VIRAL INFECTION

A deadly fibroblast response to flu

Severe viral infections in the lungs can result in acute respiratory distress syndrome (ARDS), a potentially life-threatening inflammatory condition for which there is no effective therapy. Paul Thomas and colleagues have now characterized the distinct activation states that occur in lung fibroblasts during severe influenza virus infection. They report in *Nature* that excessive fibroblast activation characterized by upregulation of the protease ADAMTS4 is associated with immunopathology, reduced lung function and higher mortality in both mice and humans during influenza virus infection.

During respiratory viral infections, much of the damage in the lungs is caused by infiltrating immune cells that kill both infected and bystander cells. As remodelling of the extracellular matrix (ECM) facilitates immune cell entry, the authors set out to define the roles of stromal cell populations during an acute respiratory infection. They performed single-cell gene-expression profiling (scGEX) on $CD45^-$ stromal cells isolated from the lungs of mice infected with influenza A virus and found that fibroblasts showed particularly dynamic patterns of gene expression. Gene-set enrichment analysis identified three main functional fibroblast subsets: resting fibroblasts, ECM-synthesizing fibroblasts (ESFibs) and inflammatory fibroblasts.

ESFibs expressed genes encoding ECM proteins but not inflammatory genes. The inflammatory fibroblasts showed high expression of genes associated with type I interferon, IL-6 or NF-κB signalling and could be further subdivided into two subsets, namely damage-responsive fibroblasts (DRFibs) and interferon-responsive fibroblasts (IRFibs). DRFibs were enriched for genes involved in tissue damage-response pathways and showed upregulation of *Itga5* and downregulation of *Cd9*, whereas IRFibs were enriched in genes involved in type I interferon response pathways and showed upregulation of *Bst2* and *Cd9*. Similar functional fibroblast subsets were also identified in human lung biopsy samples and, of note, the authors found $ITGA5^+CD9^{lo}$ fibroblasts with a damage-responsive profile in patients who had died of respiratory failure but not in healthy donors. In vitro culture experiments suggested that IL-1α, IL-1β and TNF are important for fibroblast activation during influenza virus infection.

The authors next examined how proteases produced by activated fibroblasts affect the response to influenza virus infection. They found that ADAMTS4 (which degrades the ECM proteoglycan versican) is one of the earliest proteases to be induced in the lungs of infected mice, is expressed throughout the course of infection and is almost exclusively expressed by fibroblasts. A meta-analysis of publically available scGEX human datasets indicated that DRFib-like cells show highest levels of *ADAMTS4* expression and that *ADAMTS4* is markedly upregulated

> “targeting ADAMTS4 or other ECM proteases could improve clinical outcomes in patients who have developed ARDS in response to influenza viruses, SARS-CoV-2 or other respiratory infections”

CANCER IMMUNOTHERAPY

Nanobiologic trains innate immunity for anticancer responses

The term ‘trained immunity’ describes the epigenetic reprogramming of progenitor cells in the bone marrow, which enables these cells to give rise to myeloid cells that more efficiently respond to subsequent stimuli. It is induced via the stimulation of pattern recognition receptors (PRRs) and is a form of ‘innate immune memory’, although of shorter duration and less specific than adaptive immune memory. Now, reporting in *Cell*, Priem et al. demonstrate that trained immunity can be induced with ‘nanobiologics’ and show that these have anticancer properties.

The nanobiologics were engineered from natural carrier molecules (phospholipids, cholesterol and apolipoprotein A1, which is the main protein component of high-density lipoprotein (HDL)) and functionalized with peptidoglycans such as muramyl dipeptide (MDP) and muramyl tripeptide (MTP), which can induce trained immunity by binding to the PRR NOD2. A screen of a small library of these nanobiologics resulted in the selection of a lead candidate, MTP_{10}-HDL, which efficiently induced epigenetic changes typical for trained immunity in cultures of human peripheral blood mononuclear cells. The authors had previously shown that nanobiologics have a high avidity for the bone marrow, and in vivo imaging of mice injected with MTP_{10}-HDL demonstrated that it accumulates in the bone marrow and spleen, where it is taken up by myeloid cells.

As trained immunity has been implicated in anticancer immune responses, the authors tested MTP_{10}-HDL in a mouse model of melanoma (B16F10). Treatment with MTP_{10}-HDL induced dose-dependent tumour growth inhibition, whereas the ‘bare’ nanobiologic without the peptidoglycan moiety, or treatment with the peptidoglycan alone, had no effect on tumour growth.

