during COVID-19 is known to contribute to disease severity. The efficacy of dexamethasone and anti-IL-6 biologics indicates that inhibition of this immunopathogenesis can alleviate disease, while greater understanding of this response may enable the rational selection of candidate therapies for clinical trial. We analysed blood plasma samples from 471 patients hospitalised with COVID-19 and 39 non-hospitalised patients with COVID-19. Using an immunoassay panel covering 33 biological mediators, we identified that levels of many cytokines and chemokines scale with disease severity (including IL-6, as previously described). This inflammatory response could be analysed as a network response, which was centred around IL-6 and GM-CSF. While IL-6 levels were equivalent between cases of fatal COVID-19 and historic samples from patients with fatal influenza, GM-CSF was only raised in COVID-19. This work demonstrates that GM-CSF is a central feature of immunopathogenesis that may represent a distinct feature of COVID-19.

Abstract Lay Summary

A ‘cytokine storm’ has been much discussed in COVID-19. Our results suggest that COVID-19 is not a ‘storm’, but specific aspects of the immune system appear to be highly active – understanding this response may help us select the most appropriate therapies for future trials.
Abstract

SARS-CoV-2 has circulated as a human pathogen for just over one year. Hundreds of thousands of viral genomes have been catalogued in an unprecedented sequencing effort. These contain many mutations for which the phenotypic consequence is not yet clear. Bearing in mind that we are now at a critical point in the pandemic when both natural and vaccine induced immunity is accumulating in the population, some variants may evade current immune responses, jeopardizing vaccine effectiveness. It is imperative that we are able to translate how the genomic changes will impact on the virus biology, affecting transmissibility, pathogenicity and antigenicity.

To study this we have formed a UK consortium of virologists, who aim to study the genotype to phenotype (G2P) relationship for this virus. A combination of strategies are employed, including expression of virus proteins, primarily Spike, as well as experiment with infectious virus from clinical isolates and recombinant viruses generated by reverse genetics in human cells and animal models.

We aim to describe the mechanisms by which the B.1.1.7 variant is more effectively transmitted, and to risk assess the threat posed by variants such as B.1.351 and B.1.28 P.1 and P.2 that harbour significant mutations in the regions of spike targeted by neutralizing antibodies.

Abstract Lay Summary

Vaccines are the best way for us to control the disease and spread of the virus that causes COVID. But variants of the virus have been detected against which the vaccine might be less effective. We need to study these variants very carefully to understand which ones might impact negatively on our chances to bring the pandemic to an end, so that we can focus our efforts on controlling the variants that matter. This presentation will describe our current state of knowledge on the virus variants and what is being done to risk assess the threat they pose.
ADNKA overcomes SARS-CoV2-mediated NK cell inhibition through non-spike antibodies

Abstract
SARS-CoV-2 antagonises the cellular interferon response, but whether the virus manipulates cellular immunity is unclear. An unbiased proteomic approach to determine how cell surface protein expression is altered on SARS-CoV-2-infected lung epithelial cells showed downregulation of activating NK cell ligands: B7-H6, MICA, ULBP2, and Nectin1, but no effect on surface MHC-I expression. NK ligand downregulation correlated with a reduction in NK cell activation by infected cells, and was overcome by antibody-dependent NK cell activation (ADNKA). Depletion of spike-specific antibodies confirmed their dominant role in virus neutralisation, but these antibodies played only a minor role in ADNKA compared to antibodies to other viral proteins, including ORF3a, Membrane, and Nucleocapsid. In contrast, ADNKA induced following vaccination was focussed solely on spike, was weaker than ADNKA following natural infection, and was not boosted by the second dose. These insights have important implications for understanding disease progression, vaccine efficacy, and vaccine design.
Abstract

Virus host shifts are generally associated with novel adaptations to exploit their new host species optimally. Surprisingly, SARS-CoV-2 has, at least initially, required little to no significant adaptation to humans since the start of the COVID-19 pandemic and to late 2020. For this period of time while there is moderate evidence of diversifying positive selection in SARS-CoV-2 in humans, it is limited to the early phase of the pandemic, and purifying selection is much weaker in SARS-CoV-2 than in related bat Sarbecoviruses.

The ability of SARS-CoV-2 to transmit efficiently in humans, with a harm/transmission trade-off optimal for efficient spread, seems to be through its relatively generalist nature — evidenced by frequent transmission to other mammals: minks, cats, pangolins and others. Current sampling and evolutionary signals point to the important evolution taking place in horseshoe bats prior to spillover to humans. Once in the human population, SARS-CoV-2 has spread rapidly. In most of us our multifaceted immune response controls the infection. This has not changed so much even with the "new variants", but, contrary to the start of the pandemic when the virus transmitted in an immunologically naive population, now SARS-CoV-2 is more frequently encountering previously infected individuals favouring immune escape-associated mutations. Alarmingly, SARS-CoV-2’s response to increasing host immunity (the more heavily mutated ‘variants of concern’) has brought with it increased transmissibility and possibly virulence as well, which indicates the responsible mutations are likely pleiotropic contributing to change in the viral phenotype and antigenicity. Novel variants are continuously arising with implications for vaccine strategies, for example, the new-variant lineages are increasingly accumulating changes of potential antigenic significance.

David Robertson
MRC-University of Glasgow Centre for Virus Research, UK

Abstract Lay Summary

This talk will address some key questions on SARS-CoV-2 evolution: How was this new human coronavirus so successful so quickly in early 2020? Where did it come from? Why did the ‘new variants’ with so many mutations emerge in late 2020? Why are the same ‘convergent’ mutations arising in these lineages? What next, where does the virus go from here?
CROSS-REACTIVE CORONAVIRUS IMMUNITY: T cell cross-reactivity and viral escape
Abstract

Pre-existing T cell responses that cross-recognise SARS-CoV-2 in vitro have been widely reported, but whether they respond in vivo and can play a role in mediating clearance of subclinical infection without seroconversion is less clear. We measured SARS-CoV-2-reactive T cells (IFNg-ELISpot, FACS) in a cohort of exposed health care workers who remained seronegative (ES) during the UK first wave (Weekly for 16wks: PCR, NP and S1 ELISA, neutralising Ab; n=57) and compared these to T cell immunity in a cohort matched for exposure and demographics with lab-confirmed infection (n=71), and to unexposed individuals sampled prior to the pandemic (n=53).

We observed T cell responses in ES that were higher in magnitude and breadth than seen in pre-pandemic samples, in particular to regions of ORF1 (RNA polymerase and cofactor, NSP12 and NSP7; helicase, NSP13). Interestingly, NSP12-reactive T cell responses were significantly higher in magnitude in ES cohort at 16weeks than in the SARS-CoV-2 infected cohort. Analysis of paired pre/post-exposure samples identified in vivo expansion of pre-existing SARS-CoV-2 reactive T cells, including ORF-1-specific responses in those remaining seronegative. T cells in ES were highly functional (proliferative, producing combinations of IFNg, TNF, IL2, CD40L, MIP1b) and could be mapped to shared and previously unidentified epitopes. NSP12 was the most commonly recognised antigen in unexposed individuals (UK and Singapore cohorts) and was the area of highest sequence homology with common cold coronaviruses, suggesting a potential source of these cross-reactive T cells.

T cell responses in a cohort of exposed seronegative individuals are distinguishable in magnitude and specificity from those in unexposed or infected cohorts, in particular, they are enriched for NSP12-reactive T cells, suggesting that these may play a role in protection from infection with detectable by PCR and seroconversion.

Abstract Lay Summary

Some health care workers and close-contacts remain uninfected despite exposure to SARS-CoV-2. Here we show that immune cells that recognise SARS-CoV-2, in particular the internal machinery of the virus, can be detected in these individuals where SARS-CoV-2 failed to establish a successful infection. The specific role these cells played in protecting these highly exposed individuals from infection requires further investigation.
Abstract

Evolution of SARS-CoV-2 can lead to evasion from adaptive immunity generated following infection and vaccination. Much focus has been on humoral immunity and spike protein mutations that impair the effectiveness of neutralizing monoclonal antibodies and polyclonal sera. T-cell responses following SARS-CoV-2 infection are directed against targets across the genome and may play a role in favourable outcomes during acute infection. Escape from T-cell responses has been described extensively in chronic viral infections such as HIV, as well as in several influenza epitopes.

Little is known about the potential for SARS-CoV-2 mutations to impact T-cell recognition. We have recently identified amino acid variants within several dominant SARS-CoV-2 T-cell epitopes by interrogating global sequence data. A number of variants within nucleocapsid and ORF3a epitopes have arisen independently in multiple lineages and result in loss of recognition by epitope-specific T-cells assessed by IFN-γ and cytotoxic killing assays.

Additional work by UK-CIC researchers and others have evaluated the potential impact of the spike mutations found in current variants of concern (VOC) such as lineages B.1.1.7, B.1.351 and P.1. These amino acid substitutions appear to be in regions of the spike protein that are targeted by only a small proportion of the total spike-specific T-cell response. These data demonstrate that the heterogeneity of the T-cell response may result in retained T-cell activity against current VOC, but that the potential for T-cell evasion exists and highlights the need for ongoing surveillance for variants capable of escaping T-cell as well as humoral immunity.

Abstract Lay Summary

We are increasingly seeing how the COVID-19 virus can change so that one aspect of our immunity, antibodies, may be less effective at stopping infection. We will discuss the potential for similar changes in the virus that may also affect the effectiveness of our T-cell immunity.
VACCINOLOGY: The development of vaccines against Covid-19
Abstract

The SARS-CoV2 pandemic has severely disrupted the healthcare systems and economies world-wide. It is a little over a year since the WHO declared the COVID-19 pandemic and there are multiple vaccines being rolled out in mass vaccination campaigns worldwide.

We have developed a non-replicating adenovirus-vectored vaccine (ChAdOx1 nCoV-19/AZD1222) expressing the SARS-CoV-2 spike protein. Preclinical vaccination of both small and large animals with ChAdOx1 nCoV-19 induces a strong immune response and protects against disease after challenge with homologous and variant strains of SARS CoV-2.

The safety and immunogenicity of the vaccine was assessed in a number of clinical trials run in the UK, Brazil, and South Africa. Efficacy of two doses of the ChAdOx1 nCoV-19 vaccine is high, with real-world effectiveness data demonstrating the positive impact vaccination has on preventing hospitalisation and illness in older adults. We describe, in detail, the immune response post vaccination with ChAdOx1 nCoV-19 and the new data from immunisation studies.

Abstract Lay Summary

The Oxford/AZ vaccine has been used in global mass vaccination schemes, we present and discuss the results from the trials.
Rapid COVID-19 Vaccine Development

Abstract

While the frequency of pandemic threats seems to be increasing, we fortunately have new tools and technologies to make vaccines with more precision and speed. These advances make possible a more proactive approach to pandemic preparedness and response. There are ~26 virus families associated with human infection from which the next pandemic threat will likely arise. Within each relevant virus family, a database of information with accompanying reagents, assays, and animal models could be developed for prototypic viruses based on properties of tropism, transmission routes, and other distinguishing features of pathogenesis. Candidate vaccine approaches could be designed based on virus structure, transmission dynamics, entry requirements, and replication strategy.

