mechanisms, such as degrading the bacterial genome or creating a hole in the cell membrane though the action of a pore-forming protein. But whether these systems kill cells in such ways remains to be tested. In some cases, the CBASS systems encoded a protein in which a TIR domain was fused to a STING domain similar to that in eukaryotes. The evolutionary conservation of these domains in an antiviral defence system in bacteria suggests that they might represent the ancient evolutionary origin of the eukaryotic cGAS–STING defence system.

Although some CBASS systems had only cGAS genes and components required for bacterial cell death, others had genes whose products were associated with ubiquitination, a protein-modification pathway in eukaryotic cells. In this process, a protein called ubiquitin is attached to a target by an enzyme-mediated reaction. CBASS systems included proteins that have several components associated with eukaryotic ubiquitination: E1 and E2 domains, typically found in enzymes that mediate ubiquitin activation and transfer, respectively, and JAB domains, which are found in proteins that remove ubiquitin from targets. Ubiquitination fine-tunes the length and intensity of innate immune responses in animals[13]. This provides yet another link connecting bacterial and animal antiviral responses. The ubiquitination components of the *E. coli* CBASS system were required for defence against some but not all phages, suggesting that these proteins might allow systems to recognize specific phage proteins or features, rather than being a more general property of phages – thereby refining the activity of these systems.

Antiphage defence systems in bacteria can be a target of phage-encoded inhibitor proteins. For example, phage proteins can block CRISPR–Cas defences[14]. It is highly probable that some phages have evolved ways to inhibit CBASS systems. Different CBASS systems encode a diverse set of cyclic-oligonucleotide signalling molecules and components, suggesting that cell suicide occurs through a number of mechanisms. The diversity of these CBASS-system components is probably driven by the need to evade a phage counter-attack if, for example, a phage-encoded protein could inactivate a particular cyclic-oligonucleotide signalling molecule. The selective pressure from antiphage systems that phages encounter would inevitably lead to the evolution of countermeasures in these viruses. An exciting area for future research will be to search for such phage inhibitors of CBASS systems.

One key aspect of cGAS function in bacterial defence that remains unknown is which signal the immune system detects to recognize that a viral infection is occurring. In eukaryotes, any viral double-stranded DNA in the cytoplasm can be recognized as a foreign entity because eukaryotic DNA is usually confined to the nucleus and absent from the cytoplasm. To distinguish cytoplasmic viral DNA from bacterial DNA, a bacterium lacking a nucleus would presumably require a sensor with a nuanced capacity to identify foreign DNA. One possibility is that CBASS systems recognize phage DNA specifically in the linear, relaxed state that occurs immediately after it has entered the bacterial cell. Perhaps the proteins that have E1, E2 and JAB domains in CBASS systems provide further refinement to aid the success of this aspect of phage recognition.

Cohen and colleagues' study is particularly remarkable for highlighting the striking parallels between innate immunity in eukaryotes and bacteria. The number of known bacterial antiphage systems is growing rapidly[5,15,16], and it is probable that many more such exciting connections remain to be uncovered.

Karen L. Maxwell is in the Department of Biochemistry, University of Toronto, Toronto, Ontario M5G 1M1, Canada.
e-mail:karen.maxwell@utoronto.ca

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Cancer

Teamwork by T cells boosts immunotherapy

Jonathan L. Linehan & Lélia Delamarre

Immunotherapy treatment harnesses CD8 T cells of the immune system to kill tumour cells. The finding that CD4 helper T cells contribute to the success of this treatment in mice might offer a way to improve clinical outcomes. **See p.696**

Immune cells called CD8 (or cytotoxic) T cells can target and kill cancer cells, and immunotherapies that boost this process are in clinical use. However, for reasons that are not fully clear, it is hard to predict whether a person will respond to this treatment. On page 696, Alspach *et al.*[1] report mouse studies revealing that another type of immune cell, called a CD4 cell (also known as a helper T cell), has a crucial role in aiding CD8 T cells to target tumours after immunotherapy.

