

## Considering innate immune responses in SARS-CoV-2 infection and COVID-19

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During the COVID-19 pandemic, much of the media focus has been on adaptive immunity, particularly antibody levels and memory T cells. However, immunologists have been striving to decipher how SARS-CoV-2 infection impacts our first line of defence, namely the innate immune system. In early 2022, Program staff from the NIAID at the NIH organized a workshop focusing on the innate immune response to SARS-CoV-2 infection and during COVID-19, which was chaired by Ralph Baric, Jenny Ting and John Lambris. Following the meeting, *Nature Reviews Immunology* invited some of the organizers and speakers to share their thoughts on the key discussion points.

**Q** A take-home message from the workshop was that many distinct innate mechanisms contribute to COVID-19 pathology. Do we understand why innate pathways are dysregulated in some individuals but not in others during SARS-CoV-2 infection? And what are the therapeutic implications?

**John D. Lambris (J.D.L.)** A common theme arising from the workshop was that the innate immune system, which is normally responsible for thwarting viral infections, becomes deregulated during severe COVID-19. For example, the complement, kinin–kallikrein and coagulation cascades are excessively activated in patients with severe COVID-19. My own group and others have shown that complement C3 deregulation often lies at the heart of this complex pathophysiology, amplifying many detrimental thrombo-inflammatory reactions mediated by platelets, by neutrophils undergoing NETosis (that is, releasing neutrophil extracellular traps) and by the inflamed vascular endothelium<sup>1,2</sup>. This has led to the conduct of clinical trials to evaluate anti-complement agents as stand-alone or combination treatments for COVID-19 (REF.<sup>3</sup>). However, it appears that patients do not all sustain complement deregulation to the same extent, nor do all patients respond uniformly to complement-targeting

therapies. Studies have shown that subtle genetic alterations (that is, SNPs) in complement and coagulation pathways underlie the genetic predisposition towards severe COVID-19 disease in some patients<sup>4</sup>. Additionally, other studies have shown that promiscuous auto-antibodies against type I interferons or other immune mediators can also drive this differential susceptibility to severe pathology<sup>5</sup>. That said, we are still in the early stages of uncovering the genetic and immunological determinants of this population-wide variability. Identifying reliable biomarkers for stratifying patients, applying multi-modal immunotherapies and tailoring the duration of therapeutic intervention will be key to the success of ongoing clinical efforts.

**Michael S. Diamond (M.S.D.)** As John mentions, circulating auto-antibodies that target type I interferons have been identified in several cohorts of patients with severe COVID-19 (REF.<sup>6</sup>), and these individuals show reduced interferon responses after SARS-CoV-2 infection. Remarkably, in some studies, patients with such auto-antibodies account for up to 10% of hospitalizations for severe COVID-19 and 20% of deaths<sup>7,8</sup>. These individuals are thus at substantially increased risk of severe COVID-19, organ failure and poor outcomes<sup>7,9–11</sup>. The therapeutic administration of IFN $\alpha$

will likely be ineffective in most of these patients because of the high levels of neutralizing auto-antibodies against type I interferons in their blood. While plasmapheresis can decrease the titres of such auto-antibodies and is a possible treatment<sup>12</sup>, an alternative and less invasive approach might be administration of IFN $\beta$  as only a smaller subset (~2%) of these patients have antibodies that neutralize this related type I interferon cytokine. Indeed, such a treatment approach was successfully used to prevent SARS-CoV-2 pneumonia in an individual with COVID-19 and type I interferon auto-antibodies<sup>13</sup>.

**John S. Tsang (J.S.T.)** Genetics certainly play a role in determining susceptibility to COVID-19: genetic variants in or near genes involved in viral sensing and innate defence (for example, *IFNAR2*, *OAS1* and *TLR7*) have been found to be associated with disease severity and critical illness<sup>14,15</sup>. These contribute directly to the quality and quantity of early innate responses to the infection. A related observation already noted above is the presence of anti-interferon antibodies in a small fraction of the critically ill patients, especially in men. These antibodies can block interferons and, hence, can potentially increase viral load and disease severity. These data implicate the type I and type III interferon pathways as drug targets. However, interferon therapy has not yielded clear benefits and can even prolong recovery in patients with severe COVID-19 (REF.<sup>16</sup>). The timing of administration likely plays a role as interferons are often given relatively late, that is, after patients arrived at the hospital.

