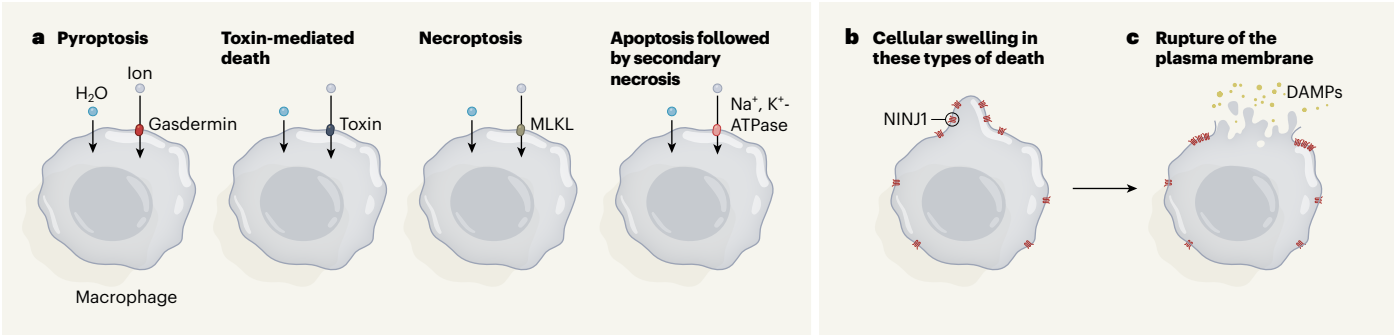

Figure 1 | Regulated rupture of the plasma membrane is an end point of multiple cell-death pathways. a, Human cells, such as macrophages, can die by a range of mechanisms, including pyroptosis, toxin-mediated death, necroptosis and apoptosis (followed by a process called secondary necrosis). A common feature of these deaths is an increase in cellular osmotic pressure, presumably arising from an ionic imbalance that drives water entry. This imbalance is triggered by ion movement through protein channels or pores, such as those formed by gasdermin proteins, toxins or MLKL channels. In apoptosis, inactivation of the Na^+, K^+-ATPase enzyme causes ion accumulation in dying cells[10]. **b**, The water entry causes cellular swelling, and bubble-like protrusions form. Kayagaki *et al.*[1] report that rupture of the plasma membrane in these types of dying cell does not occur passively, as previously thought. Instead, it is an active process that requires the protein ninjurin-1 (NINJ1). **c**, To mediate rupture of the plasma membrane, NINJ1 aggregates (oligomerizes). This rupture releases cytoplasmic content, including molecules called damage-associated molecular patterns (DAMPs), which trigger inflammation in neighbouring tissue.

of apoptotic cells. Secondary necrosis occurs if apoptotic cells are not engulfed and removed in a timely manner. Thus, NINJ1 is a common denominator at the end of many cell-death pathways.

NINJ1 is ubiquitously expressed[8], and is evolutionarily conserved, from fruit flies to humans. How might this relatively small (16 kilodaltons) protein mediate such striking effects? Its structure is predicted to contain two transmembrane helices, as well as an evolutionarily conserved extracellular helix that is needed for NINJ1 to function properly. Working out whether this helix senses a signal or serves to disrupt the membrane during cell death will require more study. Of note, this helix seems to have a mixed hydrophobic and hydrophilic (amphiphilic) character, a property similar to that of the helices found in other membrane-disrupting proteins, such as melittin or BAX.

Importantly, Kayagaki and colleagues' findings will transform cell biology in a way that goes beyond just revealing NINJ1's function. Their study underscores the enormous strength and resilience of the intact plasma membrane. It also reduces the number of events in cell biology considered to be non-specific, highlighting how stringently organisms control the fate of their cells until the very last moment of cellular existence.

Many questions remain to be answered. What signal or property is sensed by NINJ1 to activate its function in dying cells? What mechanisms, if any, exist to prevent accidental activation of NINJ1? It would be interesting to know whether NINJ1 requires other factors when mediating membrane rupture. Do other proteins with a similar function exist? And, of course, what is the structure of the membrane-rupturing entity that NINJ1 presumably forms?

Answering these questions might lead to new therapeutic strategies aimed at inhibiting NINJ1, or related proteins, that could convert necrotic death to a type of death with a less inflammatory outcome. Such treatments would thereby reduce the general level of inflammation in tissue, presumably with positive effects for chronic or acute inflammatory disorders.

