Medical research

Blocking cell death limits lung damage by influenza

Nishma Gupta & John Silke

Animals that receive an inhibitor of an antiviral cell-death response called necroptosis are less likely to die of influenza even at a late stage of infection. This has implications for the development of therapies for respiratory diseases. See p.835

If there is one lesson to be learnt from the pandemic, it is that we need to be better prepared for the next one. Influenza pandemics have killed millions in the past and could do so again. In contrast to the coronavirus SARS-CoV-2, some influenza strains have proved to be particularly lethal to younger, ostensibly healthy adults, possibly because they provoked such a strong inflammatory response in that group. On page 835, Gautam *et al.*[1] show that by inhibiting a cell-death response called necroptosis — an important innate antiviral strategy — the strength of the inflammatory response in an animal model of severe influenza infection is reduced, substantially reducing mortality.

Although it might seem obvious that an aggressive inflammatory response unleashed by the innate branch of the immune system will restrict viral growth, in lung infections the strength of the response and levels of key inflammatory molecules called cytokines are, in fact, among the clearest predictors of severe disease[2]. Intuitively, it might be supposed that this is because the strength of the inflammatory response reflects the amount of virus present (the viral load). However, viral load in samples from respiratory tissue is not a reliable predictor of disease severity[2].

The predictive power of a strong inflammatory response probably results from the fact that, in addition to helping to fight the infection, the inflammatory response can interfere with the functioning of the infected tissue. This indicates a need to maintain a fine balance between these two outcomes. The occurrence of an excessive and destructive inflammatory response, or 'cytokine storm', has been discussed in the context of COVID-19. The current study not only shows that necroptosis can drive inflammation, but also provides evidence consistent with the hypothesis that a cytokine storm is a cause, rather than a correlate, of severe disease.

Viruses require a living host cell in which to replicate, and therefore cell suicide in response to infection is a particularly effective antiviral strategy. A mechanism called apoptosis, which is mediated by enzymes called caspases, is the favoured cell-death response. This is probably because dying cells that undergo apoptosis are rapidly taken up by other cells, preventing the release of their cellular contents into the surrounding tissue and thereby enabling tight regulation of the inflammatory response. Viruses have evolved strategies to block apoptosis, which, in an evolutionary arms race, has necessitated a counter-response from the host. One such strategy is necroptosis — a type of cell death in which a dying cell breaks open — that is usually activated in response to caspase inhibition. However, necroptosis seems to come with the risk of an inflammatory response that causes more tissue damage than does cell death by apoptosis.

Necroptosis is triggered by similar stimuli to those that elicit apoptosis. These include signs of viral infection, such as the presence of viral RNA. In an influenza infection, the host protein ZBP1 binds to a type of viral RNA called Z-RNA and activates RIPK3, a type of enzyme called a kinase that can add phosphate groups to proteins, a process known as phosphorylation. RIPK3 then activates the apoptotic pathway, in a kinase-independent manner, through the protein caspase-8. During an influenza infection, in a deviation from the normal sequential process of necroptosis activation, which occurs only if apoptosis is inhibited, activated RIPK3 phosphorylates and activates a protein needed for necroptosis, the pore-forming molecule MLKL (Fig. 1).

Despite this dual activation of cell-death pathways, the apoptotic pathway is sufficient to restrict viral growth and support a normal immune response[3], which makes blocking necroptosis by inhibiting the kinase activity of RIPK3 an attractive therapeutic target during influenza infection. Unfortunately, inhibition of RIPK3 kinase activity, whether by small molecules or genetically, often has the disadvantage that it also activates apoptosis[4,5], which has dampened enthusiasm for this strategy. Gautam *et al.* report the development of a potent RIPK3 inhibitor called UH15-38, which, at low doses, inhibits necroptosis but does not activate apoptosis. Furthermore, the inhibitor does not show any off-target activity on other inflammatory signalling pathways, nor does it inhibit closely related kinases.

