Journal Club

PREPRINT WATCH

TARGETING AUTOANTIBODIES IN COVID-19

Autoantibodies to host proteins can trigger or exacerbate disease by perturbing target-dependent biological pathways, directing cell lysis and/or triggering inflammation. Although the generation of these antibodies is constrained by tolerance mechanisms, some autoantibody production is detectable in healthy individuals. An increased prevalence of more diverse autoantibodies has been reported in several inflammatory settings, including chronic viral infections. However, the breadth of antigens that are targeted and the ensuing pathophysiological effects are poorly understood.

In this preprint (non-peer-reviewed), Wang et al. used a novel high-throughput assay to quantitate circulating antibodies reactive with 2,770 secreted and cell surface-expressed human proteins in individuals infected with SARS-CoV-2 and control subjects. This work provided unprecedented insight into pre-existing and COVID-19-associated autoantibody reactivities and their contribution to pathogenesis. More proteins were targeted in infected individuals, with patients with severe COVID-19 exhibiting higher-level reactivity to the greatest number of antigens.

Notably, autoantibody reactivities found in patients with COVID-19 included many that targeted immune-relevant proteins such as cytokines (for example, type I interferons), chemokines or their receptors, as well as particular leukocyte subsets (B cells, T cells, natural killer cells and monocytes). These antibodies had immunomodulatory effects in vitro and were associated with virological and immune parameters in vivo. Blockade of key innate cytokine pathways exacerbated disease in a murine model of SARS-CoV-2 infection. Other autoantibodies in individuals infected with SARS-CoV-2 recognized tissue-associated antigens from sites including blood vessels and the brain and correlated with clinical markers of inflammation and disease severity.

The findings reported suggest pivotal immune-modulatory and effector roles for diverse autoantibodies in COVID-19 pathogenesis and prompt future work to investigate the persistence of tissue-targeted autoantibodies and their putative contribution to the long-term effects of COVID-19. It is unclear whether the remarkable breadth of autoantibody reactivities in patients with COVID-19 highlighted by this study reflects enrichment for individuals with rare pre-existing autoantibodies and/or perturbation of humoral immunoregulation in the inflammatory environment induced during infection. Understanding the mechanisms that drive these autoantibody responses could inform strategies to ameliorate severe COVID-19 and to treat 'long COVID'.

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ORIGINAL ARTICLE Wang, E. Y. et al. Diverse functional autoantibodies in patients with COVID-19. Preprint at *medRxiv* https://doi.org/10.1101/2020.12.10.20247205 (2021)
RELATED ARTICLE Pedroza-Pacheco, I. Oxford–Mount Sinai (OxMS) Preprint Journal Club. OxMS https://www.preprintclub.com/2021-jan-wang (2021)

VACCINES

MAIT cells boost adenovirus-induced CD8$^+$ T cells

Mucosal-associated invariant T (MAIT) cells are unconventional T cells that can act as innate sensors for viruses in mucosal tissues. Provine et al. now demonstrate that the ChAdOx1 virus, of recent fame as a vector for one of the first approved vaccines against SARS-CoV-2, robustly activates MAIT cells and that these cells play a central role in activating CD8$^+$ T cell responses to vaccine-encoded antigens.

MAIT cells specifically recognize microbially derived metabolites of vitamin B_2 synthesis but can also be activated via cytokines produced by virus-infected antigen-presenting cells. For example, they are known to amplify the early local immune responses to influenza infection. The authors hypothesized that these cells may also play a role in the immunogenicity of vaccines based on replication-incompetent adenoviral vectors like ChAdOx1.

Indeed, they found that stimulation of human peripheral blood mononuclear cells (PBMCs) with ChAdOx1 induced a dose-dependent upregulation of CD69, granzyme B and IFNγ in MAIT cells, indicating activation. Significant MAIT cell activation was detected after immunization of human volunteers with ChAdOx1, which correlated with an increase in plasma levels of IFNγ. RNA sequencing of human MAIT cells after ChAdOx1 stimulation revealed a strong induction of type I interferons and the IL-1, IL-12 and IL-2 family signalling pathways.