The rapid development of vaccines for COVID-19 was a direct consequence of the prototype pathogen approach for pandemic preparedness. Work on the Middle East Respiratory Syndrome (MERS)-CoV over the last 7 years was informed by structure-based immunogen-design concepts established for respiratory syncytial virus (RSV) fusion protein (F) subunit vaccines, and focused on solving coronavirus spike structures, defining mechanisms of CoV neutralization, and evaluating MERS CoV vaccine candidates in collaboration with a commercial mRNA manufacturer Moderna. Prior spike protein engineering experience resulted in rapid sequence selection and using the mRNA manufacturing platform provided rapid GMP production of a COVID-19 mRNA vaccine in record time. This candidate was tested in mice in ~25 days and humans in ~65 days from the time sequence was released. The product was tested in a 30,000 person phase 3 trial and shown to be 94% effective 10 months after sequence release and was granted Emergency Use Authorization by the FDA a month later. The proactive preparation not only facilitated rapid vaccine development and evaluation but provided stabilized spike protein for solving the atomic-level structure and reagents that were the basis for developing serological assays and isolating potent human neutralizing mAbs that have also been approved for prevention and therapy.

Rapid COVID-19 vaccine development was based on decades of prior work on other respiratory viruses to design more effective vaccines. Another 7 years of work was spent solving the atomic-level structure of the coronavirus spike protein and learning how to engineer and deliver it as a vaccine antigen. There have also been decades of work learning how to produce, preserve, and deliver mRNA as a template for protein production in human cells. Combining precision vaccine antigen design with the mRNA synthetic manufacturing approach allowed the rapid COVID-19 vaccine development witnessed in 2020.

Barney Graham
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Distinct changes in microvesicle release by monocytes and neutrophils in patients with acute SARS-COV-2 infection

Abstract
Background: In response to stimuli such as infections, microvesicle (MV) release contributes to intercellular communication and delivery of functionally relevant cargo that can exacerbate inflammation. Elevated expression of CD169 and CD177 by circulating monocytes and neutrophils respectively has been reported in COVID-19 patients. CD169 (sialo-adhesin) is a type I IFN–stimulated receptor on monocytes that binds to sialic acids on leukocytes, bacteria and viruses. CD177 expression is a marker of neutrophil activation and regulator of arrest, with greater expression associated with disease severity. Additionally, endothelial cell (EC) dysfunction is considered a hallmark of SARS-COV-2 infection and injured ECs release MVs.

Methods: Multicolour flow cytometry was used to quantify i) CD169 and CD177 expression on monocytes, neutrophils and MVs and ii) levels of EC MVs in the blood of healthy subjects, hospitalised patients with acute SARS-COV-2 infection, and convalescing patients.

Results: The proportion of CD14+/CD16- (classical) monocytes expressing CD169 was elevated in acute patients compared to healthy (acute: 42.73 ± 7.04% vs healthy: 4.94 ± 2.0%, p=0.003) and this correlated with an increased proportion of CD169+CD14+CD16- MVs in acute samples (r= 0.75, p=0.003). Additionally, CD177 expression on neutrophils correlated with expression of CD177 on neutrophil-derived MVs (r=0.653, p=0.009). Given that neutrophil responses can exert collateral EC damage, we evaluated the relationship between CD177+ neutrophils and EC-derived MVs and found a significant association between the two (r=0.58, p=0.043).

Conclusions: Increased levels of CD169+ monocytes and CD177+ neutrophils in acute infection is accompanied by increased release of MVs bearing these markers. Furthermore, there is an association between circulating CD177+ neutrophils and shedding of EC-derived MVs. Detailed assessment of these and additional markers may provide insights into pathogenesis, prognosis and potentially inform future therapeutic strategies.

Abstract Lay Summary
Microvesicles (MV) are small membrane-bound sacs produced by cells that carry proteins and nucleic acids that can be delivered from one cell to another. We have characterised the MVs produced during acute COVID-19 infection in hospitalised patients.

Our data show that MVs released from immune cells in the bloodstream of patients suffering from COVID-19 infection have increased amounts of certain surface markers that are related to inflammation. We have also detected that the cells lining blood vessels (endothelial cells) also release MVs and that this is associated with the number of neutrophils, a type of immune cell, circulating in these patients.

We hypothesise that this may be an important mechanism by which immune cells communicate with other cells including endothelial cells and that MVs may contribute to clinical complications during COVID-19 infection, including blood clotting and long-lasting tissue damage.

Our future work will focus on defining the detailed mechanisms of how MVs may play a role in these processes. We hope that a better understanding of the role of MVs may help to identify new therapies in treating complications of severe infections, including COVID-19 and other respiratory diseases.
Abstract

**Background:** The nasal epithelium is a plausible entry point for SARS-CoV-2, a site of pathogenesis and transmission, and may initiate the host response to SARS-CoV-2. Epithelial cells expressing the ACE2 receptor are abundant in the nasal mucosa. Yet, little is known about the interaction between SARS-CoV-2 and the innate immune system in this tissue. Here we provide the most comprehensive analysis to date of the host response to SARS-CoV-2, with a focus on interferons, recently shown to be critical determinants of SARS-CoV-2 outcome.

**Methods:** We applied single cell RNA sequencing and proteomics analysis to a primary cell model of human primary nasal epithelium differentiated at air-liquid interface.

**Results:** SARS-CoV-2 demonstrated widespread tropism for nasal epithelial cell types, with more efficient viral production in ciliated cells. The host response was dominated by induction of type I and III interferons and interferon stimulated gene products. Nevertheless, this response was notably delayed in onset compared to viral gene expression, and thus failed to impact substantially on SARS-CoV-2 replication. When provided prior to infection, recombinant IFNβ or IFNλ1 was capable of inducing an efficient antiviral state that potently restricted SARS-CoV-2 viral replication, preserving epithelial barrier integrity.

**Conclusion:** Collectively, these data highlight the capacity of SARS-CoV-2 to induce an antiviral response in nasal epithelial cells but suggest that timing of induction is a critical determinant of antiviral restriction. Nasal delivery of recombinant IFNs may represent a promising chemoprophylactic strategy.

Abstract Lay Summary

COVID-19 is caused by the virus SARS-CoV-2, which is believed to enter the body through cells of the nose. Yet, little is known about the interaction between SARS-CoV-2 and the immune system in nasal cells. We used a model of nasal cells in the laboratory to investigate their immune response to SARS-CoV-2 infection. We found that nasal cells became very easily infected. Whilst they were able to produce an immune response when infected with SARS-CoV-2, this response came too late to prevent infection. A group of immune factors identified as a major contributor to this immune response were interferons (IFNs), proteins that defend the body against viral infection and play an important role in the outcome of patients suffering from COVID-19. When we treated cells with interferons prior to infection this effectively protected them against viral replication. These findings suggest that interferons, applied to the nose, should be investigated as a treatment to prevent SARS-CoV-2 infection.
Abstract

**Background:** Peripheral blood correlates of immunity and tissue damage during development and recovery from COVID-19 are critical for patient stratification, optimal disease management, and for understanding the mechanisms driving severe pathology. Circulating microRNAs have been shown to be exceptional diagnostic and prognostic mechanism-based biomarkers in cancer and autoimmune and infectious diseases, yet their potential remains largely untapped in COVID-19.

**Methods:** We report the outcomes of a pilot longitudinal microRNA and cyto/chemokine profiling study in blood plasma from hospitalised patients during the first wave of the COVID-19 pandemic in the United Kingdom. We used NanoString miRNA profiling and LegendPlex multiplex cytokine chemokine assays.

**Results:** We identify microRNAs that are differentially present in plasma from patients with severe disease and can be used to construct a microRNA signature that can identify patients with severe disease in this cohort. We are currently validating these findings in independent cohorts. We note that the differentially expressed miRNAs have been linked to epithelial rather than immune cell dysfunction, in other pathologies such as cancer. This suggests that their increased plasma levels in severe COVID-19 might reflect tissue pathology. Crucially, levels of severe COVID-19-associated microRNAs correlate only with a subset of COVID-19-associated chemokines and cytokines supporting the existence of distinct patient subgroups with severe disease. Ongoing analyses of clinical and immunological correlates of the obtained profiles will be discussed.

**Conclusions:** Overall, our findings generate deeper understanding of COVID-19 pathogenesis and can lead to improved COVID-19 patient stratification.

**Abstract Lay Summary**

Here we explore information we can extract from blood samples and can shed light into what causes severe COVID-19, but also identify groups of patients displaying distinct immunological features. This can be used to improve and personalise treatment of people with COVID-19. To achieve this, we measured levels of cytokines and chemokines, proteins that are found in blood and are associated with the immune system. Importantly, we also measured levels of small pieces of RNA, called microRNAs, which have been shown to have outstanding potential in giving away disease secrets in many other diseases, including other infections and cancer. Indeed, we find microRNAs that are elevated in blood of people with severe disease. The identity of these microRNAs provides interesting clues with regards to how lung damage is reflected in circulation. Furthermore, we find correlations between microRNA and cytokine markers of COVID-19. Overall, our findings can lead to development of simple blood tests that identify subgroups of COVID-19 patients who can potentially benefit by specific therapeutic and care regimes.
Multiplex Immunoblot Assays Characterizing IgG and IgM Antibody Responses to Different SARS-CoV-2 Proteins in COVID-19 Patients

Abstract

Background: Rapid and simple serological assays for characterizing antibody responses are essential in the present COVID-19 pandemic. Multiplex immunoblot (IB) assays, termed COVID-19 IB assays, were developed for separately detecting IgG and IgM antibodies to different SARS-CoV-2 proteins.

Methods: Recombinant nucleocapsid protein (N) and the S1, S2 and receptor binding domain (RBD) of the spike protein of SARS-CoV-2 were used as target antigens in the COVID-19 IBs. Specificity was established with 231 pre-pandemic sera from persons with pertinent other infections and health conditions, and 32 goat antisera to different human influenza proteins. IgG and IgM COVID-19 IB assays were performed at ambient temperature on 84 sera provided at different times after a positive RT-qPCR test by 37 non-hospitalized COVID-19 patients with mild symptoms. Results were obtained in <3h.

Results: Algorithms for determining specific IgG and IgM antibody responses were developed through optimising specificity and sensitivity. The COVID-19 IBs had estimated specificity and sensitivity of 98.3% and 97.2% respectively for detecting IgG and/or IgM antibodies, meeting the US recommendations for laboratory serological diagnostic tests. The proportion of IgM antibody-positive sera from the COVID-19 patients following a RT-qPCR positive test was maximal at 83.3% before 10 days and decreased to 0% after 100 days, while the proportions of IgG antibody-positive sera reached 100% between days 51 and 65 and fell to 44.4% after 100 days. Detection of either IgG or IgM antibodies was better than IgG or IgM alone for assessing seroconversion. Both IgG and IgM antibodies detected RBD less frequently than S1, S2 and N proteins.