The notion that the innate immune response is a double-edged sword rings true here: a robust response is needed early (to limit viral load and induce robust T cell responses), but an overt late inflammatory response is damaging. Epidemiologically, greater age, male sex and some pre-existing conditions, such as severe asthma, are associated with worse COVID-19 outcomes, including death. The molecular and cellular mechanisms mediating the effects of these risk factors are only beginning to be unravelled, but the innate immune system has been implicated, especially later in the disease course.

**Jenny P. Ting (J.P.T.)** In addition to the innate pathways mentioned already, several speakers at the workshop showed that changes in myeloid cell populations are a feature of severe COVID-19. For example, the presence of myeloid-derived suppressor cells (MDSCs) does not bode well for disease outcome. Catherine Blish showed that higher polymorphonuclear cell counts in the blood are associated with severe COVID-19, while quiescent monocytes are associated with less severe disease<sup>17</sup>. Interestingly, higher MDSC counts in patients with COVID-19 correlate with reduced transcription of cytokines by peripheral monocytes. By contrast, in influenza virus infection, cytokines are typically increased. It is not clear if this difference seen in MDSCs is a cause or effect of severe COVID-19 (REF.<sup>18</sup>). Nir Hacohen presented findings on a population of myeloid cells, identified by single-cell RNA sequencing, that are similar to MDSCs<sup>19</sup>. These 'MS1' cells are increased in patients with bacterial sepsis as well as in patients with severe COVID-19. These cells are thought to be immunosuppressive and may contribute to the reduction in T cell populations that has been seen in severe COVID-19 and sepsis. The presence of granulocytic MDSCs (which are neutrophil-like MDSCs) upon hospitalization with COVID-19 is predictive of increased disease severity. Anna Smed-Sorensen showed that MDSCs are increased in the blood during COVID-19

and their numbers correlate with disease severity<sup>20</sup>. However, an increase in MDSCs in the lungs or in nasopharyngeal or tracheal tissues has not been observed. Blood-derived monocytic MDSCs from patients with COVID-19 can suppress T cells in an arginase 1-dependent fashion, leading to reduced expression of the TCR $\zeta$  chain. It is not clear why some patients have increased MDSCs in the blood. One candidate regulator is IL-6, which is associated with increased MDSC counts, and treatment with tocilizumab (which targets the IL-6 receptor) reduced the numbers of these cells. As well as monocytes and MDSCs, there is evidence for dendritic cell (DC) dysregulation in COVID-19. Stanley Perlman showed that, similarly to humans, young B6 mice are resistant to SARS-CoV-2 infection while aged B6 mice are more susceptible<sup>21</sup>. This was partly due to defective DC migration to the lymph nodes and the subsequently reduced priming of T cells in older mice due to their increased expression of prostaglandin D2 (PGD2). Interestingly, a PGD2 antagonist protected older mice from disease<sup>22</sup>.

John Lambris described the importance of the complement and thrombotic pathways, and these have certainly emerged as key components of the innate dysregulation seen in COVID-19. Dimitrios Mastellos showed that complement-deficient mice are protected from disease induced by SARS-CoV infection and from thrombosis<sup>23</sup>.

A C3 inhibitor, AMY-101, was reported to disrupt tissue factor expression in neutrophils and to reduce NETosis<sup>2</sup>. Sagi Shapira has published a key study on the adverse effects of complement and coagulation on COVID-19 outcomes, which he discussed at the meeting<sup>4</sup>. Richard Flavell presented data using the MISTRG6-hACE2 mouse model, which contains several humanized immune genes, to show that SARS-CoV-2 infects human macrophages, resulting in inflammasome activation and hyperinflammation of the lung<sup>24</sup>. Our own group found that inflammasome activation occurs as a result of myeloid cell–epithelial cell interaction in patients infected with SARS-CoV-2, and that a majority of inflammasome activation in the lungs of patients with COVID-19 occurs in myeloid cells that are not actively infected with the virus. This suggests that the inflammasome can also be activated in uninfected myeloid cells, likely by a bystander effect through virally infected lung epithelial cells. In addition, the interrogation of published single-cell RNA sequencing data shows that increased pan-inflammasome gene expression in myeloid clusters correlates with disease severity. Victor Garcia used mice with engrafted humanized lung, which rendered the mice susceptible to infection with SARS-CoV, MERS and SARS-CoV-2. This induces a strong inflammatory response in the human lung, including NF- $\kappa$ B activation, interferon response, cell death and coagulation, while prophylaxis with the antiviral EIDD-2801 inhibited the pathology<sup>25</sup>. To expand on the cell death mechanism, Thirumala-Devi Kanneganti described the concept of PANoptosis, where inflammatory cell death with features of pyroptosis, apoptosis and necroptosis occurs to cause cell death and inflammation during SARS-CoV-2 infection, leading to pathology<sup>26</sup>.