> “when MTP_{10}-HDL was added to anti-CTLA4/anti-PD1 combination therapy, a dramatic suppression of tumour growth was observed”

To investigate whether this effect was indeed mediated via trained immunity, bone marrow from MTP_{10}-HDL-treated animals was transplanted into irradiated mice that were challenged with B16F10 melanoma cells 6 weeks later. Tumour growth was suppressed in mice that had received bone marrow transplants from MTP_{10}-HDL-treated mice compared with mice that had received bone marrow transplants from control mice. Extensive epigenetic analysis of haematopoietic stem cells (HSCs) and multipotent progenitor cells (MPPs) from the bone marrow of MTP_{10}-HDL-treated animals revealed changes typical of trained

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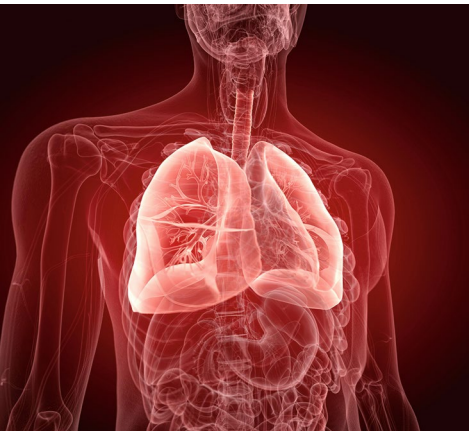

Credit: Science Photo Library/Alamy Stock Photo

in inflammatory lung diseases compared with in healthy lungs.

Compared with controls, *Adamts4*$^{-/-}$ mice showed improved survival following a lethal dose of influenza virus, and this was associated with reduced inflammation and improved lung function. The lungs of infected *Adamts4*$^{-/-}$ mice also showed higher levels of intact versican, and a series of additional experiments suggested that degradation of versican by ADAMTS4-expressing DRFibs permits the recruitment of CD8^{+} T cells, which are one of the main drivers of immunopathology during influenza virus infection.

Finally, the authors measured the levels of ADAMTS4 in respiratory tract samples from cohorts of paediatric or adult patients with moderate or severe influenza virus infection. They found that, across all age groups, levels of ADAMTS4 in the lower respiratory tract strongly correlated with increased risk for respiratory failure and death following severe influenza virus infection.

These findings indicate a crucial role for damage-responsive fibroblasts in regulating the magnitude of the immune response and the propensity to develop lung failure in response to severe respiratory infections. The authors propose that targeting ADAMTS4 or other ECM proteases could improve clinical outcomes in patients who have developed ARDS in response to influenza viruses, SARS-CoV-2 or other respiratory infections.

Yvonne Bordon

ORIGINAL ARTICLE Boyd, D. F. et al. Exuberant fibroblast activity compromises lung function via ADAMTS4. *Nature* https://doi.org/10.1038/s41586-020-2877-5 (2020)

immunity. For example, MPPs had regions of higher chromatin accessibility near the promoters of genes that regulate key cytokines such as TNF and IL-6, and transcriptomic and pathway analysis demonstrated an upregulation of genes associated with innate immune function and with metabolic rewiring in both HSCs and MPPs. MTP_{10}-HDL-treated mice had enhanced myelopoiesis and increased numbers of granulocyte–monocyte progenitor cells, and bone marrow cells harvested from these mice showed enhanced cytokine responses after re-stimulation with lipopolysaccharide.

To study potential contributions of adaptive immune cells to the anticancer activity of the nanobiologic, *Rag1*$^{-/-}$ mice, which lack T and B cells, were injected with B16F10 melanoma cells and then treated with MTP_{10}-HDL. The treatment did not appear to affect tumour growth rates, leading the authors to conclude that optimal therapeutic activity of MTP_{10}-HDL requires both 'trained' myeloid cells and an adaptive immune response.