Conclusions: The multiplex COVID-19 IB assays offer advantages for simultaneously evaluating antibody responses to different SARS-CoV-2 proteins in COVID-19. Further studies with saliva, other SARS-CoV-2 proteins, on measuring antibody titres, in clinical settings and in populations with high COVID-19 prevalence, are needed.

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COVID-19 disease is a viral respiratory infection caused by the new coronavirus, SARS-CoV-2. Manifestations of the disease include fever and persistent cough, with the outcome of infection very variable between individuals. Indeed, most people show mild symptoms that resolve by themselves and do not require medical treatment, whereas a significant minority of patients present with rapid and severe progression of the disease that requires hospital admission and results in an elevated mortality rate. The factors that determine disease outcome are still unknown.

To get a better insight into the pathogenesis of the disease and understand what is happening at the early stages of infection, we enrolled patients with COVID-19 across the whole severity spectrum and studied their immune response over the course of the disease. Our analysis has shown that an early robust immune response could be an important factor for a better prognosis. Patients with more severe disease are characterised by pronounced systemic inflammation and a reduction in circulating blood immune cells that partially resolves over the follow-up period of 60 days.
Abstract

X-linked agammaglobulinemia (XLA) is a monogenic immunodeficiency caused by mutations in Bruton’s tyrosine kinase (BTK) [1]. BTK plays a key role in B cell maturation and exerts multiple effects on the inflammation cascade including mast cell and macrophage activation, production of IL-6 and maturation and secretion of IL-1β [2]. In the COVID-19 pandemic the clinical manifestation of infection with Sars-CoV-2 in patients with innate and adaptive immune deficiencies are of significant concern. Many aspects, including pathophysiology, viral clearance and infectivity need further characterisation in this patient cohort.

We present a 22-year-old male with neonatally diagnosed XLA who attended hospital in December 2020 for assessment having become SARS-CoV-2 positive on nasopharyngeal swab. He subsequently developed cough, rigors and chest tightness and was managed in hospital with remdesivir, prednisolone and serial antibiotics. His usual immunoglobulin infusion was continued. Despite initial improvement to discharge, he remained symptomatic, testing positive in the community on Day 15 and developing new, progressive symptoms leading to rehospitalisation on Day 31. Our patient was more unwell on the second hospital admission with hypoxia, worse physiological markers of severity, more florid radiographic changes consistent with COVID-19 pneumonitis and greater derangement of biochemical and immune profiles. He was treated with IV antibiotics, dexamethasone, 10 days of remdesivir and monoclonal antibody therapy (compassionate basis prescribing). He became swab negative on day 39, within 24 hours of monoclonal antibody therapy, and started to recover clinically.

Case reports have suggested a prolonged course of illness, prolonged viral shedding, and biphasic pattern in XLA patients infected with SARS-CoV-2 [3-7]. Our case adds to limited evidence suggesting prolonged viral shedding and resurgence of clinical illness is a concern in this patient cohort. The report may signal that current PHV guidance may be insufficient to prevent onward transmission in such cases and close surveillance is required.

References

[5] Soresina et al. Two X linked agammaglobulinemia patients develop pneumonia as COVID-19 manifestation but recover. Paediatric Allergy and Immunology. 2020 Jul; 31(5) 565-569

Abstract Lay Summary

We now know that infection with COVID-19 behaves differently in different people. An example of this is the elderly population being at greater risk of severe illness when infected with COVID-19 than others. We also know that the immune system, that’s our B-cells, T-cells, and special proteins, like antibodies and cytokines, are involved in regulating how your body reacts to control and get rid of infection. What we don’t know much about, is how the body of someone who has a genetic problem affecting their immunity, such as X-linked agammaglobulinemia, a condition where antibodies are not made, may respond to catching COVID-19. This case describes the time-course and medical findings in exactly this kind of patient. This case highlights the sorts of questions doctors should consider when looking after these patients. These questions include how long to expect a patient with impaired immunity to be ill, how sick to expect them to be, and how quickly they recover, and does their infectivity period, the period during which they can pass on the illness, differ from someone with a healthy immune system and most importantly how best to treat them.
Impact of COVID-19 on mucosa-associated lymphoid tissue: new insights and perspective

Abstract

Background: Although COVID-19 primarily affects lungs, several patients have reported gastrointestinal symptoms. Presence of the virus in the intestines is corroborated by RT-PCR reactions revealing Sars-CoV2 in feces. Additionally, Sars-CoV2 actively infects intestinal organoids, which is supported by highly expression of angiotensin converting enzyme-2 by intestinal epithelium.

Methods: Here we evaluated the presence of mRNA for N1 SarsCov2 nucleocapsid protein and the expression of spike-2 protein in formalin fixed, paraffin embedded post mortem samples from 2 females and 7 males, aged from 22 to 97 years, who died of COVID-19. The mean time from initial symptoms to death was 15 days. All patients showed lymphopenia, low haemoglobin levels, and high C reactive protein during hospitalisation. The levels of mRNA for SarsCov2, normalised by expression of RNAseP, were measured in oesophagus, stomach, duodenum, ileum, colon, lungs and spleen.

Results and conclusion: At least trace amounts of virus were observed in all samples compared to pre-pandemic controls. Additionally, SarsCov2 mRNA levels in lungs and gastrointestinal tract of 4 cases including one patient, who was admitted in the intensive care with gastrointestinal symptoms, were higher and consistent with local infection. The presence of the virus was further confirmed by staining of the ileum mucosa with spike-2 COVID-19 protein that identified virus in the epithelium and subepithelial mucosal tissue. The microanatomical features of ileal Peyer’s patches and the splenic white pulp were disrupted irrespective of the level of virus in the sample measured by qPCR and immunohistochemistry. In conclusion, the architecture of lymphoid tissues in the gut and spleen are impacted by infection with Sars-CoV-2 whether infection is local or not, in ways that would impact the ability of the lymphoid tissue to mount an effective immune response.

Abstract Lay Summary

The gastrointestinal tissues constitute an essential barrier to entrance of microbial pathogens into the body. We observed that immune components of the gastrointestinal tissue are severely disorganised in samples from deceased COVID-19 patients. Such immune disruption could impact the ability of the body to control infection.
The use of highly-multiplexed Imaging Mass Cytometry (IMC) to dissect the phenotypic and spatial signatures of cellular pathology within COVID19 post-mortem lung tissues

Abstract

Background: The current COVID19 global pandemic caused by the SARS-CoV2 virus has led to 124 million infections and 2.74 millions deaths worldwide, as of March 2021. Presently, little is known why in some individuals infection proves fatal whereas others are almost completely asymptomatic. What is evident is that several organs, in particular the lung, are sites of acute immune-mediated tissue damage leading to severe complications and death. This implicates a role for the host immune system in mediating tissue damage in response to SARS-CoV2 infection. We have therefore sought to use multi-parameter digital pathology imaging methods to address the role of the host immune system in mediating tissue damage and fatal organ failure in a large cohort of post-mortem lung samples obtained from over 40 COVID19-related death.

Methods: A panel of antibodies specific for key phenotypic immune cell markers, SARS-CoV2 viral proteins and other important pathological targets was designed with each clone rigorously validated by Immuno-Fluorescence (IF) followed by pathologists’ assessment. Each validated antibody was then conjugated to one of 40 different metal isotopes and revalidated by Imaging Mass Cytometry (IMC). As a control for assessing potential technical artefacts we produced a Tissue MicroArray (TMA) composed of SARS-CoV2 infected cells, uninfected cells and tonsil tissue that we mounted on each sample slide. We ablated 3x 1 µm2 areas of tissue per slide/case using IMC and extracted single cell phenotypic and “interactome” data using a bespoke analysis pipeline.

Results: Using this approach we have found possible phenotypic and spatial signatures that link to COVID19 lung pathology, but requires further investigations.

Conclusions: We have successfully validated a panel of 40 markers for deep profiling the immune cell compartments in post-mortem lung tissue from COVID19 patients. This will be a powerful and adaptable tool for extending this analysis into other affected tissue sites.

Abstract Lay Summary

There is currently very little known as to why the SARS-CoV2 virus can cause death in certain individuals whereas in others it leads to a mild, even asymptotic disease. What is clear however is the cause of death seems to be catastrophic organ failure due to a massive overreaction by the very cells in our bodies that are supposed to protect us from infections. In many ways, the organ damage is the crime scene, and our role as UK-CIC researchers is to act as detectives, questioning and interviewing each cell type (suspect) in the affected tissues (crime scene) to see if they are responsible, and if so how we could stop them from committing these crimes to others. To do this, we are using advanced technologies that allow us to survey the affected tissues from individuals who sadly died from COVID19. These technologies are so advanced that we can ask many different questions of the cells we find at the “crime scene” to determine who they are and what they have been doing, and whether they are guilty of the crime. Importantly, the lessons learnt may help us stop these cells before they can do harm in other COVID19 patients.
Spatial profiling of immune cells in lung tissue from patients who died with a positive COVID-19 test

Abstract

Background: In comparison to the extensive published studies evaluating immune profiles in blood and bronchoalveolar lavage fluid, characterisation of immune cell phenotypes within the tissues of patients with COVID-19 has been limited largely to whole scale transcriptional analysis or standard immunohistochemistry. The analysis of tissue immune responses in autopsy cases holds significant promise for validating peripheral biomarkers of disease progression and for identifying mechanisms of immune-mediated pathology.

Methods: We used nanoString GeoMx digital spatial profiling to examine expression of 62 protein targets in autopsy lung tissue. Patients in this cohort (n=8) died in the community with a confirmed positive COVID-19 test at time of death. Based on histopathological picture, death in three patients was attributed to diffuse alveolar damage (DAD) and likely due to COVID-19. In the other five patients, death was deemed possibly related to COVID-19 and DAD was not observed. For each patient, we examined 8-16 200um x 200um geometric regions of interest (ROIs; mean cell count per ROI of 191 ± 117; 112 ROIs «20,000 cells in total). Based on signal-to-isotype ratios, we included 45/62 protein targets reflective of the major immune subsets and key signalling pathways.

Results: Patients were separated from each other applying standard approaches to dimensionality reduction (t-SNE and PCA) to non-transformed ROI data. Unsupervised clustering based on all ROIs showed some separation between patients with DAD and those with other severe lung pathology or with minimal lung pathology. Analysis using decision tree classification indicated a possible role for STING expression as a discriminator between patient groups.

Conclusions: Though requiring validation in a larger autopsy cohort, the data suggest utility of spatial profiling for stratifying COVID-19 patients.

