Immunology

An antiviral response beyond immune cells

Tomás Gomes & Sarah A. Teichmann

Fibroblast, epithelial and endothelial cells are more than just the scaffold of an organ – it emerges that they communicate with immune cells and are primed to launch organ-specific gene-expression programs for antiviral defence. **See p.296**

Immune-system responses to disease-causing agents rely on a complex web of interactions between immune cells that are underpinned by robust regulatory mechanisms. Most of our understanding of the immune system revolves around these cells, yet cells generally thought of as having a mainly structural role can also respond to invading organisms. On page 296, Krausgruber *et al.*[1] report a multi-organ examination of gene-expression programs for such structural cells in mice, revealing the roles of these cells in signalling networks used for defence purposes. The authors found that the response of structural cells to external invaders is regulated and tailored to the particular organ in question.

Structural cells, such as fibroblasts and endothelial and epithelial cells (Fig. 1), are present in most organs and provide more than just support[2,3]. Fibroblasts form part of the connective tissue and help to maintain the extracellular matrix material that surrounds cells. Endothelial cells line the interior of vessels such as blood vessels and, along with epithelial cells, which are present on the surface of organs, can be involved in responses to infection, either directly or through interactions with immune cells[3].

To understand the role of these three types of cell in immune responses, Krausgruber and colleagues isolated them from 12 different tissues in healthy mice. The authors used RNA sequencing to determine the genes expressed by the cells, and searched for known immune-associated genes. Krausgruber *et al.* also characterized the cells' chromatin – the complex of DNA and protein in the nucleus – to pinpoint genomic regions that were poised to start gene expression. This was done using a method called ATAC-seq to determine genome-wide 'open' chromatin accessibility, and the authors identified active promoter regions by tracking a type of modification called H3K4me2 on the DNA-binding histone 3 protein. Together, these methods opened a window on the transcriptional regulatory circuits that govern the identity and function of these cells.

Although the three cell types can be defined by the expression of genes corresponding to specific marker proteins found on the cell surfaces, the three cellular lineages also presented features that were characteristic of their local organ environment. Across the genome, the data sets for gene expression, open chromatin and active promoters indicated that the different cell types in an organ were more similar to each other than was a given cell type to the same cell type in different organs. This is a crucial observation that provides a foundation for future studies on the specific role that structural cells have in the function of each organ.

The authors searched the gene-expression data of structural cells to see which receptors and ligand molecules they expressed, and then matched the cells to possible interaction partners by mining previously published RNA-sequencing data for immune cells. They then assembled a computationally derived network that unveils possible cell-type- and organ-specific interactions involving structural and immune cells, and defines the baseline for routine interactions between immune cells and structural cells, from which further cellular crosstalk would develop on infection.

To understand more about how structural cells might prepare to trigger a gene-expression program for defence purposes, the authors assessed their gene-expression data together with the chromatin-accessibility profiles of the corresponding gene promoters (DNA sequences that aid gene expression). An open chromatin region encompassing a gene's promoter is known to be a reliable indicator of expression of the gene[4]. The authors used these combined data to look for outliers – genes that had an open accessible promoter but low levels of expression, on the assumption that such genes have what Krausgruber and colleagues describe as unrealized potential. This

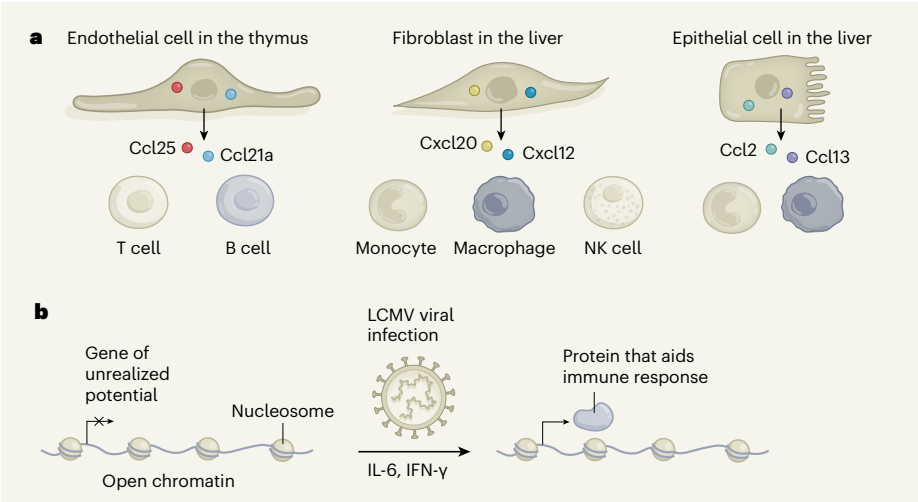

Figure 1 | Structural cells are poised for organ-specific defence responses. a, Krausgruber *et al.*[1] analysed three cell types – fibroblasts, and endothelial and epithelial cells – that are usually considered to have a structural role in organs. They found that, in mice, these cells signal to and interact with cells of the immune system (such as T cells, B cells, monocytes, macrophages and NK cells) to provide organ-specific defence responses. The authors report that structural cells express genes encoding chemokine proteins (for the examples given, the chemokines were Ccl25, Ccl21a, Cxcl10, Cxcl12, Ccl2 and Ccl13) that can attract immune cells. Structural cells also express other genes encoding ligands and receptors (not shown) that might aid communication with immune cells. The molecular interaction patterns identified were usually unique to each organ. **b**, Krausgruber *et al.* used RNA sequencing to profile gene expression in structural cells, and also assessed the state of chromatin (DNA wrapped around structures called nucleosomes) in the cells. Some genes were poised for expression – they had chromatin in an open state, and the authors described these genes as having unrealized potential. After infection with lymphocytic choriomeningitis virus (LCMV), these genes were expressed in a process that was often aided by the cytokine proteins IL-6 and IFN-γ (possibly secreted by immune cells). These genes were activated in a cell-type- and organ-specific manner, and constituted a key part of the early response of structural cells to infection.

indicates genes that are probably poised for a rapid response when infection occurs. The approach highlighted a group of genes encoding a substantial number of immune-associated proteins, and examples of these were most evident in structural cells from the skin, liver and spleen. These genes are worthy of further study that focuses on how the structural cells that express them respond to infection and protect the organ that is their home.