**Q** *From the opposite end of the spectrum, do we clearly understand yet what a 'good' innate immune response to SARS-CoV-2 looks like?*

**M.S.D.** The innate immune system is a first line of defence against many viruses, including SARS-CoV-2. Innate immune responses, which include type I, type II and type III interferon signalling cascades and interferon-induced genes, limit viral entry, translation, replication and assembly, and accelerate the development of adaptive immunity by providing co-stimulatory signals and upregulating the expression of key proteins (for example, MHC antigens). While few specific interferon-stimulated

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genes (ISGs) have been documented to have direct antiviral activity against SARS-CoV-2, some have been described, including ISG15, which augments MDA5 signalling after recognition of SARS-CoV-2 viral RNA<sup>27</sup>, and LY6E, which appears to block fusion and entry of coronaviruses into cells<sup>28</sup>. Larger-scale genetic screens evaluated the effect of human ISGs on SARS-CoV-2 replication<sup>29</sup>. These studies suggested that SARS-CoV-2 is inhibited by a network of ISGs that may regulate endoplasmic reticulum-associated protein degradation, lipid membrane composition, and vesicular transport and egress. Cellular innate immune responses can also trigger inflammation and programmed cell death that limit viral infection and promote clearance<sup>26</sup>. However, excessive innate immune activation can lead to systemic inflammation and severe disease. Thus, a balance must be achieved that rapidly restricts SARS-CoV-2 infection without resulting in excessive inflammation and tissue injury.


**J.P.T.** The use of the terms ‘good’ versus ‘bad’ to describe the innate immune response has to be exercised with caution. It may depend on the stage and severity of disease, the viral load, the extent of the response, the age of the infected individual, and other factors. These variables have not yet been thoroughly tested in the context of SARS-CoV-2 infection. Thus, a seemingly straightforward anti-viral cytokine, such as type I interferon, can be beneficial during the early stage of infection but becomes pathogenic during severe COVID-19 disease.

However, increased expression of certain chemokines may contribute to a good innate immune response to SARS-CoV-2. Human genome-wide association studies assessing susceptibility to SARS-CoV-2 identified a genetic susceptibility locus in patients with COVID-19 that contains several chemokine receptor genes (including *CXCR6* and *CCR9*)<sup>30</sup>. Using collaborative cross mice, Ralph Baric identified a susceptibility locus on mouse chromosome 9 that encodes the syntenic chemokine receptor genes<sup>31</sup>. His group found that *CXCR6*-deficient mice and *CCR9*-deficient mice show more severe disease in a mouse model of COVID-19, suggesting that these chemokine receptors may have protective functions.

Another innate immune cell we have not yet discussed is the natural killer (NK) cell. Joachim Schultze presented data showing that NK cells have antiviral activity against SARS-CoV-2 but become functionally impaired in severe COVID-19 (REF.<sup>32</sup>).