Abstract Lay Summary

Tissue damage during COVID-19 may occur as a direct effect of virus infection on host cells, but the prevailing evidence suggests that this is also a consequence of hyperactive or mis-directed immune responses that damage our own cells. Whilst we can observe how the immune system changes during COVID-19 by studying blood cells, only by looking in the tissue where damage actually occurs can we identify the immune mechanisms responsible. Identifying the cells and molecules responsible for immune-mediated tissue damage will help us to identify new treatment options, particularly for severely ill patients. We have, therefore, used advanced molecular techniques to “profile” the types of immune cells found in lung tissue taken post-mortem from patients that died with a positive COVID-19 test. Although at an early stage, our results indicate that we can use these techniques to classify patients by cause of death and identify potential mechanisms that discriminate between different patient groups. Applying this approach to a larger number of patients will allow us to test the robustness of these findings and to select immune pathways that might be amenable to disruption in order to prevent fatal COVID-19.
Disrupted resolution mechanisms contribute to altered phagocyte responses in COVID-19

Abstract

Background: Resolution mechanisms play a central role in both tissue maintenance and the return to homeostasis following injury and/or infections. Specialized pro-resolving lipid mediators (SPM) are essential fatty acid-derived signalling molecules that modulate immune cell responses to promote inflammatory resolution and limit disease severity. Notably, little is known about the relationship between the expression and activity of SPM pathways, circulating phagocyte function, and disease severity in patients infected with novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) leading to coronavirus disease 2019 (COVID-19). Here, we investigated the link between circulating SPM concentrations and phagocyte activation status and function in COVID-19 patients compared with healthy volunteers.

Methods and Results: Lipid mediator profiling demonstrated an activation of SPM pathways in patients with COVID-19 that becomes dysregulated with increasing disease severity. Plasma SPM concentrations were correlated with both circulating phagocyte activation status and function. Perturbations in plasma SPM concentrations and phagocyte activation were retained after the resolution of COVID-19 clinical symptoms. Treatment of patients with dexamethasone upregulated both the expression of SPM biosynthetic enzymes in circulating phagocytes as well as plasma concentrations of these mediators. Furthermore, incubation of phagocytes from COVID-19 patients with SPM rectified their phenotype and function. This included a downregulation in the expression of activation markers, a decrease in pro-coagulation factor and inflammatory cytokine expression, and an upregulation of bacterial phagocytosis.

Conclusions: Taken together, these findings highlight the role of altered resolution mechanisms in the disruption of phagocyte responses and the propagation of systemic inflammation in COVID-19.

Abstract Lay Summary

The immune system protects the body against outside threats such as bacteria and viruses. In patients with severe coronavirus disease 2019 (COVID-19), the immune system can stop working properly as it becomes hyperactive and causes damage to the body’s own tissues. However, the human body also produces substances that can calm down the immune system and promote a return to health. We wanted to know if these immune system-calming substances, also known as specialized pro-resolving mediators (SPM), become disturbed during COVID-19, since such knowledge could lead to better therapies for fighting the disease.

Our research shows that the levels of such protective molecules are increased in patients with mild COVID-19, while they are decreased in patients with more severe disease. We found that these changes are retained even after the end of symptoms associated with COVID-19 infections. Intriguingly, dexamethasone a drug found to protect COVID-19 patients from developing severe symptoms and dying, increases the production of such substances. We also found that SPM rectified the behaviour and function of immune cells taken from COVID-19 patients.

Taken together, our results highlight the importance of SPM in COVID-19 suggesting that targeting these pathways may represent a new way to treat COVID-19 associated inflammation.
Abstract

Prominent early features of COVID-19 include severe and often clinically silent hypoxia, and a pronounced reduction in B cells, the latter important in defence against SARS-CoV-2. This brought to mind the phenotype of mice with VHL-deficient B cells, in which Hypoxia-Inducible transcription Factors (HIFs) are constitutively active, suggesting that hypoxia might be driving B cell abnormalities in COVID-19. In moderate/severe COVID-19 we demonstrated a previously unrecognised breadth of early, persistent defects in B cell subsets, and confirmed the phenotypic similarity with B cell VHL-deficient mice. This was corroborated by hypoxia-related transcriptional changes in COVID-19 patients, and by B cell abnormalities in mice kept in hypoxic conditions, including rapid declines in marginal zone and germinal center B cells. Thus hypoxia might contribute to B cell pathology in COVID-19, and other diseases associated with hypoxia. This may impact on COVID-19 outcome, and could be remediable through early oxygen therapy.

Abstract Lay Summary

Patient’s suffering from COVID-19 develop a profound decrease in their B cells. B cells are important in the immune response against COVID-19, generating antibodies that protect against the virus. We wished to explore the relationship between the reduction in B cells and oxygen status of patients. We found patients requiring higher respiratory support had greater reductions in B cells. These findings were further supported in a mouse model where mice kept in low oxygen had a fall in B cells which improved upon removal from this environment. Thus low oxygen levels might contribute to a reduction in B cells seen in COVID-19 which might be remediable through early oxygen therapy.
Severe COVID-19 and Rheumatoid Arthritis share pro-inflammatory SPP1 myeloid pathway

Abstract

Background: Our recent analysis of synovial tissue macrophage (STM) uncovered heterogeneous, functionally distinct subpopulations governing synovitis (MerTKneg) and resolution (MerTKpos) of Rheumatoid Arthritis (RA). Whilst RA is not a viral respiratory syndrome, it constitutes chronic inflammation of the joint synovium, often accompanied by cardiovascular and lung co-morbidities. This pathology is driven by proinflammatory cytokines (TNFa, IL-6, IL1b) produced by MerTKneg STM clusters (CD48highS100A12pos and CD48posSPP1pos). The striking similarities in the immunopathogenesis of RA and severe COVID-19, led us to hypothesise that comparable macrophage subsets, to those functionally divergent synovial subpopulations, exist in the lung and play a role in directing COVID-19 inflammation or resolution.

Methods: We performed comparative scRNAseq analysis of COVID-19 bronchoalveolar lavage (BALF) macrophages with RA synovial tissue (ST) macrophages and evaluated the plasma concentration of their shared functional mediators in cross-sectional and longitudinal COVID-19. In addition, scRNAseq of whole blood stimulated with SPP1 assessed the function of SPP1 pathway in driving severe COVID19 pathology.

Results: Distinct BALF FCN1pos and FCN1posSPP1pos macrophage clusters emerging in severe COVID-19 patients were closely related to ST MerTKneg CD48highS100A12pos and CD48posSPP1pos clusters driving synovitis in active RA. Healthy lung resident alveolar FABP4pos macrophages shared a regulatory profile, including TAM (Tyro, Axl, MerTK) receptors pathway, with synovial tissue MerTKposTREM2pos macrophage that govern RA remission. This pathway was substantially altered in BALF macrophages of severe COVID-19. Plasma concentrations SPP1 and S100A12 (produced by common proinflammatory macrophages) were high in severe COVID-19 and their raised plasma concentrations predicted the need for intensive care unit transfer and persisted in long COVID-19. scRNAseq of SPP1 stimulated whole blood revealed that SPP1 drives activation of CD14pos monocytes and persistence of PDL-1pos neutrophils, as seen in severe COVID19 PBMC.

Conclusion: Severe COVID-19 pneumonitis appears driven by SPP1-expressing macrophage subpopulations resembling those that drive RA synovitis.
We present a case report of a patient with secondary immunodeficiency with persistent PCR SARS-COV-2 positivity over 8 months. This coincided with the development of a severe, progressive and steroid-unresponsive inflammatory organising pneumonia. This 63-year-old gentleman has a medical background significant for systemic lupus erythematosus, viral hepatitis B and follicular lymphoma for which he had been managed with Rituximab maintenance therapy. He was found to be pan-lymphopenic and hypogammaglobulinaemic most likely secondary to Rituximab which had been discontinued 2 months prior to his COVID-19 diagnosis.

Spontaneous seroconversion and co-incident viral clearance occurred 8 months after first PCR positivity. This was associated with significant clinical, physiological and radiological recovery along with B cell reconstitution. This is an important reminder of the immunosuppressive effect of Rituximab, an anti-CD20 monoclonal antibody, which should be avoided where possible during the COVID-19 pandemic where infection risks outweigh benefits. Reassuringly, with time and close monitoring alone, immunoglobulins and lymphocyte counts may recover to the pre-morbid baseline without the need for medical intervention with antibody replacement or anti-viral medications such as remdesivir.

From a respiratory perspective, this adds to the growing literature that severe organising pneumonia may occur as a complication of persistent COVID-19 infection. This may be a reversible process that does not invariably progress to fibrosis.

It is important to continue to expand our understanding of the complex interplay between immunosuppressive treatments, immunodeficient states, chronic viraemia, potential infectivity and complications such as inflammatory lung disease. This knowledge will have wide ranging impacts on treatments, vaccinations and the management of disease sequelae.

COVID-19 interstitial lung disease resolved with delayed seroconversion in a patient with secondary immunodeficiency

Abstract Lay Summary

We report an interesting and important case of a patient who experienced moderate COVID-19 infection. His illness persisted for many months after discharge from hospital. He was found to have a weakened immune system from a drug called Rituximab which is used to treat lymphoma. He was therefore unable to effectively clear the infection which caused additional inflammation in the lungs, known as organising pneumonia, and made him more unwell.

With time his immune system started working effectively and he was able to produce antibodies to clear the virus. Alongside this his lung disease resolved. We considered other treatments that may have been necessary to help his immune system respond, had it not done so by itself, which we suggest should be considered for other patients in a similar position.

The case shows us that coronavirus can persist when the immune system is not working properly and can cause severe lung disease. This helps us to understand the disease in other patients, predict their longer-term recovery and what treatments they may benefit from. We need to study similar cases to gain a greater understanding of how the virus, immune system, drugs and lung disease affect each other.
Abstract

Macrolide antibiotics, including azithromycin and clarithromycin, may have potential antiviral and immunomodulatory properties in addition to their antibacterial effects. As such they have been used as COVID-19 therapies although azithromycin has been found ineffective in randomised controlled trials.

We performed a retrospective cohort study and used cox regression and Kaplan-Meier analysis to investigate differences between patients whose antibiotic treatment included, and did not include clarithromycin. We then investigated the effect of incubating 15µg/ml SARS-CoV-2 S1+S2 spike protein with 10µg/ml antibiotics (amoxicillin, azithromycin, clarithromycin, and combined amoxicillin with clarithromycin) ex vivo for 6 hours on healthy volunteer lymphocyte intracellular cytokines (IL-2, IL-6, IL-10, IFNα, IFNγ, and TNFα), and monocyte phagocytosis (pHRodo) using flow cytometry to further understand the differences.

Results shown as mean difference (MD) % change analysed using Kruskal-Wallis test.

100 COVID-19 intensive care or high dependency unit patients were included of which 65 received clarithromycin. Clarithromycin was associated with a mortality benefit on cox regression (Exp(B) 0.56 [0.31-0.99]; p=0.045) and weakly associated with reduced 30-day mortality on Kaplan-Meier (51.5% vs. 32.4%; p=0.07).

Spike protein caused an increase in intracellular cytokines in CD4 cells (IL-2 and IFN-α), and CD8 cells (IL-2, IFNα, and TNFα).

Amoxicillin suppressed cytokines in CD4 (IFNα: MD -25.8; p=0.008) and CD8 lymphocytes (IFNα: -26.6%; p=0.005, TNFα: -25.2%; p=0.01), azithromycin and clarithromycin suppressed CD8 cytokines (IFNα: -25.9%, p=0.01; and 24.8%; p=0.01, TNFα: -26.5%; p=0.006; and -26.0%; p=0.007).