Next, the authors sought to investigate how these MAIT cells are activated. They found that ChAdOx1 mainly infects monocytes, conventional dendritic cells and CD123$^+$ plasmacytoid dendritic cells (pDCs). Using in vitro analysis and various knockout mice,

CANCER IMMUNOLOGY

Mutant p53 chills tumours by turning off cGAS

The tumour microenvironment can be referred to as 'hot' or 'cold' depending on whether it contains immune cells with anti-tumour or pro-tumour functions. A recent study in *Cancer Cell* has found that mutant p53 (mtp53) proteins can promote tumorigenesis by inhibiting the cGAS–STING signalling pathway and rendering tumours immunologically cold.

Cancer cells have aberrantly high levels of cytoplasmic DNA and these can be detected by cGAS; this leads to downstream formation of STING–TBK1–IRF3 complexes, in which IRF3 is phosphorylated and activated by TBK1. Activated IRF3 enters the nucleus to upregulate type I interferons or can translocate to the mitochondria to induce apoptosis, and these IRF3-driven responses protect against tumorigenesis.

However, in some cancer cells the cGAS–STING pathway cannot activate IRF3 (despite high levels of cytoplasmic DNA) and instead promotes metastasis.

Ghosh et al. assessed whether mtp53 proteins with mutations affecting the DNA-binding domain (which typically inactivate the tumour suppressor function of p53 and cause gain-of-function oncogenic activity) interfere with the cGAS–STING

Credit: Yvonne Bordon/Springer Nature Limited

they determined that pDC-derived IFNα and monocyte-derived IL-18 can activate MAIT cells. Unexpectedly, pDC-derived IFNα also stimulated the production of TNF in monocytes, which proved critical for inducing IFNγ production in MAIT cells. Interestingly, species C adenoviruses, including Ad5 (another viral vector used in vaccines against SARS-CoV-2), did not infect pDCs and only weakly activated MAIT cells.

In humans, immunization with ChAdOx1 induces a significant increase in IFNγ-producing T cells, which correlates with MAIT cell activation. Mice that lack MAIT cells were found to have significantly reduced $CD8^+$ T cell responses (but not $CD4^+$ T cell responses) following vaccination with ChAdOx1-based vaccines.

These observations suggest that MAIT cells occupy an important bridging position between innate and adaptive immunity. It is not yet clear how exactly MAIT cells facilitate $CD8^+$ T cell expansion, although the authors speculate that it may be via the local production of the chemokine CXCL10, which is known to promote $CD8^+$ T cell priming. Overall, these insights may inform the further optimization of adenoviral vectors for the induction of T cell-mediated immunity.

Alexandra Flemming

ORIGINAL ARTICLE Provine, N. M. et al. MAIT cell activation augments adenovirus vector vaccine immunogenicity. *Science* **371**, 521–526 (2021)

pathway. Knockdown of mtp53 proteins in various cancer cell lines resulted in activation of TBK1, IRF3, STING and *IFNB1* expression, whereas overexpression of mtp53 in cancer cells or in human fibroblasts suppressed these responses. Data from human breast cancer samples showed that the presence of mtp53 correlated with a decrease in interferon-associated mRNAs. Therefore, mtp53 proteins impede the cGAS–STING pathway.

The authors found that mtp53 blocks TBK1 function; mtp53, but not wild-type p53, interacted with TBK1 and prevented the formation of STING–TBK1–IRF3 complexes. Consequently, mtp53 prevents IRF3-mediated responses in cancer cells. In a mouse model of breast cancer, tumour cells expressing mtp53 grew faster and larger than p53-deficient counterparts. Notably, this effect was not seen in immunodeficient mice. The authors found that mtp53 suppresses tumour infiltration by cytotoxic T cells and natural killer (NK) cells and instead favours accumulation of M2-like macrophages that support tumour growth. Additional experiments suggested that these effects were due to loss of *IFNB1* expression in $mtp53^+$ tumours.