As already discussed above, type I interferons exert anti-SARS-CoV-2 effects. Published work from several of the presenters at the Workshop, including Michael Diamond and Benjamin tenOever, has shown the importance of interferons for controlling SARS-CoV-2 (REFS.<sup>33,34</sup>). Qian Zhang presented data from human genetic studies showing that inborn errors in TLR and IRF are associated with severe COVID-19 (REF.<sup>35</sup>) and TLR7-deficient plasmacytoid DCs (from patients with deleterious *TLR7* variants) fail to produce interferons upon SARS-CoV-2 infection. However, she pointed out that these are rare variants and cannot explain the large numbers of people who succumb to fatal COVID-19. She highlighted the work from her group and others, already noted above, on how anti-interferon auto-antibodies have been found in ~20% of patients with severe COVID-19 (REF.<sup>34</sup>). These antibodies pre-exist before the development of COVID-19, and their frequency increases with age. All of this helps us to conclude that, at least early in infection, interferon production is important to avoid the development of severe COVID-19.

**J.D.L.** Defining the proper set of immunological markers or baseline immune features that distinguish a ‘good’ from a ‘maladaptive’ innate immune response to SARS-CoV-2 remains a hotly debated topic in the field. In attempting to identify the probable ‘culprits’, we should consider that the timing, hierarchy and functional redundancy of all these responses are of paramount importance. As most of these innate responses are engaged at different times during infection, the readouts we collect after a patient’s admission to the clinic may be largely biased or even misinformative. Furthermore, the magnitude and duration of such responses may vary between individuals due to inherent genetic or immunological traits. Therefore, only through the dynamic and longitudinal monitoring of these responses are we likely to gain insights into those spatiotemporal patterns that are most relevant to the patient’s prognosis or clinical outcome. Additionally, the rigorous genetic testing and immunological phenotyping of individuals who contract the virus but do not develop clinically overt symptoms may help immensely in this direction.

 *Many groups have used systems biology approaches to identify innate immune biomarkers that predict disease severity following SARS-CoV-2 infection. How powerful are these tools and do you*

*think they can realistically be used to guide therapeutic decisions in the clinic?*

**J.S.T.** There are broadly two types of systems biology approaches. I refer to them as ‘top down’ and ‘bottom up’. The premise of the first is that there is much that we do not yet know about a biological process like the innate immune response to COVID-19; therefore, we and many others in the field use unbiased approaches, such as multimodal single-cell profiling and circulating protein analysis, to examine tens and thousands of parameters at once in individual patients over time. These data, together with computational approaches, can lead to new hypotheses, for example, on the molecules and cells involved and their association with disease outcomes. Such top-down approaches have provided a great deal of information, including biomarkers of disease severity and outcomes such as depressed type I interferon signatures and certain dysregulated innate immune cell phenotypes associated with severe disease (for example, low HLA-DR expression and MDSC-like phenotypes in monocytes), as highlighted in presentations from Catherine Blish, Nir Hacohen and Bali Pulendran. These systems studies also pointed to the importance of timing. For example, through multimodal single-cell profiling and circulating protein analysis, a late disease juncture (approximately on days 17–23 post-symptom onset) was identified during which the inflammatory response undergoes a dramatic shift in certain patients with severe disease and fatal outcomes<sup>36</sup>.

While top-down approaches are good at identifying relevant variables, narrowing down to those that truly matter, for example, those that are causally connected to disease outcomes, is challenging. Statistical approaches have been applied to distil a few ‘primary’ correlates of disease severity out of tens and hundreds, with the notion that many correlates are likely bystanders. Interestingly, such analyses inadvertently pointed to the importance of innate immune cells in COVID-19 outcomes, for example, plasmacytoid DCs and NK cells<sup>36</sup>. Top-down systems immunology approaches can lead to clinically actionable patient stratification strategies given the extensive inter-patient heterogeneity. For example, even among hospitalized patients with similar clinical severity scores upon admission, their molecular and cellular profiles can be quite distinct and can be linked to differences in outcomes<sup>36–39</sup>.

The bottom-up systems biology approaches I alluded to earlier aim to

develop quantitative, mechanistic and dynamic models to better understand how the interactions among the underlying variables (including those identified by top-down approaches) can causally lead to the observed behaviours and outcomes.

