immune crosstalk. By conducting a detailed analysis of the major tumor-infiltrating immune cell subsets, Hangai et al. found that TCTP-induced CXCL1 and CXCL2 production is mainly restricted to M-MDSCs and that TCTP was primarily detected by TLR2, but not TLR4. Consistent with this, the TLR signaling adaptor protein MyD88, but not the innate signaling pathways mediated by MAVS and STING, was mandatory for TCTP-induced transcription of *Cxcl1* and *Cxcl2*, indicating that RNA- and DNA-recognition pathways are dispensable under these circumstances. TCTP-dependent accumulation of immunosuppressive PMN-MDSCs is coincident with a dramatic reduction in CD8$^+$ T and NK cells within the tumor microenvironment (Fig. 1). Adoptive transfer of PMN-MDSCs purified from TCTP-proficient tumors accelerated the growth rate of TCTP-deficient tumors in mice. On the flipside, targeted depletion of PMN-MDSCs with anti-Ly6G limited the outgrowth of IL-2ss–TCTP-engineered tumor cells that secrete TCTP. Taken together, these findings suggest a causal link between TCTP-triggered accumulation of PMN-MDSCs and weakened tumor immunosurveillance mediated by CD8$^+$ T and NK cells. Besides deleting immunosuppressive PMN-MDSCs, blocking TCTP emerges as a feasible therapeutic approach to switch off necrotic cell death–induced tumor outgrowth.

One may speculate on the translational implications of these interesting preclinical data. It would be fascinating to evaluate the cancer-prognostic value of either TCTP levels in the serum and/or tumor tissues or amplification of the *Tpt1* allele. It would be also quite useful to determine the therapeutic window of TCTP blockade for patients with cancer. TCTP is also known as a histamine-releasing factor. Histamine is primarily stored in the granules of mast cells in tissues and basophils in the blood. Since histamine and its receptors also exhibit immunomodulatory activities in cancer[11,12], it is possible that more downstream mediators and immune cell populations may participate in necrosis-associated, TCTP-induced tumor progression. ❐

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Competing interests

The author declares no competing interests.

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STROMAL IMMUNOLOGY

Inflating the role of stromal cells in CD8$^+$ T cell memory

Lung fibroblastic stromal cells support inflating memory CD8$^+$ T cells after vaccination with an adenovirus vector through the creation of organized lymphoid structures that support the metabolic fitness of these expanded antigen-specific T cells.

Katharine E. Block and Stephen C. Jameson

Adenovirus vectors (Ad) are versatile vaccine platforms that can effectively induce both humoral and cellular immunity[1], as exemplified by the success of various widely used Ad-based SARS-CoV-2 vaccines[2]. A surprising feature of some Ad vaccines is their ability to induce memory CD8$^+$ T cells that increase in frequency over time (thus called "inflating" memory), which can enhance and extend protective immunity. It was known that this outcome reflected the persistence of Ad in tissues and sustained antigen presentation, but which cells display the Ad-derived antigens and drive CD8$^+$ T cell memory inflation? The counterintuitive answer, as revealed by the paper from Cupovic and her colleagues[3] in this issue of *Nature Immunology*, is that Ad infection of lung fibroblastic stromal cells provides a source of persistent antigen and reprograms the microenvironment niche to produce and sustain inflating CD8$^+$ T cell memory, providing a striking example of how stromal cells regulate adaptive immunity (Fig. 1).

As vividly illustrated by the SARS-CoV-2 pandemic, the ability to generate safe, potent and flexible vaccines has huge consequences for protection from infectious diseases. Ad vaccines meet these objectives and have been widely deployed. Intriguingly, it was observed some years ago that CD8$^+$ T cell responses to some Ad-encoded antigens showed memory "inflation"[4]. While "conventional" CD8$^+$ T cell responses follow the textbook expansion and contraction of the antigen-reactive population, resulting in a stable but small memory pool, memory inflation entails the accumulation of antigen-specific memory cells for weeks or months before stabilization at impressive frequencies (often >10% of the entire CD8$^+$ T cell pool in blood and at even higher abundance in tissues such as liver and lung)[4,5]. Inflating (also called inflationary) memory had initially been associated with persistent viral infections capable of latency, such as cytomegalovirus in humans and mouse cytomegalovirus (MCMV) in mice, and it was presumed