The interplay of innate and adaptive immunity in anticancer responses was further explored by combining MTP_{10}-HDL treatment with checkpoint inhibitors, which enhance T cell responses. A combination of CTLA4- and PD1-targeted checkpoint inhibitors had no anti-tumour effects in the B16F10 melanoma model; however, when MTP_{10}-HDL was added to anti-CTLA4/anti-PD1 combination therapy, a dramatic suppression of tumour growth was observed, with complete responses in 4 of 10 animals. Further analysis revealed that MTP_{10}-HDL not only enhances myelopoiesis and sensitizes myeloid cells for activation but also appears to affect the tumour microenvironment by shrinking the population of immunosuppressive tumour-associated macrophages.

MTP_{10}-HDL was well tolerated in non-human primates. The authors suggest that peptidoglycan-functionalized nanobiologics may be particularly useful for sensitizing tumours to checkpoint therapy.

Alexandra Flemming

ORIGINAL ARTICLE Priem, B. et al. Trained immunity-promoting nanobiologic therapy suppresses tumor growth and potentiates checkpoint inhibition. *Cell* **183**, 786–801 (2020)

IN BRIEF

COVID-19

Intestinal attenuation of COVID-19 inflammation

Gastrointestinal (GI) symptoms are observed in patients with COVID-19, but the link between GI immune responses and disease outcomes is unclear. This preprint shows that COVID-19 severity and mortality, and levels of circulating inflammatory cytokines, are reduced in patients with GI symptoms. The SARS-CoV-2 receptor ACE2 was highly expressed in small intestinal enterocytes and viral particles were detected in these cells in patients with COVID-19. GI inflammation was absent in patients with COVID-19, as shown by a reduction of cellular inflammatory subsets and downregulation of inflammatory pathways. This study provides a basis for exploring the mechanisms involved in attenuation of SARS-CoV-2-associated GI inflammation to aid a comprehensive understanding of organ-specific immune responses in COVID-19.

ORIGINAL ARTICLE Livanos, A. E. et al. Gastrointestinal involvement attenuates COVID-19 severity and mortality. Preprint at *medRxiv* https://doi.org/10.1101/2020.09.07.20187666 (2020)

COVID-19

IL-18-dependent MAIT cell activation in COVID-19

Flament et al. report a marked reduction of circulating mucosal-associated invariant T (MAIT) cells in patients with severe COVID-19, compared with controls sharing co-morbidities. These MAIT cells had very high levels of activation that correlated with disease severity. Among T cells, alterations in MAIT cells preferentially associated with mortality, and high CD69 expression correlated with poor outcome. Severe inflammation, particularly high levels of IL-18, was associated with increased cytotoxicity of circulating MAIT cells. Co-culture studies of in vitro SARS-CoV-2-infected macrophages with MAIT cells suggest a two-step process of MAIT cell activation, through type I IFN and later IL-18. Together with other reports, this preprint supports a pivotal role for MAIT cells, through an IL-18-dependent mechanism, in the pathology of COVID-19.

ORIGINAL ARTICLE Flament, H. et al. Outcome of SARS-CoV-2 infection linked to MAIT cell activation and cytotoxicity: evidence for an IL-18 dependent mechanism. Preprint at *medRxiv* https://doi.org/10.1101/2020.08.31.20185082 (2020)

COVID-19

At the heart of COVID-19

Cardiac damage, even after recovery, has been reported in COVID-19, with nearly 50% of mildly ill patients having echocardiogram abnormalities. This preprint investigated the cellular alterations that occur after SARS-CoV-2 infection in vitro of cardiomyocytes derived from human induced pluripotent stem cells. The authors show that cardiomyocytes can be infected by SARS-CoV-2 and that this results in marked cytoskeletal, inflammatory and proteasomal alterations at the transcriptional level. Infected cardiomyocytes increase cytokine production and have pronounced myofibrillar fragmentation. Fragmentation was also observed in uninfected cardiomyocytes in vitro and in post-mortem cardiac tissue. Cytoskeletal fragmentation in the absence of infection might indicate putative effects of pro-inflammatory cytokines and stress responses on long-term cardiac changes in COVID-19.

ORIGINAL ARTICLE Pérez-Bermejo, J. A. et al. SARS-CoV-2 infection of human iPSC-derived cardiac cells predicts novel cytopathic features in hearts of COVID-19 patients. Preprint at *bioRxiv* https://doi.org/10.1101/2020.08.25.265561 (2020)

Aljawharah Alrubayyi, Ester Gea-Mallorquí and Felix Clemens Richter
OxImmuno Literature Initiative, University of Oxford, Oxford, UK
e-mail: covid19lit@medsci.ox.ac.uk