It also represents a mindset for thinking quantitatively about the emergent behaviour of the system, for instance, how network features, such as feedback and feedback loops, might play a role. Bottom-up approaches have been less attempted in COVID-19 research compared with the top-down approaches because the former are significantly more challenging to develop in practice (some examples include REFS.<sup>40,41</sup>); for example, there are many unknowns, including reaction rates, where the cells are and when they interact. However, bottom-up approaches and thinking hold promise for providing causal, mechanistic understanding and quantitative insights on interventional targets and prevention strategies; for example, quantitative models are needed to better understand how the early innate response may provide feedforward signals to attenuate (or exacerbate) later inflammatory responses. We have qualitative knowledge about potential mediators of these feedforward circuits, such as endogenous glucocorticoids and certain cytokines, but a quantitative understanding of how these signals are integrated by cells to impact clinical outcomes remains a major knowledge gap. I believe some of the key challenges in developing emergent-phenomena quantitative models can be overcome with upcoming advances in measurement technologies, disease models using organoids and animals, and computational methods.

**J.D.L.** A main takeaway from this workshop was the appreciation of the sheer complexity and multi-dimensionality of this disease, extending from the molecular and cellular levels to the tissue and organismic levels. In this regard, multi-omics platforms and systems biology approaches have contributed significantly to our understanding of the pathophysiology of COVID-19 by leveraging large data sets and interrogating potential predictive biomarkers. However, there are still some hurdles to be overcome regarding the limited use of such technologies in tissue compartments other than the blood or bronchoalveolar lavage fluid. While there is potential for leveraging systems biology in clinical decision-making algorithms, we are still far from implementing this scenario in the clinic.

**Q** *What do we know concerning the roles of innate immune sensors, viral pathogen-associated molecular patterns (PAMPs) and host damage-associated molecular patterns during SARS-CoV-2 infection?*

**M.S.D.** Cytosolic RIG-I-like receptors (RLRs), including RIG-I and MDA5, detect non-self RNA PAMPs and trigger a MAVS–TBK1–IRF3 signalling cascade that induces type I and III interferon responses. SARS-CoV-2 is recognized by both RIG-I and MDA5, although the primary RLR sensor used is influenced by post-transcriptional RNA modifications to the SARS-CoV-2 genome. While RIG-I generally recognizes the 5′-end of non-capped RNAs, other motifs may be detected. Indeed, RIG-I reportedly binds the 3′-untranslated region of SARS-CoV-2 RNA through a unique mode that aborts MAVS-dependent signalling and cytokine production<sup>41,42</sup>. Nonetheless, RIG-I binding to the SARS-CoV-2 genome inhibits viral RNA-dependent RNA polymerase and replication. However, recognition of the 3′-end of SARS-CoV-2 by RIG-I may be inhibited by N-6-methyladenosine (m<sup>6</sup>A) post-transcriptional modification<sup>43</sup>. An MDA5-mediated antiviral interferon response against SARS-CoV-2 requires viral replication as the negative-strand RNA is likely the primary recognition target<sup>44</sup>. The SARS-CoV-2 negative-strand RNA may form hairpin structures providing the double-stranded RNA motif typically recognized by MDA5. However, a specific motif of the SARS-CoV-2 viral RNA recognized by MDA5 remains undefined.

**J.P.T.** MDA5 was one of the pattern recognition receptors that was mentioned a few times at the workshop. Bali Pulendran showed that, in mice vaccinated with the Pfizer-BioNTech mRNA COVID-19 vaccine, MDA5 is needed for effective vaccination<sup>45</sup>. This was reinforced by studies by Michael Gale, who showed that MDA5, and not RIG-I, is the important innate immune receptor that responds to SARS-CoV-2 RNA in experiments using cell lines and mice<sup>46</sup>. Qian Zhang mentioned the importance of TLR7 in humans<sup>47</sup> and Benjamin tenOever discussed how the extensive production of double-stranded RNA by SARS-CoV-2 as well as mitochondrial DNA from damaged host cells can serve as PAMPs to amplify inflammation during SARS-CoV-2 infection<sup>48</sup>. Thirumala-Devi Kanneganti showed the importance of TLR2 in mediating adverse inflammatory responses during SARS-CoV-2 (REF.<sup>49</sup>). Our own work

and that of others showed the importance of the inflammasome as a sensor for viral infection. Last but not least, Katherine Fitzgerald described novel findings of an RNA-binding protein, CNBP, that binds viral RNA and attenuates infection by both influenza virus and SARS-CoV-2 (REF.<sup>50</sup>).