Finally, the authors showed that overexpression of TBK1 restores IRF3 activation and function in $mtp53^+$ tumour cells. In the mouse breast cancer model, inducible overexpression of TBK1 in $mtp53^+$ tumour cells suppressed tumour growth, and this was associated with robust tumour infiltration by cytotoxic T cells and NK cells and a reduction in M2-like macrophages.

The authors conclude that suppression of the cGAS–STING pathway by mtp53 promotes an immunologically cold tumour microenvironment. They suggest that therapies that activate TBK1 could be used in patients with $mtp53^+$ tumours to restore anti-tumour immune responses.

Yvonne Bordon

ORIGINAL ARTICLE Ghosh, M. et al. Mutant p53 suppresses innate immune signaling to promote tumorigenesis. *Cancer Cell* https://doi.org/10.1016/j.ccell.2021.01.003 (2021)

Journal Club

PREPRINT WATCH

TAKING CAR T CELLS UP A SYNTHETIC NOTCH

Therapeutic T cell engineering has facilitated precise targeting of adoptively transferred T cells against cancer cells. The most notable of these are T cells expressing chimeric antigen receptors (CARs), antibody–T cell receptor hybrids that, upon recognition of a surface-expressed antigen, provide activation signals to mediate cell killing. CAR T cell therapy has offered complete and durable control of B cell malignancies by targeting a homogeneously expressed antigen, CD19. However, in solid tumours such as glioblastoma, tumour-specific candidate CAR targets are expressed by only a subset of malignant cells, leading to outgrowth of resistant clones after initial tumour regression. Other targets lack tumour specificity, which can lead to off-tumour effects. Furthermore, constitutive CAR expression can lead to T cell exhaustion and relapse after remission.

In this preprint (non-peer-reviewed), Choe et al. harness their recently designed combinatorial T cell circuits to elicit specific, complete and durable antitumour responses in a mouse model of glioblastoma. They generated 'prime-and-kill' T cells expressing synthetic Notch receptors that sense a priming antigen, the glioma-specific EGFRvIII. When primed, cleavage of the Notch intracellular domain is induced, which drives transcription of CARs specific for EphA2 or IL-13Rα2, antigens that are homogeneously expressed on, but not exclusive to, glioma cells. Prime-and-kill CAR T cells killed not only cancer cells expressing both the priming antigen and CAR antigen but also cancer cells lacking the priming antigen, with as little as 10% of tumour cells expressing the antigen necessary for priming. Both prime-and-kill CAR T cells and T cells expressing conventional EGFRvIII-specific CARs induced in vivo regression of xenografts with heterogeneous EGFRvIII expression, but only the prime-and-kill CAR T cells persisted in a naive-like state and mediated prolonged tumour regression. Priming with the brain-specific antigen MOG showed similar potency against glioma xenografts, demonstrating the modularity of this approach.

This strategy has the potential to extend the success of CAR T cell therapy towards solid tumours. In a companion preprint (non-peer-reviewed), Hyrenius-Wittsten et al. applied the same technique to mesothelioma, priming cells with the mesothelioma-specific antigen ALPPL2 to mediate killing of MCAM-expressing tumours. The technical and regulatory complexities of manufacturing engineered T cells will likely be a hurdle to clinical translatability, but the impressive preclinical efficacy highlights promise for solid tumours.

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ORIGINAL ARTICLES Choe, J. H. et al. Multi-antigen recognition circuits overcome challenges of specificity, heterogeneity, and durability in T cell therapy for glioblastoma. Preprint at *bioRxiv* https://doi.org/10.1101/2021.01.07.425632 (2021) | Hyrenius-Wittsten, A. et al. Enhanced solid tumor recognition and T cell stemness with SynNotch CAR circuits. Preprint at *bioRxiv* https://doi.org/10.1101/2021.01.06.425642 (2021)
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