The authors confirmed that they had indeed identified genes poised for a role in an immune response by infecting mice with lymphocytic choriomeningitis virus (LCMV) and then monitoring gene expression by RNA sequencing of structural cells. LCMV is a well-studied virus that affects most organs, and this allowed Krausgruber and colleagues to distinguish organ-specific from global defence responses. Eight days after infection, up to 57.9% of the genes of unrealized potential had been activated in structural cells, with notably high responses in fibroblasts and endothelial cells in the liver, spleen, lungs and large intestine.

Furthermore, the authors found that an antiviral response was evident in these gene-expression profiles. When infected and non-infected animals were compared, the infected animals had higher levels of expression of transcription factors and immune-associated signalling proteins called cytokines that are involved in pathways associated with expression of the antiviral protein interferon. In response to the viral infection, structural cells also expressed small proteins called chemokines that attract immune cells. This was a surprise, because chemokine secretion has been mainly associated with immune cells. The authors propose that their predicted interaction network between immune cells and structural cells is altered on LCMV infection, and suggest that, on infection, structural cells in various organs increase interactions with immune cells such as monocytes, macrophages and B cells.

To dissect the effects of signalling in response to LCMV infection, the authors injected individual cytokines, of types detected in the antiviral response, into the bloodstream of mice that did not have an LCMV infection. Krausgruber *et al*. then sequenced the RNA in structural cells from the organs with the greatest previously observed response to LCMV. They found that gene-expression changes were more evident in fibroblasts and endothelial cells than in epithelial cells. Dissecting the gene-expression response to each cytokine revealed the portion of the antiviral program that it controls. Among other interactions, this revealed that the cytokines IL-6 and IFN-γ, possibly produced *in vivo* by immune cells, are responsible for eliciting much of the antiviral response of spleen endothelial cells by driving the expression of genes with unrealized potential.

Although gene-expression programs involved in the immune response have been reported previously for some structural cells, Krausgruber and colleagues' work underscores these cells' decisive role in coordinating organ-specific and organism-wide immune responses. It also indicates how functionally relevant candidate genes can be pinpointed using a combination of cell-communication networks and analysis of chromatin-mediated regulation. One of the ultimate goals of this research field could be to develop cell-type-targeted therapies that modulate immune responses. This could greatly benefit cancer research, for example, because cancer-associated fibroblasts have a role in promoting tumour progression[5].

Future studies will probably focus on the defence responses of other types and subtypes of human cells in studies linked to the Human Cell Atlas initiative[6], which is generating detailed molecular profiles for all human cells to fully describe cell-type diversity. Single-cell approaches could assist in profiling the RNA transcripts in all cell types and states of entire organs, in steady-state and post-stimulus scenarios. The use of a new method called spatial transcriptomics (which monitors gene expression in intact tissue sections rather than in dissociated cells), together with information about chromatin status, could disentangle the entire cellular chain of events, from the detection of infection to the defence response and immune-cell recruitment, and then finally to the removal of the infectious agent. By profiling structural cells in different mouse organs, Krausgruber *et al*. have unlocked a trove of knowledge about antiviral defences, which might be relevant to other species and facilitate new ways to target human diseases.

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Cancer evolution

Strands of evidence

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DNA damage can cause mutations due to failure of DNA repair and errors during DNA replication. Tracking the strand of the DNA double helix on which damage occurs has shed light on processes that affect tumour evolution. **See p.265**

How a cancer evolves and how mutations are generated are highly intertwined processes, and both are nearly impossible to observe directly. Instead, we are usually restricted to making inferences about them using data from a single snapshot in time after a cancer has formed. Aitken *et al*.[1] show on page 265 that, for a cell that has undergone DNA damage, such a snapshot provides remarkably rich information when the two DNA strands that form the double helix are considered independently.

DNA resembles a ladder, with the two 'side rails' often called, respectively, the Watson and Crick strands. These are fused together by 'rungs' of two complementary nucleotide base pairs: either cytosine (C) paired with guanine (G) or adenine (A) paired with thymine (T). When a cell divides, each daughter cell inherits either the Watson or Crick strand from the parent; this provides a template from which the other, complementary strand is replicated. Damage to a base can trigger a repair process, but if repair is not swift enough, the damaged base might be mispaired with an incorrect base during DNA replication. At the next round of cell division, when a daughter cell with such a mispaired base prepares to divide, the base complementary to the mispaired base will be added to the newly synthesized strand. This leads to a double-stranded mutation at the base pair corresponding to the original damaged base (Fig. 1).

Standard practice for genome sequencing is to consider mutations without paying attention to which of the strands received the original damage. However, when a chemical change occurs that damages a base, creating a site referred to as a lesion, this lesion is on only one of the two DNA strands of the affected base pair. Aitken and colleagues had the insight to see that, because