Mutations in tumour cells can give rise to abnormal proteins, fragments of which – termed neoantigens – are displayed on the surface of cells bound to major histocompatibility complex (MHC) molecules. If a neoantigen is recognized by a CD8 T cell, this cell can target and kill any tumour cells that express the neoantigen. However, this cytotoxic response can be blocked, for example by an immunosuppressive environment surrounding a tumour. Immunotherapy treatments called immune-checkpoint blockade or immune-checkpoint therapy can counteract such problems to enable CD8 T cells to unleash an effective immune response against the tumour.

Much immunotherapy research focuses on CD8 T cells. However, there is emerging evidence that CD4 T cells might have a key role in tumour-targeting immune responses[2,3].

Alspach and colleagues sought to identify the minimal immune-stimulating neoantigen requirement to drive an effective immune response in mice that were given an immunotherapy treatment. The authors studied mice that had a type of tumour to which the immune system does not normally respond, and they engineered such tumours to express neoantigens. The neoantigen termed mLAMA4 is recognized by CD8 T cells[4], and the neoantigen termed mITGB1, recognized by CD4 T cells, was identified by the authors using a computational prediction method. In the absence of immunotherapy, the expression of these two neoantigens, either alone or together in a tumour, was insufficient to trigger an effective immune response against the tumour. However, if both neoantigens were expressed in animals receiving immunotherapy, the tumour regressed.

To check whether this response was simply

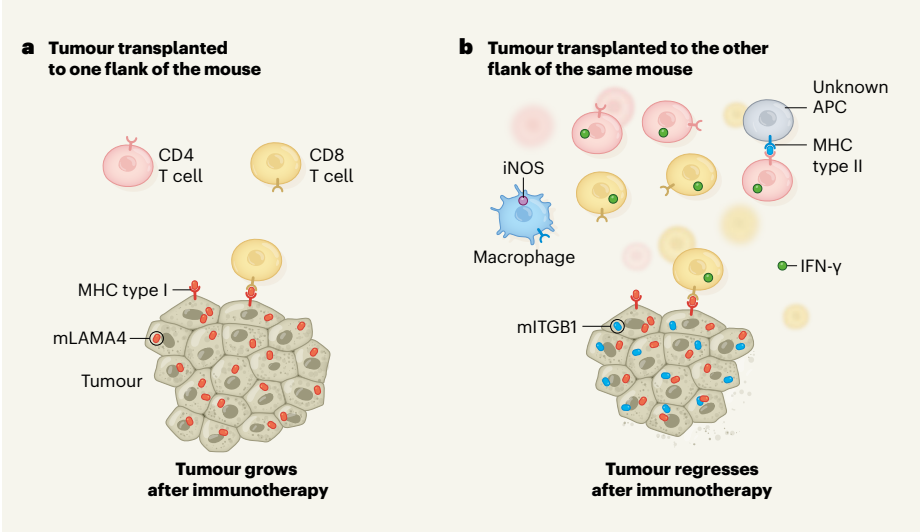

Figure 1 | T-cell collaboration drives an effective anti-tumour response to immunotherapy. Alspach *et al.*[1] studied how mice responded to immunotherapy that boosts tumour destruction by CD8 T cells. The mice received transplanted tumours in each flank that expressed abnormal protein fragments called neoantigens. The animals were then treated with immunotherapy. The neoantigen mLAMA4 is presented by type I major histocompatibility complex (MHC) molecules and recognized by immune cells called CD8 T cells, and the neoantigen mITGB1 is presented by type II MHC molecules and recognized by other immune cells called CD4 T cells. **a**, A tumour that expressed mLAMA4 attracted few immune cells and grew. **b**, By contrast, a tumour on the animal's opposite flank that expressed mLAMA4 and mITGB1 regressed, indicating the importance of activating both CD8 and CD4 T cells at the tumour site to generate a successful response to immunotherapy. The robust immune response generated included the accumulation of CD4 and CD8 T cells (which produce the signalling protein IFN-γ) and macrophage cells (which expressed the protein iNOS). The tumour cells lacked type II MHC molecules; therefore, an as-yet-unidentified antigen-presenting cell (APC) probably presents mITGB1 to CD4 T cells.

dependent on neoantigen quantity, rather than the need for neoantigen recognition by both types of immune cell, the authors engineered mouse tumours to express two different neoantigens that are recognized by CD8 T cells, but not by CD4 cells. These tumours did not respond to immunotherapy, demonstrating that a successful immune response depends on the presence of neoantigens that trigger responses from both CD4 and CD8 T cells.