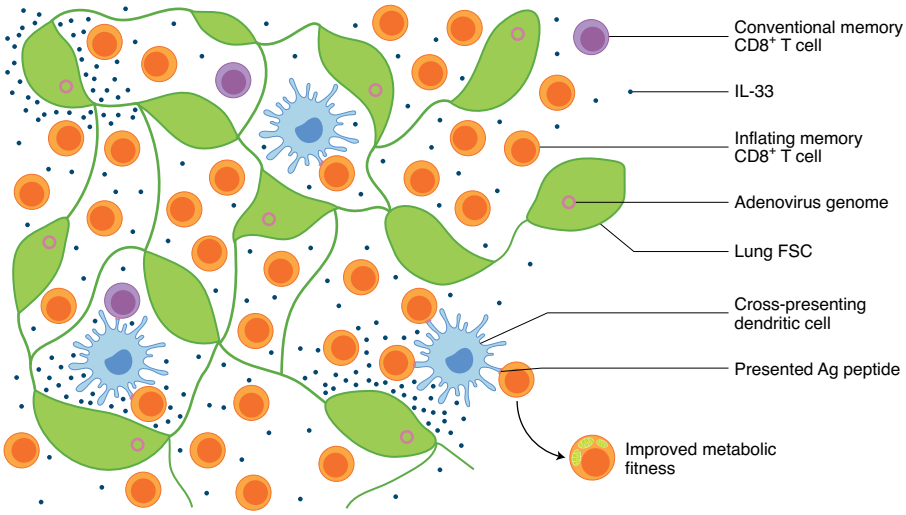

Fig. 1 | Lung fibroblastic stromal cells create a supportive niche for inflating memory CD8⁺ T cells after adenovirus vaccination. Cupovic et al. have identified a powerful system for sustaining high frequencies of memory CD8⁺ T cells in response to Ad, coordinated by FSCs; these cells both serve as a key source of Ad-infected cells and organize lymphoid structures that promote the sustained metabolic fitness and protective functions of inflating memory CD8⁺ T cells. The authors targeted Ad (light pink) infection to Ccl19⁺ FSCs (green) and found that these cells in the lungs were required for the development of inflating memory CD8⁺ T cells (orange) but not conventional memory CD8⁺ T cells (purple). Cross-presentation by professional antigen-presenting cells (blue) was required for this phenomenon, as well as FSC-derived IL-33 (dark blue).

to reflect durable antigen presentation from viral reservoirs, attributable to the complex lifestyle of these herpesviruses[5,6]. The induction of memory inflation by replication-deficient Ad vectors was therefore surprising, but careful analysis showed that Ad-vaccine genomes could be detected in multiple tissues many months after a single injection in mice and was associated with unexpectedly prolonged antigen expression[4]. It may be anticipated that memory inflation relies on professional antigen-presenting cells (APCs) serving as the primary depot for antigen. But there were some hints suggesting otherwise. A quirk of memory inflation is that it occurs for some epitopes but not others—even if the epitopes are from the same protein[5]. For example, among CD8⁺ T cells responding to β-galactosidase (β-gal, the model system used by Cupovic et al.), the response to the epitope β-gal$_{96}$ was inflated, while the response to the epitope β-gal$_{497}$ followed the "conventional" pattern[3,4]. In previous studies, members of this group showed that induction of conventional but not inflating CD8⁺ T cell memory depended on the expression of immunoproteasome components (involved in antigen processing) that are selectively expressed by professional APCs[4]. While not definitive, this finding suggested that the critical antigen-expressing cell involved in driving memory inflation may not be a professional APC after all.

The new report by Cupovic et al. utilized a sophisticated approach to resolve this issue. The authors employed a replication-incompetent Ad5-based vector in which β-gal expression is dependent on the action of Cre recombinase. Using mice expressing Cre in different cell populations, the authors quickly found that targeting β-gal expression in myeloid cell populations that would contain most APCs did not drive inflating or conventional CD8⁺ T cell responses[3]. Instead, when β-gal expression was induced only in Ccl19⁺ cells, strong inflationary (and conventional) responses ensued. Ccl19 is expressed by fibroblastic stromal cells (FSCs) in diverse tissues (including the spleen, liver and lung), some of which are known to support the survival of lymphocytes and provide a framework for immune encounters[7]. These new findings suggest an additional role as a source of durable viral antigen expression for CD8⁺ T cell memory inflation, something that may also occur in MCMV infections[8]. Extending these studies, Cupovic et al. showed that human FSC tissue explants were preferentially infected by Ad (including vectors using the same Ad5 system employed in the mouse experiments, as well as ones involving the ChAdOx1 backbone used in the current Oxford–AstraZeneca COVID-19 vaccine[9]), indicating the translational relevance of their findings[3].

Interestingly, it appears that lung FSCs are critical for supporting memory inflation in mice. Capitalizing on their Ccl19-Cre system, the authors developed a strategy to both label the relevant FSCs and deplete them at will with diphtheria toxin (DT). Intranasal DT treatment after Ad infection caused the loss of Ccl19-expressing FSCs selectively in the lungs, which compromised the inflating response to β-gal$_{96}$ to the same degree as that observed with the systemic depletion of Ccl19-Cre⁺ FSCs with intraperitoneal DT treatment. FSC depletion by any route did not derail the conventional response to β-gal$_{497}$. Loss of these lung FSCs led to reduced immune cell infiltrates in the Ad-infected lung, with CD8⁺ T cells most strongly affected, and an impaired ability to resist the metastasis of a β-gal-expressing tumor to the lungs.