Amoxicillin with clarithromycin had an additive effect in CD4 (TNFα: -25.2%; p=0.005) and CD8 lymphocytes (IFNα: -33.6%; p=0.001, TNFα: -30.9%; p=0.001). Amoxicillin but not macrolides enhanced classical monocyte phagocytosis both with (11.0%; p=0.04) and without (12.5%; p=0.02) clarithromycin.

Our results suggest that whilst there is evidence of a mortality benefit with clarithromycin, the role of its immunomodulatory properties may be confounded by its effect on treating undiagnosed superimposed bacterial infections.

Abstract Lay Summary

A number of medications have been repurposed in the fight against COVID-19 including macrolide antibiotics (azithromycin and clarithromycin), normally used to treat bacterial infections because they may have both antiviral effects and help our immune system to fight the infection. Whilst randomised trials have not shown a mortality benefit, our own patient data suggested a benefit with clarithromycin. We undertook further experiments in healthy volunteers to try and identify how clarithromycin might be helping our immune system.

We took healthy volunteer immune cells and added COVID-19 and antibiotics to them. After 6 hours we analysed the cells to see how their cytokine (important signalling molecules involved in fighting infections) levels changed. We also looked to see if their ability to capture bacteria was altered.

We found that whilst clarithromycin decreased the levels of two important cytokines involved in clearing COVID-19 virus, amoxicillin, another commonly used antibiotic had a similar effect.

Our results suggest that whilst clarithromycin may help our immune system fight COVID-19, its benefit may still be in its traditional role of treating co-existing bacterial infections.

Clarithromycin may have immunomodulatory effects in patients with COVID-19

Clarithromycin may have immunomodulatory effects in patients with COVID-19.
Abstract

The COVID-19 pandemic poses an unprecedented public health challenge and we are only now beginning to realise the longer term consequences of infection. Whilst our understanding of the acute immune response to SARS-CoV-2 infection is improving rapidly, the long-term impacts on the immune system are poorly understood. To address this knowledge gap we have performed detailed immune phenotyping on PBMCs from 46 hospitalised patients with severe COVID-19 requiring ICU admission, three months post hospital discharge and 45 age-matched controls. We found significantly increased immunesenescence in the COVID-19 survivors including: a reduced frequency of naïve (CD45RA+ve CCR7+ve) T cells; increased TEMRA senescent (CD28-ve CD57+ve) T cells and an increased functional Th17 polarisation (RORγ+ve). Additionally, COVID-19 survivors display elevated markers of T cell activation (CD69, CD25) and exhaustion (PD1). As senescent T cells are pro-inflammatory and have been implicated in driving a range of age-related diseases, they may contribute to the symptoms of Long COVID. As our cohort are aged below 60 years (mean age 53.4) the data suggest a state of accelerated immune ageing in COVID-19 survivors. Our observations imply an urgent need for testing of immunesenescence reversing strategies in COVID-19 survivors.

Abstract Lay Summary

Ageing is accompanied by an impairment in our ability to mount a robust immune response to pathogens and remodelling of the immune system, termed immunesenescence. In this study we have observed that COVID-19 infection patients who developed severe disease and were admitted in ICU display an immune profile similar to an aged immune system three months post-infection. Suggesting that severe COVID-19 infection results in long term impact on the immune system, providing the evidence base for testing immunesenescence reversing strategies in COVID-19 survivors.
Abstract

**Background:** The elderly care-home population may be at heightened risk of SARS-CoV-2 re-infection or vaccine failure due to rapidly waning antibodies. However, where antibody titres have waned, persisting antigen-specific memory B cells could provide rapid immune protection upon re-exposure. We therefore analysed SARS-CoV-2 spike-specific B cell memory in a cohort of care-home residents 5 months post infection, comparing with a younger cohort of care-home staff.

**Methods:** Infected residents (n=22, median 86yrs) and staff (n=11, median 56yrs) of care-homes recruited during SARS-CoV-2 outbreaks in April 2020 underwent serological assessment for live virus neutralising antibody (NAb) in May (T1) and September 2020 (T2), alongside evaluation of spike/S1/RBD-specific memory B cell phenotype and function by flow cytometry and ELISPOT at T2.

**Results:** We observed a significant decrease in NAb titres between T1 and T2, with 36.4% staff and 42.9% residents seroreverting to undetectable NAb. NAb titres were closely associated with the frequency of spike and RBD bait-binding B cells, with higher numbers found in those maintaining NAb. However, spike- and RBD-bait-binding B cells with a classical, IgG+ memory phenotype remained detectable regardless of age and waning of NAb (28/33 and 26/33 respectively), even in some who had seroreverted. Similarly, functional recall to spike, S1 and RBD was detected by ELISPOT, including in some seroreverted individuals, with no significant differences in responses between those with or without NAb. However, RBD-specific memory B cell responses were significantly reduced in seroreverting residents compared to staff.

**Conclusions:** The persistence of functional SARS-COV-2 spike-specific memory B cells suggests protective immunity can persist >5 months post-infection of care home residents and staff despite NAb waning, even in some seroreverters. However, care-home residents that had lost detectable NAb had minimal memory responses to RBD, with potential implications for risk of reinfection and vaccine strategies in the older age group.

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Abstract Lay Summary

The COVID-19 pandemic has significantly affected elderly care home populations with outbreaks seen worldwide. The majority of the research into the effects of infection with COVID-19 on the immune system and the impact of this on the ability to respond to future infection has been conducted in younger individuals and hospitalised patients. We sought to understand the impact of past COVID-19 infection in care home residents infected during the first wave of the pandemic in the UK 5 months after their initial infection. Studying blood samples we identified B cells specific for the SARS-CoV-2 virus that causes COVID-19 in elderly residents and younger staff members. These B cells have the potential to respond promptly, providing protection from repeat or severe infection. The cells were present in individuals who no longer had protective antibodies detectable in their blood, but at lower levels than those with detectable protective antibodies. When stimulated the cells from older residents had more limited responses than those of younger staff members, highlighting that immune responses of older individuals may have limited ability to respond to repeat infection following natural infection or vaccination, and emphasising the need to continue monitoring for infection in care home environments.
It is important to understand the immune response in individuals representing different types of infection presentation. Specific to COVID-19 – a new infection – it is important to examine the tenure of immune response in participants with the full spectrum of infection/disease status. Ideally, one would also do this in the context of pre- and post-pandemic biosamples and with health and lifestyle data available across the life course of participants involved. Established population-based cohorts offer a solution to this challenge. Although, have in general been understudied by the basic science community for immunological research. The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective population-based cohort study which recruited pregnant women in 1990-1992 and has followed these women, their partners and their offspring ever since. ALSPAC reacted to the COVID-19 pandemic by deploying four online questionnaires (March, May, October 2020 and January 2021) accompanied by Serological Lateral Flow Testing in October and currently (Easter 2021). In addition, linkage to Public Health England Pillar I and II testing results has been obtained for all participants who have consented or for whom we have NHS Confidentiality approval group permitted Section 251 access. Combining this data has allowed for the identification of 125 symptomatic and asymptomatic SARS-CoV-2 cases. These individuals, along with symptom matched and random controls (matched on age and gender to a total of 325 individuals) were identified for repeat clinics where biosamples have and will continue to be collected for detailed immunological analysis. This opens up population-based resources to immunological analysis and the possibility of examining the immunological response in both symptomatic and asymptomatic cases. Moreover, this demonstrates the possibilities made available through this resource, the interplay between population-based and laboratory-based science and how understanding the T cell and antibody response to infection can be related to policy relevant traits.
Abstract

Background: SARS-CoV-2 infection or vaccination with spike protein or mRNA are able to induce neutralising antibodies and prevent COVID-19 disease. Recently, SARS-CoV-2 variants have emerged with the ability to escape antibody neutralisation in vitro, and protective immunity in vivo. Defining correlates of protection has been complicated by variability in the assays used to measure neutralising antibody titres. Whilst plaque reduction neutralisation tests (PRNT) remain the gold standard, low throughput and long turnaround times limit their utility. Non-standardised assays using spike-pseudotyped viral particles are therefore commonly employed, but correlate imperfectly with results from wildtype virus. There is therefore an urgent unmet need for a simple, high-throughput method to quantitate neutralisation of authentic SARS-CoV-2 isolates.

Methods: In this study, we exploit the expression of SARS-CoV-2 Papain-Like protease to detect authentic viral infection. Our assays rely on the cleavage of a specific oligopeptide linker, leading to activation of a circularly permuted firefly luciferase-based reporter. We stably express this biosensor with ACE2 in a highly permissive HEK293T cell line. Replication of wildtype SARS-CoV-2 virus is quantitated 24 hrs after infection by luminometry in a 96- or 384-well plate format, without the need for antibody reagents.

Results: First, we characterise our biosensor using recombinant viral proteases. Then, we confirm its ability to detect endogenous Papain-Like protease expression during infection with wildtype SARS-CoV-2. Next, we generate a stable reporter cell line, and confirm that it accurately quantitates infectious SARS-CoV-2 virus. Finally, we show that the reporter cell line may be activated by different SARS-CoV-2 isolates, and used for titration of neutralising activity in sera.

Conclusions: We have developed a sensitive luciferase-based reporter cell line for quantitation of authentic SARS-CoV-2 infection, including new variants of concern. Neutralising antibody activity may be measured using a simple luminescent readout, in a standard format suitable for large-scale screening.

A luciferase-based reporter cell line for quantitation of authentic SARS-CoV-2 infection and neutralising antibody activity

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Abstract Lay Summary

Antibodies from previous infection or vaccination are able to protect against COVID-19. Recently, new variants of coronavirus have emerged, which may be able to escape these antibodies. It is difficult to be certain how important these variants are, because there is no standard way to measure their susceptibility to antibodies in the laboratory. The traditional way to do this is laborious and time-consuming, limiting its usefulness. Different laboratories therefore use a range of quicker, easier techniques. Unfortunately, they don’t always yield the same results. In this study, we develop a fast, reliable alternative. Our method exploits a particular protein made by the virus when it infects cells. This protein is an enzyme, which means it is able to cut other proteins. We therefore design a special protein, which can be made to emit light, but only when it is cut by the viral enzyme. The amount of light indicates how much virus is present, and whether it can be blocked by antibodies. This is really easy to measure using standard equipment, and can be done on a massive scale. It should therefore help different laboratories predict whether new variants of coronavirus will be resistant to vaccination or previous infection.
Abstract

The rise of SARS-CoV-2 has rapidly redirected research efforts around the globe. The Surrey COVID-19 Collaboration has collected blood, saliva and sebum samples from COVID-19 patients in Surrey hospitals for use in diagnostic and immunology-related research. Sequencing the B-cell repertoire of these patients at different time points highlights the SARS-CoV-2 footprint on the repertoire and how this develops in acute versus convalescent samples. We compare these data with similar repertoire data on other anti-viral immune responses including Ebola, Respiratory Syncytial Virus and Yellow Fever Vaccination to highlight similarities and differences in the viral footprints. We also highlight other collaborations enabled by our biobank, for example a consortium with the Mass Spectrometry community. (Spick et al 2021; https://doi.org/10.1016/j.eclinm.2021.100786).

