**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Three recent preprints (not peer-reviewed) have sought to functionally characterize Omicron in comparison to previous variants of concern.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.

**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 ILC3s 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 Ptges2 (encoding COX2) by ILC3s. Ptges2, together with constitutive expression of Ptgs2 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 Mg2+ 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, Mg2+ 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 Mg2+. Indeed, expression of activation markers, cell–cell clustering, induction of glycolysis and degranulation by memory T cells all showed a Mg2+-dependent dose response. By contrast, naive T cell activation in vitro is not affected by Mg2+ 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

**ORIGINAL ARTICLES**

Meng, B. et al. SARS-CoV-2 Omicron spike mediated immune escape, infectivity and cell-entry fusion. Preprint at bioRxiv. 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/10.1101/2021.12.17.473248v2 (2021) | 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/10.1101/2021.03.01.21268111v1 (2021)

**RELATED ARTICLE**

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of conformation-dependent antibodies specific for LFA-1 revealed that a conformational change in LFA-1 (stalk extension and headpiece opening) is stabilized by binding of Mg²⁺ to metal-ion-dependent adhesion sites. Moreover, Mg²⁺-dependent conformational changes were required for LFA-1 outside-in signalling, leading to FAK phosphorylation, calcium flux and downstream effector functions in T cell blasts.

Turning to mouse models, they show that T cells from wild-type mice given a Mg²⁺-low diet for 2 weeks have reduced activation following injection of anti-CD3, similar to T cells from LFA-1-deficient mice. Conversely, efficient control of cancer growth could be achieved in mice transferred with wild-type tumour-specific T cells and supplemented with intratumoural Mg²⁺. Combination of intratumoural Mg²⁺ and PD-1 blockade provided the most effective tumour control. In a Listeria infection model, dietary Mg²⁺ depletion reduced the efficiency of memory CD8⁺ T cells to clear the infection, which could be rescued by administration of a Mg²⁺-spiked bacterial inoculum. Furthermore, LILCs from human tonsil had increased HBEGF expression in response to PGE2 in vitro, and there was a reduction in the number of HB-EGF-producing ILC3s in inflamed intestinal tissue of patients with IBD compared with controls.

These data advance our knowledge of how ILCs contribute to barrier tissue homeostasis and they complement the role of ILC2-derived amphiregulin, another member of the EGF family, in epithelial barrier repair.

Kirsty Minton

ORIGINAL ARTICLE Zhou, L, et al. Group 1 innate lymphoid cells produce the growth factor HB-EGF to protect the intestine from TNF-mediated inflammation. Nat. Immunol. https://doi.org/10.1038/s41590-021-01110-0 (2022)

In the context of immunotherapy, Mg²⁺ enhanced T cell cytotoxicity induced by the bispecific T cell engaging antibody blinatumomab (directed against CD19 and CD3); an effect that was abrogated by the presence of antibody that prevents LFA-1 headpiece opening. Moreover, in mice, dietary Mg²⁺ restriction blunted chimeric antigen receptor (CAR) T cell-mediated tumour rejection. Interestingly, retrospective analysis of a CAR T cell clinical trial and a cohort of patients treated with PD-1 blockade revealed that patients with lower serum Mg²⁺ levels had reduced overall survival and progression-free survival compared with patients with normal serum Mg²⁺ levels.

These findings suggest that Mg²⁺, by increasing LFA-1 outside-in signalling, enhances T cell effector function and may be harnessed to improve efficiency of T cell immunotherapies.

Lucy Bird

ORIGINAL ARTICLE Lötscher, J, et al. Magnesium sensing via LFA-1 regulates CD8⁺ T cell effector function. Cell https://doi.org/10.1016/j.cell.2022.01.014 (2022)

Immune checkpoint blockade and chimeric antigen receptor T cell therapies have proven safe in treating many types of cancer. However, their limited efficacy raises the need to discover other cellular processes that could be harnessed to develop better treatments. Tumour-infiltrating T cells and the formation of tertiary lymphoid structures (TLSs) have been recently correlated with a favourable clinical outcome in different types of cancer. However, the cellular and molecular mechanisms that promote T cell infiltration and TLS formation in the tumour microenvironment are still not fully understood.

In this preprint (not peer-reviewed), Ukita et al. investigated the contribution of infiltrating lymphocytes and TLSs in two different cohorts of patients with high-grade serous ovarian carcinoma. The study suggests that tumour infiltration with CD8⁺ T cells and B cells, and TLS formation, correlate with a beneficial clinical prognosis. For the first time in ovarian cancer, TLSs were shown to colocalize with the presence of CXCL13, a chemokine that is crucial for the formation of lymphoid structures and that is also found in other types of cancer with better outcomes, including superior responses to immunotherapy, such as breast cancer and melanoma. Using RNA in-situ hybridization in tissue samples from initial surgical specimens without prior chemotherapy, the authors further identified a subset of CD4⁺ T cells as the source of CXCL13 in the early stages of TLS development, whereas follicular dendritic cells were shown to be responsible for the maintenance of mature TLSs.

Furthermore, in vitro differentiation of naive CD4⁺ T cells co-cultured with various ovarian cancer cell lines elucidated the role of TGFβ in promoting the differentiation of CXCL13-producing CD4⁺ T cells, which adopted a peripheral helper cell phenotype through expression of PD1 and lack of CXC5 expression. Finally, the authors confirmed the contribution of CXCL13 to TLS formation in a mouse model of ovarian cancer by administering recombinant CXCL13, which was associated with an increased survival rate.

Overall, this study describes key cellular mechanisms that promote and sustain TLS formation in ovarian cancer, which is associated with improved clinical prognosis. Future characterization of the phenotype and function of CXCL13-producing CD4⁺ T cells and the interactions between humoral and cellular components in the tumour microenvironment may elucidate the role of these cells in anti-tumour responses, thus informing strategies to improve ovarian cancer treatment.

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HARNESSING CXCL13 IN OVARIAN CANCER

ORIGINAL ARTICLE Ukita, M, et al. Tertiary lymphoid structures induced by CXCL13-producing CD4⁺ T cells increase tumour infiltrating CD8⁺ T cells and B cells in ovarian cancer. Preprint at bioRxiv https://doi.org/10.1101/2021.12.01.470493 (2022)

RELATED ARTICLE Brenna, E, et al. Preprint Journal Club. PreprintClub https://www.preprintclub.com/2021-eng-ukita(2022)