



Credit: Science Photo Library/Alamy

Next, the authors turned their attention to plasmacytoid dendritic cells (pDCs), which are a known source of type I IFNs. Depletion of pDCs reduced the effect of microbiome disruption on CHIKV viraemia, and gene expression analysis showed an altered antiviral, but not basal, immune response in pDCs. In particular, the type I IFN response by pDCs following stimulation with ligands for Toll-like receptor 7 (TLR7) was decreased by antibiotic treatment compared with controls. The response also depended on MyD88 expression in pDCs, suggesting that signals from the gut microbiome support a TLR7–MyD88 signalling axis to generate type I IFNs that limit monocyte infection and virus dissemination.

The authors developed targeted-IP-multiplex-light-absolute-quantitative mass spectrometry (TIMLAQ-MS) to simultaneously measure the dynamics of ITAM phosphorylation of the different TCR signalling subunits in a single measurement. This revealed distinct patterns and kinetics of phosphorylation of the different subunits. In particular, the ITAM of CD3 $\epsilon$  had high levels of monophosphorylation, which is known to be associated with the recruitment of inhibitory molecules. Indeed, monophosphorylated CD3 $\epsilon$  was found to bind to the inhibitory tyrosine kinase CSK, which is known to downregulate LCK activity. This indicated that CD3 $\epsilon$  may allow for negative feedback regulation of TCR signalling.

As CAR T cell therapies can induce excessive cytokine release, the authors tested whether adding CD3 $\epsilon$  to current CAR constructs may allow for better-tuned signalling. Using the clinically approved CD19.28Z CAR construct, which contains signalling domains derived from CD28 and CD3 $\zeta$ , the authors inserted the entire cytoplasmic domain of CD3 $\epsilon$  at the membrane-proximal

position. They found that cells containing this construct produced significantly lower levels of cytokines upon stimulation than cells with the parent construct. The authors also found that the BRS motif of CD3 $\epsilon$  can recruit p85, the regulatory subunit of PI3 kinase, to promote persistence of CAR T cells. Thus, CD3 $\epsilon$  affects CAR signalling via multiple mechanisms. In a mouse xenograft model of B cell lymphoma, CAR T cells with the CD3 $\epsilon$  module showed improved anti-tumour function. Similar results were obtained when CD3 $\epsilon$  was inserted into another CAR construct that is currently tested in mesothelin-expressing solid tumours. Overall, these results indicate that the TCR is a self-restraining signalling complex and that the CD3 $\epsilon$  cytoplasmic domain may serve as a useful module to tune CAR T cells for anticancer immunotherapy.

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**ORIGINAL ARTICLE** Wu, W. et al. Multiple signaling roles of CD3 $\epsilon$  and its application in CAR-T cell therapy. *Cell* <https://doi.org/10.1016/j.cell.2020.07.018> (2020)

Lucy Bird

**ORIGINAL ARTICLE** Winkler, E. S. et al. The intestinal microbiome restricts alphavirus infection and dissemination through a bile acid-type I IFN signaling axis. *Cell* <https://doi.org/10.1016/j.cell.2020.06.029> (2020)

## IN BRIEF

### COVID-19

#### Preventing escape from neutralizing antibodies

Convalescent plasma (CP) and monoclonal antibodies (mAbs), two approaches being evaluated for COVID-19 therapy, are vulnerable to antibody-resistance mutations in SARS-CoV-2 that maintain viral fitness. This preprint describes the use of replication-competent chimeric viruses to generate spike protein escape mutants to four CP samples and three mAbs. Viral RNA from resistant cultures was used to identify shared and treatment-specific escape mutations. The mutations identified in this study are currently found at low frequencies in sequencing databases, but this methodology could be used to monitor emerging virus variants and predict their impact on mAb treatments. These findings support the use of combination mAb regimens and the design of vaccines targeting conserved B cell and T cell epitopes to prevent mutational escape.

**ORIGINAL ARTICLE** Weisblum, Y. et al. Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. Preprint at [bioRxiv](https://doi.org/10.1101/2020.07.21.214759) <https://doi.org/10.1101/2020.07.21.214759> (2020)

### COVID-19

#### IgGs drive COVID-19 myeloid hyperinflammation

Recent studies have identified an aberrant myeloid cell activation programme in patients with COVID-19. In this preprint, Hoepel et al. elucidate a mechanism by which alveolar macrophages facilitate hyperinflammation. Serum IgGs, in complex with spike protein, from patients with severe COVID-19 were shown to induce a potent pro-inflammatory response in human macrophages through multiple Fc $\gamma$ Rs, but mainly through Fc $\gamma$ RII. Blockade of this pathway using an inhibitor of the kinase SYK counteracted the pathological production of IL-6, IL-1 $\beta$  and TNF. Interestingly, in vitro exposure of endothelial cells to serum IgGs from these patients resulted in loss of barrier integrity and increased coagulopathy. These results improve our understanding of the abnormal myeloid response in COVID-19 and identify a potential therapeutic target.

**ORIGINAL ARTICLE** Hoepel, W. et al. Anti-SARS-CoV-2 IgG from severely ill COVID-19 patients promotes macrophage hyper-inflammatory responses. Preprint at [bioRxiv](https://doi.org/10.1101/2020.07.13.190140) <https://doi.org/10.1101/2020.07.13.190140> (2020)

### COVID-19

#### ACE2 is not induced by interferon

Interferon (IFN) is emerging as a promising therapeutic for COVID-19. Yet it was also proposed that IFN induces transcription of the SARS-CoV-2 entry receptor ACE2, potentially increasing viral infectivity. In this preprint, Onabajo et al. show that it is actually a shorter transcript of ACE2, coined dACE2, that was previously detected by RNA sequencing upon exposure to IFN. Expression of dACE2, but not the canonical ACE2, is induced by viral infection as well as treatment with type I, II and III IFNs. dACE2 is also highly expressed in several tumour tissues, owing to an inflamed microenvironment that resembles virus-infected tissues. Importantly, dACE2 cannot bind to the SARS-CoV-2 spike protein receptor-binding domain and lacks carboxypeptidase activity, dispelling concerns that IFN treatment might enhance viral infection.

**ORIGINAL ARTICLE** Onabajo, O. O. et al. Interferons and viruses induce a novel primate-specific isoform dACE2 and not the SARS-CoV-2 receptor ACE2. Preprint at [bioRxiv](https://doi.org/10.1101/2020.07.19.210955) <https://doi.org/10.1101/2020.07.19.210955> (2020)

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