Abstract Lay Summary

The rise of SARS-CoV-2 has rapidly redirected research efforts around the globe. The Surrey COVID-19 Collaboration has collected blood, saliva and sebum samples from COVID-19 patients in Surrey hospitals for use in diagnostic and immunology-related research. Sequencing the B-cell repertoire (the viral binding port off an antibody) of these patients at different time points highlights the SARS-CoV-2 footprint on the repertoire and how this develops in acute versus convalescent samples. We compare these data with similar repertoire data on other anti-viral immune responses including Ebola, Respiratory Syncytial Virus and Yellow Fever Vaccination to highlight similarities and differences in the viral footprints. We also highlight other collaborations enabled by our biobank, for example a consortium with the Mass Spectrometry community. (Spick et al 2021; https://doi.org/10.1016/j.eclinm.2021.100786).
Background: Immunological Memory (IM) describes the ability of the immune system to mediate a more rapid and robust immune response on second exposure to the same pathogen. If robust IM is not maintained, then individuals risk being re-infected with the same pathogen at a later time point. It is unknown whether long-lived IM is established following recovery from COVID-19 or how this is influenced by factors such as age. The Avon Longitudinal Study of Parents and Children (ALSPAC) provides a unique opportunity to study the duration of IM in young adults (~28yo) and their parents.

Methods: Peripheral blood mononuclear cells, serum and saliva were collected from 325 ALSPAC participants in December 2020. This includes 125 ‘cases’ selected on the basis of a previous positive PCR test and/or positive Serological Lateral Flow Test (LFT). Remaining participants (who tested negative by PCR or serological LFT) were assigned to control groups based on whether they reported either matched symptoms or no symptoms. The same participants will be invited back for repeat sampling in April and August 2021.

Results: In-depth analysis of T-cell responses is underway to examine the magnitude and breadth of SARS-CoV-2 specific T-cell responses. Preliminary data demonstrates robust T-cell responses against specific proteins in samples from ‘cases’ taken at the 1st sampling time point. The longevity of these T-cell responses will be determined using samples taken from the same individuals at the 2nd and 3rd sampling time points. SARS-CoV-2 specific serum and salivary antibodies will also be quantified at each sampling time point and neutralization capacity examined using pseudovirus assays.

Conclusions: The comprehensive analysis of antibody and T-cell responses against SARS-CoV-2 in this longitudinal cohort study will allow us to determine the characteristics and duration of SARS-CoV-2 immunity following recovery from symptomatic and asymptomatic COVID-19 in young adults and their parents.

Abstract Lay Summary
When we get viral infections, cells from our immune system spring into action and help us get rid of the infection. After we have recovered, some of these cells remain in our bodies and protect us from getting ill with the same infection again. This is called ‘memory’ and can last a long time after we have recovered from infection. We are trying to find out how long ‘memory’ lasts after recovery from COVID-19. We are studying ‘memory’ in young adults who have had COVID-19 as they make up a significant proportion of the COVID-19 infections that have occurred. To do this we are collecting blood samples at multiple time points from young adults who are part of a unique study in the South West of England. Then we are looking at T-cells and antibody responses and how long they persist for after recovery from COVID-19. This work will help us to work out how long individuals might be protected from getting COVID-19 again after recovery from the infection. In future work, it will be important to extend this study to determine how long ‘memory’ lasts for after vaccination to enable vaccine strategies to be shaped in the future.
Given the widespread impact of COVID-19, ensuring the research of UK-CIC is relevant and accessible to the public is crucial to the overall success of the project. The Patient and Public Involvement (PPI) Panel have led this effort, with their lived experiences and knowledge providing an invaluable source of expertise to UK-CIC researchers, demonstrating the importance and benefits that PPI can bring to basic research.

Research direction in UK-CIC has been influenced by interactive discussions with the PPI panel, ensuring outputs provide relevant public benefit in a constantly evolving situation. By actively engaging with their communities, the PPI representatives have brought UK-CIC research to a much wider audience in an accessible way highlighting the usefulness of basic immunology research to the public.

Here we present the ways through which collaboration between patient and public representatives and research is fostered along with evidence of the impact that this collaboration can achieve within and beyond UK-CIC. We hope this inspires UK-CIC researchers to continue engaging with the panel.
Identification of ISGs restricting SARS-CoV2

Abstract

First identified in late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the third coronavirus that emerged in human populations in the past two decades. Emergence of SARS-CoV in 2002 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 were characterized by high case-fatality rates and limited human-to-human transmission. In comparison, SARS-CoV-2 is highly transmissible between humans and clinical symptoms range from asymptomatic infections to lethal COVID-19. Similar to SARS-CoV-1, strong and early type I interferon (IFN) responses confer protection from severe COVID-19 disease. In contrast, delayed IFN responses, inborn errors in interferon pathways and anti-interferon antibodies are associated with severe disease.

To dissect how IFN inhibits SARS-CoV-2 and identify the IFN stimulated genes (ISGs) that inhibit SARS-CoV-2, we screened >500 human ISGs for their ability to inhibit SARS-CoV-2 in vitro. We identified 6 genes with potent anti-SARS-CoV-2 activity, with 2'-5'-oligoadenylate synthetase 1 exhibiting potent antiviral activity. The Restriction was virus specific as inhibition was observed for SARS-CoV-2 and encephalomyocarditis virus (EMCV), whereas the replication of OC43, VSV, Influenza A virus and ZIKA virus remained unaffected. We further show that the restriction of viral replication was due to activation of an RNaseL-dependent pathway of RNA degradation.

The human 2'-5'-oligoadenylate synthetase 1 gene encodes multiple protein isoforms through alternative splicing, with 2 predominant protein isoforms produced. A single-nucleotide polymorphism (SNP) at the splice acceptor position of exon 7 has been shown to result in higher levels of an isoform that has been associated with improved COVID-19 disease outcome. We present a molecular explanation for the improved survival of patients expressing the anti-CoV isoform and present the evolutionary pathways that likely rendered SARS-CoVs sensitive to this pattern recognition pathway.

Abstract Lay Summary

SARS-CoV-2, the virus that causes COVID-19 is extremely sensitive to type I interferons (IFNs). IFNs are secreted in response to infections and initiate a signalling cascade that instructs cells to increase the levels of their antiviral defences. These heightened defences mean that SARS-CoV-2 no longer replicates efficiently in IFN treated cells. This has led to the therapeutic potential of IFNs being intensively investigated in relation to COVID-19. We sought to understand the molecular basis of this antiviral protection in order to better understand why some individuals experience more severe COVID-19 than others and understand the mode of action of therapeutic IFNs. We conducted a genetic screen to identify defences that target SARS-CoV-2 and identify an antiviral defence that provides a molecular explanation underlying why some people are particularly susceptible to severe COVID-19 disease. In addition, we present an evolutionary explanation that potentially explains why SARS-CoV-2 is so sensitive to this antiviral defence.
Abstract

Background: Following a single dose of BNT162b2 mRNA vaccine, higher antibody titres are observed in individuals with previous SARS-CoV-2 infection than in infection-naive subjects. T-cell responses however are less well defined.

Methods: We sampled healthcare workers (HCWs) enrolled in the UK PITCH study, before and after BNT162b2 mRNA vaccination. We measured spike-specific antibody, and quantified T-cell responses by IFNγ ELISpot assay and intracellular staining of peripheral blood mononuclear cells (PBMC), comparing SARS-CoV-2-naïve individuals to those with prior infection.

Results: HCWs aged 22 to 71 years received one (n=216) or two (n=21) vaccine doses. After a single dose, the spike-specific T-cell response was 6-fold higher in previously-infected vs. naïve individuals (median 340 vs. 58 SFU/106 PBMC, p<0.0001; fresh PBMC, n=86). The T-cell response in previously-infected individuals after one vaccine dose was equivalent to naïve individuals receiving two vaccine doses (median 158 vs. 165 SFU/106 PBMCs, p=0.65; cryopreserved PBMC, n=117). Anti-spike IgG levels following a single dose in those previously infected (median 512.9 antibody units/ml (AU/ml)) were 6.8-fold higher vs. naïve individuals following one dose (median 75.0 AU/ml, p<0.0001) and 2.9-fold higher than naïve individuals given two doses three weeks apart (179.9 AU/ml, p=0.03). Following vaccination, plasma from individuals with prior infection demonstrated higher in vitro neutralisation of the B.1.351 variant of concern compared to naïve individuals.

Conclusion: Following a single BNT162b2 dose, HCWs with a prior history of SARS-CoV-2 infection have significantly higher T-cell and antibody responses than naive individuals.

Funding: UK Department of Health and Social Care and UK Coronavirus Immunology Consortium.
The burden of nosocomial covid-19 in Wales: results from a multi-centre retrospective observational study of 2518 hospitalised adults

Abstract

Objectives: To define the burden of nosocomial (hospital-acquired) novel pandemic coronavirus (covid-19) infection among adults hospitalised across Wales.

Design: Retrospective observational study of adult patients with polymerase chain reaction (PCR)-confirmed SARS-CoV-2 infection between 1st March – 1st July 2020 with a recorded hospital admission within the subsequent 31 days. Outcomes were collected up to 20th November using a standardised online data collection tool.

Setting: Service evaluation performed across 18 secondary or tertiary care hospitals.

Participants: 4112 admissions with a positive SARS-CoV-2 PCR result between 1st March to 1st July 2020 were screened. Anonymised data from 2518 participants were returned, representing over 60% of adults hospitalised across the nation of Wales.

Main outcome measures: The prevalence and outcomes (death, discharge) for nosocomial covid-19, assessed across a range of possible case definitions.

Results: Inpatient mortality rates for nosocomial covid-19 ranged from 38% to 42% and remained consistently higher than participants with community-acquired infection (31% to 35%) across a range of case definitions. Participants with nosocomial-acquired infection were an older, frailer, and multi-morbid population than those with community-acquired infection. Based on the Public Health Wales case definition, 50% of participants had been admitted for 30 days prior to diagnostic testing.

Conclusions: This represents the largest assessment of clinical outcomes for patients with nosocomial covid-19 in the UK to date. These findings suggest that inpatient mortality rates from nosocomial infection are likely higher than previously reported, emphasizing the importance of infection control measures, and supports prioritisation of vaccination for covid-19 negative admissions and trials of post-exposure prophylaxis in inpatient cohorts.

Abstract Lay Summary

Hospitals are intended as places of healing, however admission can expose patients to infection. Little is known about the outcome of patients who are infected with the novel pandemic coronavirus SARS-CoV-2 (causing covid-19) during their hospital stay. We studied the outcome of 2518 adults hospitalised with a positive SARS-CoV-2 PCR test result between March to July 2020. This represents over 60% of patients with covid-19 across the nation of Wales during the first wave. Those with hospital-acquired covid-19 infection were older, frailer, and more likely to die in hospital compared to those who caught covid-19 in the community. Strikingly, over half of those dying with hospital-acquired covid-19 had been admitted for over a month before infection was recognised. Inpatient vaccination has long been considered a missed opportunity for improving influenza vaccine uptake, however in time of analysis was not routinely part of the roll-out of vaccination within Wales. This report has been presented to the Welsh Technical Advisory Group and Directors of Nursing Group, leading to recommendations to ministers. Inpatients without a diagnosis of covid-19 within priority groups and those being admitted for a planned procedure at increased risk (e.g. cancer surgery) are now being offered vaccination.
Abstract

**Background:** T cells have been shown to play key protective but also pathogenic roles in COVID-19. Whereas antigen-specific T cells can clear SARS-CoV-2-infected cells, T cell exhaustion, senescence, or aberrant proliferation have all been linked to COVID-19 pathogenesis.