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Coronavirus

Surprising effects of antibodies in severe COVID

Matteo Gentili & Nir Hacohen

Defects in the immune defences induced by the protein interferon are associated with some severe cases of COVID-19. An analysis of patients' blood samples sheds light on how antibodies might contribute to these defects. **See p.124**

Infection with the SARS-CoV-2 virus can lead to diverse outcomes, ranging from no symptoms to varying degrees of disease severity, spanning mild illness to death. What determines the degree of severity is unclear, but mounting evidence points to exacerbated and abnormal responses in the innate branch of the immune system as a main driver of major illness. Combes *et al.*[1] present a study on page 124 investigating the hallmarks of COVID-19 severity.

The authors analysed cells, including immune cells, in blood samples from 21 people with COVID-19 and 25 uninfected individuals who were either healthy or had a lung injury or breathing difficulties. They monitored gene expression during the course of the infection as patients went on to develop either what was categorized as mild–moderate COVID-19 (which required a short hospital stay without the need for intensive care or mechanical ventilation) or severe COVID-19 (requiring intensive care and mechanical ventilation). The authors found that the cells of people with

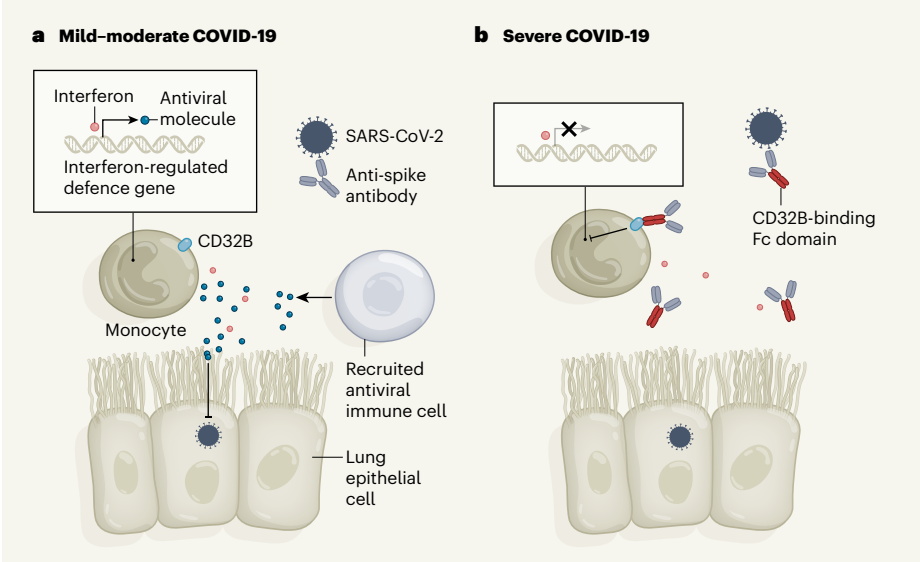

Figure 1 | Antibodies that affect immune defence in severe COVID-19. Combes *et al.*[1] analysed blood samples from people who had COVID-19 of differing degrees of severity. **a**, In what the authors classify as mild–moderate cases of this illness, patients made antibodies that recognize the spike protein of the SARS-CoV-2 virus. The authors report that immune cells called monocytes from these individuals express genes that are regulated by the protein interferon. These genes encode molecules that aid antiviral defence by boosting immune responses or by suppressing the virus at sites of infection, which might include the epithelial cells that line the lungs. The CD32B receptor, which is found on monocytes, can dampen such immune responses if it is bound by another protein. **b**, The authors report that individuals with severe COVID-19 have anti-spike antibodies in which a region of the antibody, called the Fc domain, binds to CD32B. This interaction hinders the expression of interferon-regulated genes.

mild–moderate COVID-19 expressed a distinct set of genes whose expression depends on what are known as type I interferon proteins. Interferons, molecules that are also called cytokines, drive the expression of genes that have a role in antiviral defence.

This interferon-regulated gene-expression signature was not observed in the cells of people with severe COVID-19. Instead, the cells had a gene-expression signature described as an inflammatory S100A12 myeloid-cell program (S100A12 is a protein expressed as part of this program). A S100A12 signature was previously identified[2] as being associated with severe COVID-19. Interestingly, a similar program is associated with another form of severe disease called sepsis, which derives from an aberrant immune response to bacterial infection[3].