**J.D.L.** From a complementologist's perspective, diverse PAMPs originating from both the surface and the interior of the SARS-CoV-2 viral particle have been shown to trigger activation by all three complement pathways. Such PAMPs include surface carbohydrate moieties, viral envelope glycoproteins (for example, spike protein) and the nucleocapsid protein (N-protein)<sup>3</sup>. Of note, the spike protein may fuel complement dysregulation on host surfaces by interfering with the binding and activity of endogenous complement regulators<sup>51</sup>.

**Q** *A full session of the meeting was devoted to the animal models that have been developed to study SARS-CoV-2 infection. One question raised was, why do we need these models when we currently have so many humans infected with SARS-CoV-2 across the globe?*

**J.S.T.** Animal (and human organoid) models are essential. First, models can provide precise timing and tissue information that are otherwise impossible or very challenging to obtain in humans. This is particularly important for studying the early innate response to SARS-CoV-2 as we often only have access to human data after symptom onset and from blood. Animal models also allow us to establish causality directly, for example, by using infection dose, genetics and other 'knobs' to modulate disease severity. Systems biology approaches, both top-down and bottom-up, can be applied to examine which tissues contribute to the blood-based signatures associated with disease severity and outcomes. However, the limitations of animal models, including biological differences between animals and humans, need to be factored into the analysis and interpretation. 'Dirty' and humanized models can help mitigate some of these challenges. Multiple animal and organoid models may also need to be used.

**J.P.T.** Obviously, both human studies and mouse models have their limitations but these two are complementary approaches. Since there are so many infected and vaccinated people, the pandemic provides a unique opportunity where one can establish what an antiviral response or vaccine response looks like as well as the gene expression and



protein expression patterns observed in those with severe versus moderate disease versus those without infection. However, in human studies, it is harder to determine the mechanism of the response, whether the response is beneficial or detrimental, direct or indirect, or is a cause or effect. In mice, one can answer these questions directly. However, gene expression, structure and regulation in mouse tissues are clearly different from that in humans and modelling the severe COVID-19 disease is still difficult to achieve in mice. Humanized mice, such as those described by Wahl et al.<sup>25</sup> and Sefik et al.<sup>52</sup>, where either human cells or tissues are transplanted into immune-deficient mice or where human genes or gene sequences are knocked into mice, will be useful. Other animal models comprising human components will be of interest such as those with transgenic human *ACE2* genes, which have been used to reveal coronavirus pathogenesis for over a decade<sup>53,54</sup>.

**M.S.D.** Animal models will continue to be more important as we move further along the course of the pandemic, especially in terms of understanding the disease pathogenesis of viral variants or vaccine-mediated efficacy against them. As an example, the question of pathogenicity of the Omicron variant of SARS-CoV-2 arose based on observational human studies. However, the initial human studies were complicated by pre-existing immunity in populations due to vaccination or prior infection with earlier variants. Studies in naive rodents by several groups have shown that Omicron causes less pathogenicity in the lower airway<sup>55,56</sup>, which correlates with data suggesting altered use of TMPRSS2 and differential viral infection in certain airway cell types<sup>57</sup> (in preprint<sup>58</sup>).

Another key use of animal models is for therapy and vaccine testing. While studies eventually will have to be performed in human trials, animals help to serve as a down-selection process before the initiation of costly human clinical trials. For vaccines, we may need to learn how the sequence of immunological events (immunization, natural infection, boosting) impacts the breadth, magnitude, and durability of immune responses and whether homologous-matched or unmatched vaccines are necessary to optimize such responses. Such questions can be tested in the controlled setting of animal experiments, but it will become increasingly more difficult to address these questions in human populations as their immunological histories become complex due to prior vaccinations and infections.

**J.D.L.** We should acknowledge the importance of developing humanized mouse models of SARS-CoV-2 infection that closely mimic disease progression in humans — these are valuable platforms for screening potential therapeutics or gaining preclinical proof-of-concept for vaccine leads and for responding to new variants of concern. However, we should also stress that the human disease caused by SARS-CoV-2 is so diversified in its clinical spectrum with systems-wide manifestations that it cannot be emulated to a satisfactory extent by rodent models.