**Methods:** We performed long intergenic non-coding RNA (lincRNA)-anchored analysis of scRNA sequencing experiments to explore T cell transcriptomes in patients with COVID-19. We reasoned that lincRNAs can be used as transcriptome-intrinsic reference points during single cell profiling because they are both components of the transcriptome and effector molecules. Several lincRNAs (e.g. XIST, MALAT1, HOTAIR) mediate their functions in immune cells through dynamic and promiscuous interactions with multiple RNA-binding proteins, therefore our approach can provide insight into the transition from transcriptional to post-transcriptional profiles of immune cells.

**Results:** By analysing datasets from bronchoalveolar lavage fluid and PBMCs from people infected with SARS-CoV-2, we find that lincRNA-anchored profiles can efficiently separate patients with severe COVID-19 from those with mild disease, and healthy controls. We identify both core and severe COVID-19-specific lincRNA-associated gene signatures. Our analysis gave insight into poorly understood aspects of immune function in COVID-19, including type I interferon responses, sex hormone responses in CD4+ and CD8+ T cells. Crucially, a list of genes that anti-correlate with MALAT1 in T cells from BAL and PBMC datasets identifies proliferative CD4+ and CD8+ T cells in severe COVID-19. We have previously shown that MALAT1 plays a role in T cell differentiation and function. Further validation of these findings in independent cohorts is ongoing.

**Conclusion:** Our findings suggest that MALAT1 suppression in conjunction with up-regulation of a specific gene set is a hallmark of lung proliferating T cells, which in turn are feature frequently observed in severe COVID-19.

Abstract Lay Summary

In a properly working immune system, immune cells such as T cells are essential in protection from coronavirus infection through targeted clearance of infected cells. On the other hand, during the overwhelming inflammation observed in patients with severe disease, abnormal function of T cells is a driver of COVID-19 development. Here, we describe a novel approach into studying single cells to unravel what drives harmful function of T cells in COVID-19. We discover a specific signature that persists in T cells from people with severe COVID-19. This signature is characterised by a correlation of levels of several genes with expression of single gene. Intriguingly, this is not a gene that is used as a template to make protein, rather it is a part of DNA that is used with the sole purpose of creating an RNA called MALAT1. We believe that our findings reveal a key feature of COVID-19-promoting T cells, shedding further light into potential ways of treating people with severe disease.
Abstract

Background: Neutrophil recruitment to the lung and excessive neutrophil activity in the alveolar space and airways contribute to lung damage and acute respiratory distress syndrome (ARDS) in severe COVID-19. In response to infection neutrophils undergo NETosis to release NETs, which are web-like DNA structures coated in cytotoxic and microbicidal proteins. NETs are highly pro-inflammatory and can cause significant host tissue damage. Inhibiting NETosis is emerging as a potential therapeutic target to reduce lung injury and inflammation in patients with severe COVID-19. PKC is a key enzyme involved in the cellular mechanism of NETosis. Here we demonstrate that the PKC-β inhibitor, ruboxistaurin, which has previously been shown to be safe for use in human clinical trials of other diseases, reduces elevated NETosis in patients with COVID-19.

Methods: Neutrophils were isolated from peripheral blood from hospitalised patients with COVID-19 or healthy controls. Cells were pre-incubated for 1 hour with either ruboxistaurin (200 nM), dexamethasone (10 µM) or media control and stimulated for a further 3 hours with either LPS (5 µg/ml) or PMA (100 nM). NETs were quantified by sytox green fluorescence using a plate reader and supporting images captured using fluorescence microscopy.

Results: Neutrophils from patients with COVID-19 undergo significantly increased NETosis in response to LPS compared to healthy controls (p< 0.01). NETosis in response to PMA was similar between patients and controls. Ruboxistaurin significantly reduced both LPS-induced and PMA-induced NET Formation (p< 0.001; p< 0.0001). Dexamethasone was without effect on NETosis. Analysis of patient clinical data highlighted a significant positive correlation between LPS stimulated NETosis and neutrophil lymphocyte ratio (p< 0.05).

Conclusion: Increased NETosis may contribute to lung damage and ARDS in severe COVID-19. Inhibiting PKC-β with ruboxistaurin is a novel approach to reduce NETosis in COVID-19 and may have therapeutic potential in the longer term.

Abstract Lay Summary

COVID-19 disease, caused by the SARS-CoV-2 virus, can cause severe lung damage, leading to respiratory failure, the requirement for mechanical ventilation and death. In response to the infection, cells of the immune system become highly stimulated and travel to the lung, where a sub-set of cells, called neutrophils, release toxic proteins in a web-like structure called neutrophil extracellular traps (NETs). NETs are generated to kill the infecting pathogens, including viruses. However, increased NET release may cause tissue damage to the lung, making breathing more difficult. Investigating new therapies to reduce the release of NETs could lessen lung damage in patients with COVID-19. In this research we isolated neutrophils from blood from hospitalised volunteers with COVID-19 or healthy controls and investigated in the laboratory if an experimental medicine, named ruboxistaurin could reduce NET formation. We showed that ruboxistaurin significantly reduced NET formation. To understand if ruboxistaurin would be a useful treatment further investigations, including clinical trials, are needed. Importantly this medicine has been used safely in trials for other human diseases. The next step in our research will focus on understanding the wider impacts of ruboxistaurin on other immune cell functions.
CSF1R defines the Mononuclear Phagocyte System lineage in human blood in health and COVID-19

Abstract

Mononuclear Phagocytes defend tissues, present antigens and mediate recovery and healing. To date we lack a marker to unify mononuclear phagocytes in humans or that informs us about their origin. Here, we reassess Mononuclear Phagocyte ontogeny in human blood through the lineage receptor CSF1R, in the steady state and in COVID-19. We define CSF1R as the first sensitive and reproducible pan-phagocyte lineage marker, to identify and enumerate all conventional monocytes, and the myeloid dendritic cells. In the steady state CSF1R is sufficient for sorting and immunomagnetic isolation. In pathology, changes in CSF1R are more sensitive than CD14 and CD16. In COVID-19, a significant drop in membrane CSF1R is useful for stratifying patients, beyond the power of cell categories published thus far, which fail to capture COVID-19 specific events. Importantly, CSF1R defines cells which are neither conventional monocytes nor DCs, which are missed in published analysis. CSF1R decrease can be linked ex vivo to high CSF1 levels. Blood assessment of CSF1R+ cells opens a developmental window to the Mononuclear Phagocyte System in transit from bone marrow to tissues, supports isolation and phenotypic characterisation, identifies novel cell types, and singles out CSF1R inhibition as therapeutic target in COVID-19 and other diseases.

Abstract Lay Summary

The Mononuclear Phagocyte system (MPS) is a dispersed homeostatic organ of embryonic and bone marrow origin. MPS cells constitute an important innate cell network that provides tissues as needed, with inflammatory or pro-resolution suppressor cells. Here, we reassess Mononuclear Phagocyte ontogeny in human blood through the Colony Stimulating Factor 1 receptor CSF1R, in the steady state and in COVID-19. We define CSF1R as the first sensitive and reproducible pan-phagocyte lineage marker, to identify and enumerate all conventional monocytes, and the myeloid dendritic cells. This clarifies the cellular targets of the drugs that are being developed for CSF1R, with profound clinical implications. In COVID-19, the CSF1R system is the most informative MPS marker and makes it possible to distinguish patients from other pulmonary diseases. Dissecting the contribution of CSF1R expression to human blood and tissues, will enable us to develop anti COVID-19, anti-cancer and anti-inflammatory strategies, rescuing the MPS for therapy.
Abstract

End-stage kidney disease (ESKD) patients are at high risk of severe COVID-19. We measured 436 circulating proteins in serial blood samples from hospitalised and non-hospitalised ESKD patients with COVID-19 (n=256 samples from 55 patients). Comparison to 51 non-infected patients revealed 221 differentially expressed proteins, with consistent results in an independent cohort of 46 COVID-19 patients. 203 proteins were associated with clinical severity, including IL6, markers of monocyte recruitment (e.g. CCL2, CCL7), neutrophil activation (e.g. proteinase-3) and epithelial injury (e.g. KRT19). Machine learning identified predictors of severity including KRT19, PARP1, PADI2, and CCL7. Survival analysis with joint models revealed 69 predictors of death. Longitudinal modelling with linear mixed models uncovered 32 proteins displaying different temporal profiles in severe versus nonsevere disease, including integrins and adhesion molecules. These data implicate epithelial damage, innate immune activation, and leucocyte-endothelial interactions in the pathology of severe COVID-19 and provide a resource for identifying drug targets.

Abstract Lay Summary

COVID-19 varies from a mild illness in some people to fatal disease in others. Patients with kidney failure are at very high risk of severe COVID-19. We measured approximately 500 proteins in the blood of kidney failure patients with COVID-19 with repeat sampling throughout the illness. We showed that severe COVID-19 has a protein ‘signature’ indicating activation of the innate immune system, increased immune cell communication and movement, and lung and blood vessel injury. Using machine learning and statistical modelling, we were able to identify protein markers that predict severe disease and death. Together with similar studies, these findings shed light on the biological pathways that drive severe COVID-19. By identifying the proteins that are elevated in severe disease, these data provide a resource to help identify potential drug targets for the treatment of severe COVID-19.
Background: SARS-CoV2 infection is associated with perturbations in immune-cell subsets and altered immune function, most marked in hospitalised COVID-19 patients. Early reductions in lymphoid and some myeloid populations in the blood are reported, accompanying robust systemic inflammation and dynamic shifts in transcriptional programming. Much interest has surrounded the productive anti-viral and pathological features of early immune-cell dysregulation in severe disease, however the rate and extent to which immune system homeostasis is re-established following infection, and associated long-term health implications, remain to be understood.

Methods: Immunophenotyping, serum profiling and whole blood transcriptomics was performed on 45 healthy participants and 201 COVID-19 patients across a spectrum of disease severities (asymptomatic to intubated) at serial timepoints beyond 9 months post symptom onset. The rate of immune-cell recovery was assessed in patients stratified by persisting or resolving systemic inflammation, and whole blood RNASeq used to identify biological functions differing from health throughout disease and subsequent recovery.

Results: Early lymphopenia in hospitalised COVID-19 patients was largely resolved by one-month post symptom onset in those not requiring assisted ventilation. In severe disease, certain B cell, activated CD4+ and non-classical T cell populations remain suppressed alongside prolonged systemic inflammation, returning gradually to healthy levels by 6 months post infection. Although early transcriptional activation of interferon and TNF/IL-6 cytokine signalling pathways wane, late activation of OXPHOS-, ROS- and heme-related metabolic pathways become apparent in those with severe disease, with transcriptional changes remaining prominent beyond 100 days post infection.