The authors' analysis reveals that the CD4 T cells that responded to mITGB1 had the hallmarks of a type of CD4 T cell called a T helper type 1 cell, which can increase the number and cell-killing activity of CD8 T cells[2]. The authors confirmed that, if tumours expressed both mLAMA4 and mITGB1, this indeed caused an increase in the number and cytotoxic activity of CD8 T cells, compared with the case for animals with tumours that expressed only mLAMA4. Alspach and colleagues also showed that, if animals were first vaccinated with dying tumour cells and were then implanted with a growing tumour that expressed mLAMA4 and mITGB1, the transplanted tumours were most efficiently rejected if the vaccine contained tumour cells that expressed both mLAMA4 and mITGB1 in the same cell.

To determine whether CD4 T cells have a role beyond just enhancing the priming of CD8 T cells, as occurs during vaccination, the authors investigated whether mITGB1 is required at the tumour site for an active immune response. They implanted mice with a tumour that expressed both mLAMA4 and mITGB1 on one flank and with a tumour that expressed only mLAMA4 on the opposite flank, and treated the mice with immunotherapy (Fig. 1). As expected, the tumour that expressed mLAMA4 and mITGB1 was targeted by the immune system and regressed, but the tumour that expressed only mLAMA4 continued to grow slowly. In comparison with the mLAMA4- and mITGB1-expressing tumour, the growing tumour was infiltrated by fewer CD4 T cells, and by fewer CD8 T cells that could recognize mLAMA4.

These results highlight the need for a tumour to express neoantigens that are recognized by both CD4 and CD8 T cells to generate a productive response to immunotherapy. Together, these data demonstrate that CD4 T cells not only aid the priming of CD8 T cells, but also collaborate with CD8 T cells at the tumour site to maintain an effective anti-tumour response during immunotherapy. The mechanism enabling this collaboration remains to be determined.

The authors suggest that interferon-γ (IFN-γ), a type of immune-signalling protein called a cytokine, might be one necessary component enabling this collaboration. IFN-γ is produced by CD8 T cells and CD4 helper T cells, and can help to tackle an immunosuppressive tumour environment[5].

Tumours that are responsive to immunotherapy are associated with the presence of activated immune cells called macrophages that express the protein iNOS (ref. 6). Alspach and colleagues observed that tumours that expressed mLAMA4 and mITGB1 had an impressive 83-fold increase in the presence of iNOS-expressing macrophages in comparison with tumours that expressed only mITGB1. CD4 helper T cells alone are not sufficient to drive this iNOS expression in macrophages, and CD8 T cells are also required, suggesting an interplay between these three types of cell. Consistent with these findings, previous work[7] indicates that macrophage activation triggered by IFN-γ from CD4 T cells leads to the inhibition of tumour growth.

The tumour cells studied by the authors express type I MHC molecules that present neoantigens to CD8 T cells, but they do not express type II MHC molecules that present neoantigens to CD4 T cells. The identification of the immune cells that present neoantigens, such as mITGB1, to CD4 T cells in this system should be a topic for future research. Antigen-presenting immune cells, such as macrophages or dendritic cells, that capture material from dead tumour cells and present it on type II MHC molecules are probably involved. Indeed, dendritic cells are required[8] for the maintenance of an immunotherapy response in an IFN-γ-dependent manner.

Future studies could investigate whether immunotherapies that target both CD4 and CD8 T cells should be developed for clinical use. An obstacle to understanding and harnessing the responses of CD4 T cells for immunotherapy has long been the difficulty in identifying neoantigens that trigger such responses, as well as the need for adequate tools to monitor these responses. Alspach and colleagues' work, along with that of others[9,10], suggests that this is now changing.

Jonathan L. Linehan and **Lélia Delamarre** are at Genentech, South San Francisco, California 94080, USA.
e-mail: delamarre.lelia@gene.com

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