These findings do not, however, exclude a key role for professional APCs in the process of memory inflation. Indeed, the authors found that β-gal expression in FSCs was insufficient to provoke memory inflation in mice lacking dendritic cells capable of antigen cross-presentation, suggesting a hand-off of β-gal antigens from FSCs to APCs. But does that mean that Ad-infected FSCs simply act as a passive source of antigen for supporting memory inflation? Further work suggested not. While investigating how Ad infection altered the differentiation state of various lung Ccl19-Cre⁺ populations, Cupovic et al. discovered the appearance of a distinct population of fibroblasts that began expressing the alarmin interleukin (IL)-33. In situ imaging studies showed that these cells developed a network that included CD8⁺ T cells and subsequently appeared to nucleate the formation of inducible bronchus-associated lymphoid tissues near blood vessels. This population of IL-33⁺ FSCs also expressed elevated levels of Ad-encoded *lacZ*, suggesting that they included cells still infected with Ad. Expression of IL-33 turned out to be critical for enabling these lung cells to support memory inflation—ablation of the *Il33* gene in Ccl19-Cre cells was as potent as DT-mediated elimination of those cells in reducing the inflating β-gal$_{96}$-specific population (without affecting the conventional β-gal$_{497}$ response), indicating that IL-33 production by these lung FSCs was essential for their ability to support inflating memory.

And how do inflating memory CD8⁺ T cells benefit from encounters with

IL-33-expressing lung FSCs? Tracking the residual β-gal$_{96}$-specific response after eliminating Ccl19$^+$ FSCs or FSC-derived IL-33 revealed qualitative changes in their acquisition of the typical inflating-memory phenotype and function and a marked loss in mitochondrial maintenance and activity (including reductions in both the mitochondrial membrane potential and expression of electron transport chain components). Hence, deprivation of the factors and niches provided by these lung FSCs compromises the fitness and function of inflating memory CD8$^+$ T cells. It will be interesting to discover whether maintenance of the β-gal$_{96}$-specific memory population collapses if these stromal cells (or their ability to make IL-33) are eliminated after inflating memory has already been established, which would test whether the continued presence and function of FSCs is required.

Many additional questions remain, and the answers will have substantial implications for the therapeutic potential of Ad and other vaccination approaches. For one, why do responses to only some epitopes lead to CD8$^+$ T cell memory inflation? These new findings suggest that initial antigen processing by FSCs dictates which epitopes are subsequently presented by professional APCs. If so, can re-engineering of the context of an epitope make it more amenable to being processed by these cells, and hence the target for inflating memory? This strategy may provide a way to produce large numbers of functional memory CD8$^+$ T cells against desired epitopes to control infectious diseases (for example, to target a panel of HLA-restricted CD8$^+$ T cell epitopes for SARS-CoV-2 vaccination) or to generate robust responses to tumor (neo)antigens. Also, it is unclear whether similar IL-33-producing FSC populations are involved in generating inflating memory following CMV or MCMV infections, although it is noteworthy that IL-33 has been shown to promote CD8$^+$ T cell memory inflation following MCMV infection[10]. The cells responding to IL-33 and the mechanism by which the cytokine promotes inflating memory responses to Ad also remain to be elucidated, although it is known that virus-specific CD8$^+$ T cells express the IL-33 receptor and that IL-33 enhances conventional memory CD8$^+$ T cell responses[11].

The ability to promote and sustain high numbers of protective memory CD8$^+$ T cells has prompted strategies to leverage CMV vectors as part of the vaccination strategy against confounding viruses like HIV, with promising results in the rhesus macaque/SIV model[12]; however, concerns about the safety of attenuated herpesvirus–based vaccines will probably endure. The possibility of using replication-incompetent adenoviral vectors as a platform for generating similar responses is certainly appealing. As illustrated by Cupovic et al., defining the mechanisms involved in Ad vaccine–mediated induction of CD8$^+$ T cell memory inflation will be key to developing these approaches, as well as to understanding the basic biology of T cell memory dynamics. ❐

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Competing interests

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STROMAL CELLS

Common heritage of fibroblasts

Meta-analysis of single-cell RNA sequencing datasets identifies core fibroblasts present in all organs that may give rise to more specialized organ-specific subtypes.

Agne Antanaviciute, David Fawkner-Corbett and Alison Simmons

Fibroblasts are essential structural cells within organs. They secrete matrix and provide an anatomical framework through which vascular, neural, immune and organ-specific structures are interwoven. The extent of fibroblast heterogeneity within and between organs has long been debated, and information categorizing fibroblast progenitors and lineages is incomplete. In recent years, a series of single-cell RNA sequencing (scRNA-seq) and biological studies have demonstrated that different subtypes of fibroblasts exist in different organs in both mice and humans[1–4]. Questions remain as to whether a common lineage-wide fibroblast exists in all organs, how and why different subtypes emerge within organs and how pathogenic subtypes appear in disease. In *Nature*, Buechler et al.[5] sought to investigate fibroblast origin by interrogating fibroblast diversity across murine organs. This involved conducting a meta-analysis of a variety of murine scRNA-seq fibroblast datasets in health to define which fibroblast signatures are organ-specific and which may be common across tissues and thus indicative of a lineage-wide core population.

Based on the analysis of more than 120,000 fibroblasts derived from 28 datasets, the authors provide an atlas composed of 10 clusters in steady-state. A number of these clusters represented organ-specific types of fibroblasts, such as fibroblastic reticular cells (FRCs), red pulp fibroblasts, mesenchymal stromal cells, osteo-lineage