Conclusions: Immune-cell recovery is apparent within six months following SARS-CoV2 infection, variably dependent on disease severity and the resolution of systemic inflammation. Transcriptional shifts detected in late-stage severe disease hint at the emergence of yet uncharacterised metabolic dysfunction and persistent immune changes, and may provide insight into the long-term health consequences of SARS-CoV2 infection and fundamental mechanisms of immunological recovery.

Abstract Lay Summary
The immune response to infection with the novel coronavirus SARS-CoV2 has been well studied, however less is known about how, and at what rate, the body recovers post infection. To address this, we have collected repeated blood samples from a large cohort of COVID-19 patients (with disease severity ranging from very mild or asymptomatic infection to that requiring assisted respiratory support) at first symptoms to 9 months post symptom onset. Characterisation of immune system changes over a substantial span of time following a severe viral insult has revealed that the time required for immune-recovery surpasses that taken for symptoms to wane. Furthermore, long-term changes to biological processes are apparent and may hint at prolonged health consequences of SARS-CoV2 infection.
Impact of age, sex and ethnicity on specific and non-specific host responses to the SARS-CoV-2 infection

Abstract

Background: As the pathobiology of COVID-19 is multifactorial, an integrated approach combining patient demographics, routine biochemistry, and markers of specific and non-specific host immune response may provide important insights.

Methods: Demographic and clinical data, treatments and WHO COVID-19 severity scale outcome data were collected from adult patients positive for SARS-CoV-2 within 5 days of hospital admission (n=86). Comparison was made against healthy (n=7) and infected but asymptomatic (n=11) volunteers. Serum samples were analysed for inflammatory mediator levels, SARS-CoV-2 antibody levels and pseudotype neutralisation assays.

Findings: Of 86 patients admitted, 44 patients had mild disease, 22 progressed to critical illness, and 20 subsequently died in hospital. Patients who did not seroconvert at presentation had higher mortality than those who did (p=0.025). Significant differences in serum cytokine levels were seen between survivors and non-survivors for IL-6 (p=0.020), IL-10 (p=0.032), and IP-10 (p=0.030). Notably, male patients had higher cytokine levels than females but no differences were seen with age or ethnicity. Advancing age (adjusted Hazard Ratio 1.064 (1.019- 1.112); p=0.005), lower SpO2:FiO2 ratio (adjHR 0.994 (0.990- 0.998); p=0.004), and days from symptom onset to hospital presentation (adjHR 0.85 (0.728- 0.993); p=0.004) were independent predictors of mortality.

Interpretation: Numerous cytokines were more pronounced in males, which may explain some of the marked sex variances in treatment responses noted in COVID-19 randomised controlled trials. Age, and a more aggressive respiratory illness trajectory but not seroconversion on hospital admission were risk factors for mortality.

Funding: UCLH Biomedical Research Centre, UCL Coronavirus Response Fund, and MRC UKRI.

Abstract Lay Summary

Understanding the immunology of COVID-19 is key to developing treatment options and identifying specific differences between patient groups can help target which patients would benefit from which treatments.

We collected data for 86 patients including demographics (age, sex, ethnicity) and clinical findings (blood tests, observations, inflammatory markers, viral markers) during the first 5 days of hospital admission, and patient outcomes. We compared these with 11 infected but asymptomatic volunteers and 7 healthy controls.

Of the 86 patients, 44 had mild disease, 22 progressed to critical illness, and 20 died in hospital. Patients who did worse had lower levels of natural antibodies against COVID-19, and differences in cytokines including IL-6, IL-10 and IP-10.

Males had higher cytokine levels than females, but no differences were seen with age or ethnicity. Increasing age was also associated with an increased chance of death, as was a higher oxygen requirement and duration of illness. Numerous cytokine levels were higher in males which may account for the gender differences seen in recent randomised trials.
 Importance: For a targeted therapeutic strategy to show outcome benefit, there should be a strong biological and pathogenic rationale to underpin and direct personalised treatments. Relevant biological disease features and biomarkers identify patients for the correct therapeutic, at an appropriate time, dose and duration for maximal efficacy.

Objective: We evaluated whether serum levels of a range of proposed COVID-19 therapeutic targets discriminated between patients with mild or severe disease.

Design, setting and participants: A search of clinicaltrials.gov identified COVID-19 immunological drug targets. We subsequently conducted an observational study investigating the association of serum biomarkers within the first 5 days of hospital admission relating to putative therapeutic biomarkers, with illness severity and outcome.

Main outcomes and measures: We measured levels of ten cytokines/signalling proteins related to the most common therapeutic targets (GM-CSF, IFN-α2a, IFN-β, IFN-γ, IL-1β, IL-1ra, IL-6, IL-7, IL-8, TNF-α), immunoglobulin G (IgG) antibodies directed against either COVID-19 spike protein (S1) or nucleocapsid protein (N), and neutralization titres of antibodies.

Results: 477 randomized trials, including 168 different therapies against 83 different pathways were identified. 86 patients were recruited, 44 (51%) with mild disease and 42 (49%) with severe disease. Six of the ten markers (IL-6, IL-7, IL-8, IFN-α2a, IFN-β, IL-1ra) discriminated between patients with mild and severe disease, although most were similar or only modestly raised above that seen in healthy volunteers. A similar proportion of patients with mild or severe disease had detectable S1 or N IgG antibodies with equivalent levels between groups. Neutralization titres were higher among patients with severe disease.

Conclusions and relevance: Some therapeutic and prognostic biomarkers may be useful in identifying COVID-19 patients who may benefit from specific immunomodulatory therapies, particularly IL-6. However, biomarker absolute values were either appropriately elevated or within the normal range, implying that these immunomodulatory treatments may be of limited benefit.
Abstract

Background: Multisystem inflammatory syndrome in children (MIS-C) is a life-threatening disease occurring several weeks after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. MIS-C shares many clinical features with Kawasaki Disease (KD), a rare childhood vasculitis, but also notable differences. MIS-C therapy is largely based on KD treatment protocols but whether these diseases have the same underpinning immunological perturbations is unknown.

Methods: We undertook a detailed high-dimensional immune characterisation of MIS-C patients presenting at a tertiary paediatric hospital, comparing them to KD patients and healthy children. Using scRNA sequencing, mass cytometry and cytokine analysis we characterised the immunological changes occurring in MIS-C before treatment, after infusion with standard-of-care intravenous immunoglobulin (IVIG) and at time of discharge.

Results: Clinical laboratory data analysis showed neutrophilia in MIS-C patients correlates with inflammation, cardiac dysfunction and disease severity. Acute MIS-C and KD patients shared the same profile of highly activated neutrophils, classical monocytes and CD8+T-cells, and elevated levels of pro-inflammatory (IL6, IL18, IP10, MCP1) but also anti-inflammatory (IL-10, IL1-RA, sTNFR1, sTNFR2) cytokines. In contrast with KD, MIS-C patients had increased CD27- IgD- double-negative B-cells but lacked expansion of intermediate/non-classical monocytes.

Disease recovery in MIS-C and KD patients treated with IVIG was associated with increased CD163+ expression on classical monocytes, appearance of a novel highly immature neutrophil population and decreased levels of pro- and anti-inflammatory cytokines with a transient increased in arginase in a MIS-C and a KD patient.

Conclusions: Our data suggest the immunopathology of MIS-C and KD are fundamentally similar. IVIG treatment was associated with multiple anti-inflammatory changes supporting its use in MIS-C. Acute MIS-C patients possessed elevated levels of IL1-RA and sTNFR, natural inhibitors of IL-1 and TNF-a respectively, suggesting that inhibition of IL-6 may be the preferred option if targeted therapy is required.

Abstract Lay Summary

Multisystem inflammatory syndrome in children (MIS-C) is a new disease that emerged during the COVID-19 pandemic. MIS-C resembles another childhood disease called Kawasaki Disease (KD). Treatment for MIS-C is based on that used for KD but little is known if the same diseases processes are operating in both illnesses. We performed a deep analysis of the immune systems of children with MIS-C, KD and healthy children. We found that both MIS-C and KD have multiple immune features in common during acute illness, although there were some notable differences that could potentially explain important differences between the two diseases. Importantly, we also found that patients with MIS-C or KD behave similarly when treated with the medicine (intravenous immunoglobulin, IVIG) most commonly used to treat both diseases. Our data support using IVIG to treat MIS-C and suggest other agents that could be used in cases where IVIG by itself did not resolve MIS-C symptoms.
Abstract

Background: The potential for anti-interferon autoantibodies to influence the interferon response in severe COVID-19 was highlighted in a recent study by Bastard et al. [1]. A UKRI National Centre for Cytokine Autoimmunity was established to answer several important questions about anti-interferon autoantibodies. Its aim is to further investigate these autoantibodies, including defining their association with gender, ethnicity and clinical manifestations of COVID-19 infection, and changes in their titre over time.

Methods: Study participants were recruited as part of several prospective UK studies, including ISARIC4C, with PCR-confirmed diagnosis of COVID-19. Screened healthcare workers were grouped according to whether they were asymptomatic or symptomatic, and hospital patients classified by the level of respiratory support needed. In addition, age and gender matched controls were recruited, and patients with stored samples from the pre-COVID era identified in the BioResource. All study participants were screened for anti-cytokine antibodies using an established Luminex method [2]. Serum samples positive for anti-interferon antibodies were further analysed with a functional assay to quantify interferon neutralisation, as determined by flow cytometric assessment of STAT1 phosphorylation.

Results: Distinct patterns of anti-cytokine antibody reactivity were revealed in different groups of patients with COVID-19. One of these patterns included the presence of high titres of anti-interferon alpha and/or omega antibodies in a subset of male patients with severe COVID-19, as seen by Bastard et al, but other patterns and differences in kinetics were also seen. An effort to investigate whether these antibodies are linked to genetics or specific ethnicity is ongoing.

Conclusions: Neutralising anti-interferon autoantibodies are found in around 10% of patients with severe COVID-19. Once their impact on pathogenesis has been defined, their removal in some individuals might be of therapeutic benefit.

References


Abstract Lay Summary

COVID-19 has killed around 2.5 million people worldwide and seems to target men and specific ethnic groups. Messenger molecules called interferon are very important for fighting the infection. However, a study showed that around 10% of patients that become very ill from COVID-19, have molecules (called antibodies) that stop interferon from working. Interestingly, around 90% of these patients were men.

A National Centre for Cytokine Autoimmunity has been established to answer important questions about the antibodies against interferon in COVID-19.

Patients with mild to severe COVID-19 were recruited from centres across the UK. Blood and clinical information was collected from the participants, including healthy controls. The blood was screened for antibodies against interferon and the positive samples were further tested for blocking antibodies. Different patterns of antibodies were found in different groups of patients. A subgroup of patients with severe COVID-19 had high levels of antibodies against interferon in their blood. Once the impact of these antibodies on COVID-19 disease has been defined, it might be that their removal could benefit some patients.