An interferon-regulated gene-expression program can be crucial to defence against viral infection, so the lack of activation of this program in people with severe COVID-19 provided a hint that defective initiation of this pathway might contribute to the observed differences in disease severity. Combes *et al.* therefore set out to determine the reason for the differences. The first obvious suspect was the level of an interferon protein (IFN-α) in blood plasma (blood lacking its cellular content). The authors found no notable difference in IFN-α levels with differing disease severity. However, there are other types of interferon protein that the authors did not measure.

The authors next turned their attention to antibodies. Antibodies against SARS-CoV-2 have a protective role in the natural immune response to this virus, and antibodies targeting the virus have been used as COVID-19 treatments. Indeed, part of the rationale for using the vaccines currently available is to drive the generation of such antibodies. The authors found that the level of antibodies against the SARS-CoV-2 spike and nucleocapsid proteins was higher in people with severe disease than in those with mild–moderate COVID-19. Moreover, high antibody levels were negatively correlated with the presence of cells expressing an interferon-regulated gene-expression program.

To search for a missing link between antibody levels, interferon and COVID-19 severity, Combes and colleagues used an *in vitro* system. They took immune cells from the blood of healthy people, and exposed them to blood plasma samples from people with mild–moderate or severe COVID-19. The authors then stimulated the immune cells with IFN-α, to determine whether an antiviral response developed. They found that the presence of plasma from people with severe disease blocked the induction of interferon-responsive genes. However, if this plasma was treated to deplete it of antibodies, interferon-mediated gene expression was restored in these immune cells.

Intriguingly, in a previous study[4] of 987 individuals with severe COVID-19, 135 (13.7%) had anti-interferon antibodies that could blunt the induction of interferon-induced genes. However, given that most individuals in that study did not have anti-interferon antibodies, the presence of such antibodies alone could not fully explain the development of severe COVID-19. Indeed, Combes *et al.* found anti-interferon antibodies at a similarly low frequency in the samples they had obtained from patients.

In an attempt to explain the enigma of a dampened interferon response in severe COVID-19, the authors considered various aspects of antibody function. An antibody consists structurally of two functional units: a variable region that recognizes the disease-causing agent and a constant region (termed Fc) that engages Fc receptors on the surface of immune cells (Fig. 1). This latter interaction can help to shape the immune response. During the course of a disease, the characteristics of the antibodies produced change to regulate immune defences. One aspect of these changes is an alteration in the antibody Fc component that affects which Fc receptors are engaged. For example, engagement with the Fc receptors CD64, CD16 and CD32 can regulate how the immune system eliminates bacterial and viral infections[5].

Combes and colleagues investigated whether Fc-receptor engagement has a role in blunting interferon-mediated responses in severe COVID-19. Using immune cells from healthy donors exposed to IFN-α and plasma from people with severe COVID-19, they individually blocked CD64, CD16 and CD32 Fc receptors. Only CD32 blockade enabled the expression of interferon-regulated genes.

The CD32 Fc receptor exists in two forms – CD32A and CD32B. CD32A engagement activates the immune system, whereas CD32B dampens immune responses[6]. Combes and colleagues showed that the inhibition of interferon-regulated gene expression was due to CD32B. They thus conclude that people with severe COVID-19 develop antibodies that engage with CD32B Fc receptors and thereby blunt interferon-mediated defence responses.

In support of this conclusion, a previous study[7] demonstrated that people with moderate and severe COVID-19 develop a diverse antibody response in terms of the Fc regions recruited, with the presence of spike-specific antibodies that engage CD32B being a main predictor of disease severity. What determines this difference in antibody type between severe and moderate COVID-19 remains to be discovered.

It is tempting to speculate that changing the Fc domain to one that engages CD32B is a mechanism used by the immune system to shut down an intense immune response to SARS-CoV-2. It would be interesting to investigate whether such mechanisms are involved in other types of viral infection, and, if so, whether they have a detrimental or beneficial

role. Of note, there are also reported examples of SARS-CoV-2 infection generating antibodies that turn against the host. People with COVID-19 can develop antibodies that target nucleic acids[8] and host proteins[9].

It is important to remember that we do not yet know whether, in people with severe COVID-19, this antibody-mediated phenomenon is detrimental (by suppressing a natural antiviral pathway, allowing uncontrolled virus replication) or beneficial (by reducing toxic effects of a potent antiviral response). That said, these results raise the possibility that therapy to block CD32B would partially restore interferon responses in people with severe disease. However, before considering therapeutic applications, the following steps should be taken. These results need to be confirmed in a larger group of patients, the process should be examined in other types of tissue in which the virus is found (rather than just in blood samples), and a fuller explanation is needed of the mechanisms that underlie these findings.