Daily injections of UH15-38 were well tolerated by mice, with no obvious indications of toxicity and, crucially, the inhibitor was highly effective in reducing mortality in models of influenza infection. Moreover, UH15-38 didn't increase the survival of infected mice that lack RIPK3 or MLKL, indicating that the effect of the inhibitor is due to on-target activity. UH15-38 also protected mice much more effectively than did another RIPK3 inhibitor, which actually increased disease severity, possibly owing

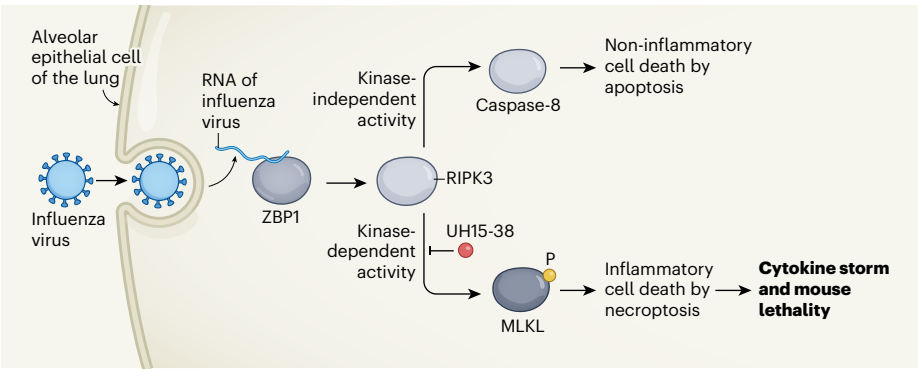

Figure 1 | An inhibitor molecule that prevents lung injury associated with influenza infection. Alveolar epithelial cells, which line the surface of the lungs, can die as part of a defensive response to prevent viral spread. The RNA of influenza virus is recognized by the protein ZBP1. This can activate the protein RIPK3, which is a type of enzyme called a kinase that can add phosphate (P) groups to target proteins such as MLKL. RIPK3 can function in a kinase-independent manner to activate the protein caspase-8, triggering a type of non-inflammatory cell death called apoptosis. RIPK3 can also launch, in a kinase-dependent manner, a more destructive type of cell death called necroptosis. This is associated with destructive inflammation and the release of inflammatory molecules (not shown) that generate what is termed a cytokine storm. Gautam *et al.*[1] report that an inhibitor of RIPK3's kinase activity, called UH15-38, can block necroptosis associated with influenza infection, reduce tissue damage and prevent lethality in mice.

to the inhibitor having a secondary ability to induce apoptosis in uninfected cells.

One of the interesting findings reported by the authors is that after the onset of infection, a particular type of lung cell called a type I alveolar epithelial cell contained around 80-fold more viral RNA than did any other type of lung cell. This fits with previous observations that destruction of this particular cell type, beginning at a threshold of destruction of 10% of these cells, is correlated with loss of lung function and lethality[6]. Consistent with the effects of UH15-38 and the potential importance for disease treatment using UH15-38, these cells express all of the required necroptotic machinery and, on infection, MLKL becomes phosphorylated, a process that can be blocked by UH15-38. By contrast, activation of caspase-8 and caspase-3 is unaffected by UH15-38.

The most striking finding presented by the authors is that UH15-38 works for at least 5 days post-infection. In earlier studies in mice, antivirals approved for use in the clinic, such as oseltamivir and zanamivir, worked best when delivered before infection (prophylactically) and did not provide notable protection if delivered 48 hours after infection commenced[7,8]. These drugs are therefore usually recommended only for at-risk patients within 48 hours of the first signs of symptoms. It would be interesting if the two types of inhibitor were tested head-to-head to determine whether the superiority of UH15-38 can be confirmed and whether the findings have relevance for clinical treatments.

Is UH15-38 particularly effective in influenza because it accumulates in the lung or because the lung is particularly susceptible to necroptosis? Both are possible. Gautam and colleagues report that the level of UH15-38 in the lung is eightfold higher than the level in blood plasma. There have been a number of reports regarding other lung conditions, including chronic obstructive pulmonary disease and asthma, in which necroptosis has been shown, at least in mouse models, to contribute to disease severity[9]. Conversely, a paper examining the role of necroptosis in SARS-CoV-2 infection, on the basis of studies of lethal infections in mice lacking MLKL, indicates that necroptosis has no role in disease severity[10], suggesting that inhibiting necroptosis will not be a panacea for all respiratory diseases.

Using this new RIPK3 inhibitor to tackle influenza infections therefore strikes a balance, reducing the force of the inflammatory response but sustaining its antiviral effect. We eagerly await clinical trials that could help with the next pandemic.

