Journal Club

PREPRINT WATCH

OMICRON ENTRY ROUTE

SARS-CoV-2 Omicron has rapidly become the dominant variant worldwide. Omicron has 30 mutations in the spike protein compared to the ancestral Wuhan-Hu-1 strain, and recent studies have detailed how these mutations contribute to viral escape from antibody responses in convalescent or vaccinated individuals. However, whether mutations in Omicron spike protein affect virus entry into host cells and host tropism has yet to be explored.

Three recent preprints (not peer-reviewed) have sought to functionally characterize Omicron in comparison to previous variants of concern. Key findings from Meng et al., using a pseudotyped virus, are that Omicron replicates less efficiently in lung organoids and lung epithelial cells compared with the Delta variant and with Wuhan-Hu-1. Peacock et al. and Willett et al. also reported significantly lower viral copy numbers following Omicron infection of lung epithelial cells compared with Delta or Wuhan-Hu-1. However, Peacock et al. also noted an increase in viral copy number in Omicron-infected human nasal airway epithelial cells. These findings hint at a mechanism that could contribute to increased transmissibility of Omicron, as well as its apparent reduced disease severity.

All three studies conclude that Omicron has a reduced ability to induce syncytia in tissue culture, which potentially has clinical significance because syncytia formation has been associated with increased disease severity. Syncytia formation usually requires viral infection through membrane fusion involving TMPRSS2. The low rate of syncytia formation with Omicron infection suggests that it may have switched to using endosomal fusion through cathepsins instead. Confirming this, Willett et al. found that infection with pseudotyped Omicron virus was reduced in cells expressing high levels of TMPRSS2 but increased in cells that only support endosomal entry. Furthermore, by blocking TMPRSS2-mediated cell-surface fusion and/or cathepsin-mediated endosomal fusion, Willett et al. and Peacock et al. determined that Omicron can use both entry routes but prefers endosomal fusion to cell-surface fusion. The ability to infect cells by both routes considerably increases the number of cell types that Omicron can infect.

Finally, Peacock et al. observed that Omicron can use ACE2 receptors from a larger range of host species than other variants, including mice and domestic poultry. This raises the possibility that SARS-CoV-2 could form a long-term reservoir in a new animal host for future human outbreaks.

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ORIGINAL ARTICLES Meng, B. et al. SARS-CoV-2 Omicron spike mediated immune escape, infectivity and cell-cell fusion. Preprint at *bioRxiv* https://www.biorxiv.org/content/10.1101/2021.12.17.473248v2 (2021) | Peacock, T. et al. The SARS-CoV-2 variant, Omicron, shows rapid replication in human primary nasal epithelial cultures and efficiently uses the endosomal route of entry. Preprint at *bioRxiv* https://www.biorxiv.org/content/10.1101/2021.12.31.474653v1 (2022) | Willett, B. et al. The hyper-transmissible SARS-CoV-2 Omicron variant exhibits significant antigenic change, vaccine escape and a switch in cell entry mechanism. Preprint at *medRxiv* https://www.medrxiv.org/content/10.1101/2022.01.03.21268111v1 (2022)
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INNATE LYMPHOID CELLS

Intestinal barrier protection

Tumour necrosis factor (TNF)-induced cell death is both a driver and consequence of chronic inflammation in many settings, including inflammatory bowel disease (IBD), and thus is a major therapeutic target of interest. A study by Zhou et al. investigates the mechanisms that protect the healthy intestine from TNF and shows a role for an epidermal growth factor (EGF) family mediator produced by group 3 innate lymphoid cells (ILC3s).

In various mouse models of ILC3 deficiency and reconstitution, high-dose delivery of recombinant TNF in vivo induced significantly greater levels of intestinal epithelial cell death in the absence of ILC3s. ILC3s are well known to protect epithelial barrier tissues through multiple pathways induced by IL-22, but this effect on TNF-induced cell death was shown to be independent of IL-22.

Using single-cell RNA-sequencing of purified ILCs from the small intestine of TNF-treated mice, the authors showed that ILC3s had upregulated expression of *Hbegf* in response to TNF. *Hbegf* was dominantly expressed by ILC3s in the mouse intestine compared with other lymphoid and myeloid populations, in particular by CCR6$^+$ ILC3s. Recombinant heparin-binding EGF-like growth factor (HB-EGF) significantly reduced TNF-induced death of an intestinal epithelial cell line in vitro. By contrast, in mice with selective knockout of *Hbegf* in ILC3s, administration of recombinant TNF led to significantly increased intestinal epithelial cell death. Thus, ILC3-derived HB-EGF protects the intestine from the damaging effects of TNF.

Further experiments showed that TNF does not directly upregulate *Hbegf* expression. Rather, TNF acted through IL-1β production to upregulate the expression of *Ptgs2* (encoding COX2) by ILC3s. *Ptgs2*, together with constitutive expression of *Ptges* by

T CELLS

Magnesium: essential for T cells

Magnesium deficiency is linked to various diseases, including infection and cancer, prompting Christoph Hess and colleagues to explore its effect on T cell function. Reporting in *Cell*, they show that extracellular Mg^{2+} is directly sensed by the co-stimulatory molecule LFA-1 on CD8$^+$ T cells, leading to outside-in signalling and augmented T cell activation and effector functions. Accordingly, Mg^{2+} sufficiency supports improved T cell activity against infections and cancer and in the context of immunotherapies.

In initial in vitro experiments, the authors noted that activation of memory CD8$^+$ T cells is blunted when cultured in media lacking Mg^{2+}. Indeed, expression of activation markers, cell–cell clustering, induction of glycolysis and degranulation by memory T cells all showed a Mg^{2+}-dependent dose response. By contrast, naive T cell activation in vitro is not affected by Mg^{2+} deficiency.

The author's search for a metal-ion-binding, cell surface molecule led them to the integrin LFA-1, which is expressed at low levels on naive CD8$^+$ T cells but high levels on effector memory T cells and T cell blasts. Use

Credit: Piotr Malczyk / Alamy