**Q** *There were interesting discussions at the workshop concerning the activation of innate immune pathways in 'bystander cells' and on whether innate immune mechanisms contribute to long COVID. Would you like to finish by sharing your thoughts on these, or any other relevant points?*

**J.P.T.** At the meeting, the term 'bystander' was used to refer to cells that are not infected by the virus, but that can still participate in the ensuing immune response and inflammation. For example, Benjamin tenOever showed that, in hamsters, many of the ISGs expressed following SARS-CoV-2 infection arise from bystanders and not from infected cells. However, an interferon signature in peripheral blood mononuclear cells is found in the absence of infectious virus but in the presence of viral RNA and subgenomic viral RNA. In fact, in tissues where virus is not found, such as the kidneys, expression of ISGs is still observed<sup>48</sup>. I already mentioned our work where SARS-CoV-2-infected primary human epithelial cells drive the activation of the inflammasomes in uninfected myeloid cells — another example of how bystanders may be important for the antiviral response and disease pathogenesis.

**J.S.T.** Given the systemic responses induced by SARS-CoV-2 infection and the dense interaction network among molecules and cells, it is natural to see responses from cells that may not be directly involved in host defence or pathogenesis. That is likely one reason why systems immunology analyses have revealed a sizable number of correlates of disease severity and outcomes. As discussed above, distilling those down to the primary correlates and causal subnetworks is an important goal of systems biology analysis.

The clinical and immunological definitions of long COVID are currently being worked out by the research community.

What has been emerging is that, what is currently classified as 'long COVID', may in fact reflect several phenomena with distinct underlying mechanisms. This is consistent with the fact that COVID-19 can affect multiple organs and systems throughout the body. Recent data suggest that innate immune mechanisms could be involved. For example, single-cell studies of patients who have recovered from COVID-19 have revealed potentially persistent changes in the transcriptional and chromatin accessibility landscape in their monocytes. It remains to be seen whether these changes can last longer than a month or two after acute disease and, if so, whether they are associated with long COVID symptoms.

We have recently conducted a systems immunology study of individuals who, on average, were 6-months recovered from mild COVID-19 (that is, non-hospitalized) but were otherwise healthy (published in preprint<sup>59</sup>). We also assessed their innate and adaptive responses to vaccination — not by using one of the COVID-19 vaccines but by using the seasonal influenza vaccine to assess whether prior COVID-19 can shift immune set-points to the extent that responses to a heterologous vaccine challenge could be impacted in an antigen-agnostic way. The answer was yes but it depends on the sex. We found numerous sex-specific innate immune-related differences, both before (at baseline) and after influenza vaccination between patients who had recovered from COVID-19 and matching healthy individuals who never had COVID-19. Whether these imprints are linked to long COVID symptoms remains to be determined, but these data suggest that COVID-19 can establish new antigen-agnostic immunological set points with functional consequences. These observations are consistent with the concept of 'trained innate immunity' pioneered by Mihai Netea and colleagues as well as with observations by Peter Aaby, Christine Benn and colleagues of the antigen-agnostic protective effects of live vaccines like BCG early in life.

**J.D.L.** We are only now beginning to 'scratch the surface' of the complex pathophysiology of long COVID. There are several indications that an insidious, systemic inflammatory response remains persistently active in patients that recover from acute COVID for many months to come<sup>60</sup>. However, we are still in an infant stage with regard to defining the specific mechanisms by which innate immune pathways, such as complement, contribute to the clinical course of these long haulers.

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#### Competing interests

M.S.D. is a consultant for Inbios, Vir Biotechnology, and Carnival Corporation and is on the Scientific Advisory Boards of Moderna and Immunome. The Diamond laboratory has received unrelated funding support in sponsored research agreements from Vir Biotechnology, Moderna and Emergent BioSolutions. J.D.L. is the founder of Amyndas Pharmaceuticals, which is developing complement inhibitors for therapeutic purposes; is the inventor of patents or patent applications that describe the use of complement inhibitors for therapeutic purposes, some of which are being developed by Amyndas Pharmaceuticals; is the inventor of the compstapin technology licensed to Apellis Pharmaceuticals (Cp05/POT-4/APL-1 and PEGylated derivatives such as APL-2/pegcetacoplan and APL-9). The other authors declare no competing interests.

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