Nishma Gupta and **John Silke** are in the Inflammation Division at the Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3052, Australia.
e-mails: silke@wehi.edu.au; gupta.n@wehi.edu.au

1. Gautam, A. *et al. Nature* **628**, 835–843 (2024).
2. Flerlage, T., Boyd, D. F., Meliopoulos, V., Thomas, P. G. & Schultz-Cherry, S. *Nature Rev. Microbiol.* **19**, 425–441 (2021).
3. Nogusa, S. *et al. Cell Host Microbe* **20**, 13–24 (2016).
4. Mandal, P. *et al. Mol. Cell* **56**, 481–495 (2014).
5. Newton, K. *et al. Science* **343**, 1357–1360 (2014).
6. Sanders, C. J. *et al. Am. J. Physiol. Lung Cell. Mol. Physiol.* **304**, L481–L488 (2013).
7. Bantia, S. *et al. Antimicrob. Agents Chemother.* **45**, 1162–1167 (2001).
8. Leyva-Grado, V. H. & Palese, P. *Antivir. Res.* **142**, 12–15 (2017).
9. Pierotti, C. L. & Silke, J. *Immunol. Cell Biol.* **100**, 79–82 (2022).
10. Bader, S. M. *et al. Cell Death Dis.* **15**, 100 (2024).

J.S. declares competing interests; see go.nature.com/3u7c0nn for details.
This article was published online on 10 April 2024.

Biomechanics

The insect-wing hinge comes into focus

Tanvi Deora

The hinge enables insects to control their wing movements, but how it works is hard to study. Multidisciplinary research, using imaging and machine-learning methods, now sheds light on the mechanism that underlies its operation. **See p.795**

Winged insects, including butterflies, wasps and beetles, are some of the most successful animals on the planet, in terms of numbers of species and of individuals. Part of this success comes from their ability to fly and from the evolution of wings, which have evolved as a new type of appendage, independently of limbs. The wing is connected to the insect body through an exquisite hinge. Although the wing hinge is an important joint, its small size, its fast movement and researchers' inability to directly observe it have made understanding how it works difficult. On page 795, Melis *et al.*[1] go a long way to solving this riddle.

Insects such as flies and bees flap their wings hundreds of times a second to perform extremely rapid, yet controlled, flight manoeuvres. These animals have evolved specialized muscles and body appendages that enable such high-frequency wing movements[2]. Wing motion is powered by a set of muscles called the indirect flight muscles, which do not attach directly to the wings, but instead attach to and deform the insect's exterior surface — its exoskeleton. These deformations are transmitted to the wing by the hinge, a complex joint that consists of a series of tiny, hardened structures known as sclerites (Fig. 1). Each sclerite transmits force to its neighbour — in a way reminiscent of a series of gears — thereby transforming tiny exoskeletal deformations into large back-and-forth wing movements.

Small steering muscles, also called direct flight muscles, attach to sclerites and apply force directly to them to fine-tune the wing movements on a stroke-by-stroke basis. Therefore, the hinge functions not only as a flexible joint between the wing and the body wall of the thorax, but also as an 'organ' with several independent elements (the sclerites). Of these, four, studied by Melis *et al.* in the fruit fly *Drosophila melanogaster*, are connected to a dozen direct flight muscles that together drive the varied wing movements.

Understanding how the joint functions is difficult, because the hinge and its associated muscles are internal structures that can't be observed directly in an insect with flapping wings, and the high frequency of wing beats further complicates matters. As a result, the key questions of how muscle activity generates sclerite movement and, as a consequence, causes changes in wing motion have been challenging to address. Melis and colleagues used an innovative approach to examine the neuroanatomical basis of how the wing hinge functions. The authors recorded the calcium activity (a readout of the cellular activity) of the 12 muscles associated with 4 sclerites and mapped this information onto the fly's wing movements, using machine learning. Their strategy thus provides a glimpse of the potential contribution of individual sclerite–muscle groups to wing motion.

The fruit fly wing hinge has conventionally been studied in dissected tissue in which physical force is applied to each observable muscle and the subsequent effect on wing movement is recorded[3]. Such experiments are, by design, limited to providing results consisting of static interpretations. Although some researchers have recorded muscle activity in live insects, such as blowflies[3,4], and have provided quantitative insights[4,5] into the function of individual muscles, the effect of the collective action of all wing muscles remains unknown.