With several anti-SARS-CoV-2 vaccines currently approved, it will be useful to determine the antibody profile that vaccination elicits, and to compare it with the profile that develops during SARS-CoV-2 infection. Such a comparison would help to reveal the checks and balances used by the immune system to help keep us alive during severe infection.

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Biochemistry

Dynamics of RNA–protein binding probed in cells

Olivia S. Rissland

An understanding of how quickly biomolecules bind and dissociate in cells is crucial for developing quantitative models of biology, but measurements of these kinetics were possible only using purified proteins *in vitro* — until now. **See p.152**

As the geneticist Theodosius Dobzhansky stated[1] in 1973, "Nothing in biology makes sense except in the light of evolution." Many modern biologists might add that nothing in molecular biology makes sense except in the light of biochemistry — without the quantitative understanding that biochemistry provides, how can biologists predict the effect of a twofold reduction in the levels of a protein during the early development of an organism, or of a tenfold increase in the concentration of another protein in cancer cells? The chasm between the streamlined experiments of biochemistry and the messy complexity of the cell has long seemed unbridgeable. Now, on page 152, Sharma *et al.*[2] report a technique that enables the biochemical analysis of molecular interactions in cells.

The authors focused on the dynamics of interactions between RNA molecules and proteins. Messenger RNA molecules are bound by various RNA-binding proteins (RBPs), which control almost every aspect of the mRNA life cycle — from the initial processing of newly made RNAs to their eventual destruction[3]. Each RBP can bind to hundreds of RNA molecules, and, in turn, each RNA can be bound by dozens of different RBPs[4]. Moreover, RNA–protein interactions are not static[5,6]. Instead, proteins can rapidly bind to their target RNAs and just as rapidly dissociate from them (Fig. 1), and these dynamics are at the core of gene regulation. In other words, the kinetics of RNA–protein interactions are a driving force of gene expression. Defining the parameters of these kinetics in cells is therefore crucial for fully understanding the regulation of gene expression.

Although RNA–protein interactions have been investigated for decades, their kinetics in cells have not been characterized. Broadly speaking, kinetic insight has been available only from *in vitro* studies using purified proteins; experiments in cells have been able to identify the RNA targets of RBPs, but lacked the precision to measure the kinetics of the interactions[5]. With the advent of high-throughput sequencing methods, *in vitro* approaches can now probe the kinetics of a protein's interactions with tens of thousands of RNA variants[7]. But these experiments are still carried out on purified proteins in the absence of the cellular milieu. In the past few years, a method called crosslinking and immunoprecipitation[8] (CLIP) has become a workhorse for the characterization of RNA–protein interactions in cells. In CLIP, a protein in complex with an RNA molecule is covalently crosslinked to the RNA using ultraviolet light; the complexes are then isolated and the crosslinked RNA is identified by high-throughput sequencing. This approach provides a catalogue of RNAs that bind to a specific RBP in the complex environment of the cell, but it provides, at best, only a snapshot of these interactions.

Sharma and colleagues now bridge the gap between *in vitro* strategies and CLIP by developing a type of CLIP that can determine the kinetic parameters of RNA–protein interactions in cells. The authors' key insight was that certain technical aspects of previously reported CLIP methods precluded such approaches from being useful for capturing kinetic parameters. The most challenging limitation is that crosslinking rates must be rapid to capture the rates at which proteins and RNA molecules associate and dissociate. Conventional UV sources cannot achieve sufficiently rapid crosslinking, and so using

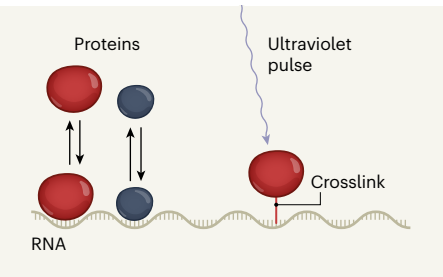

Figure 1 | A method for probing RNA–protein interactions in cells. Proteins that act on RNA molecules rapidly associate and dissociate from their target binding sites. Measurements of the rates of association and dissociation are needed for a quantitative understanding of gene regulation, but have been impossible to do in living cells. Sharma *et al.*[2] describe a method called KIN-CLIP that uses ultrafast pulses of ultraviolet light to generate covalent crosslinks between the bound proteins and RNA molecules in cells. This not only allows the RNA targets of the proteins to be identified (as was possible in previously reported crosslinking techniques), but, owing to the rapidity of the crosslinking process, also allows the kinetics of association and dissociation to be determined.