RESEARCH HIGHLIGHT

Swiss army knife T cell: one T cell many tumor targets

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T cell immunotherapies have revolutionized the treatment of many types of cancer; however, the exact mechanisms of some of these therapies remain unknown. In a recent study published in *Cell*, Dolton et al. found that in a melanoma patient who had a successful immunotherapy treatment, T cells can recognize not 1 but 3 tumor-associated antigens.

Tumor cells often have mutated (neoantigens) or abnormally expressed proteins (tumor-associated antigens, TAAs).[1,2] Antigen presentation is a process by which every nucleated cell in the body presents small samples of its proteins to the immune system in a context of peptide-loaded Human Leukocyte Antigen (HLA)[3] (Fig. 1a). Once loaded, the peptide–HLA complex gets presented on the surface of cells where it can be recognized by T cells for immune surveillance of abnormal peptides that come from intracellular pathogens, neoantigens or TAAs.[4] Each clone of T cells has a unique T cell receptor (TCR) that can recognize a unique peptide–HLA complex. In adult humans it is estimated that there are hundreds of millions of unique T cell clones circulating.[5] This incredible diversity in TCRs allows T cells to recognize a huge repertoire of peptide–HLA complexes. Killer or cytotoxic T lymphocytes (CTLs) can recognize target cells through their TCRs. This interaction often leads to apoptosis in the target cells (Fig. 1b).

In some solid tumors, T cells can be found in the tumor microenvironment. Some of these T cells have a TCR that recognizes a neoantigen or TAA. These tumor-specific T cells are often low in numbers and/or inhibited by the immunosuppressive tumor microenvironment.[6] Tumor-infiltrating lymphocyte (TIL) therapy is a form of immunotherapy where immune cells are isolated from the tumor, then activated and expanded ex vivo. After expansion, these cells get infused into the patient where they find and kill tumor cells (Fig. 1c). This type of immunotherapy has shown promising results in some patients and clinical trials are ongoing towards potential commercialization of this procedure.[6] Characterizing the antigens recognized by TILs, especially in patients experiencing durable remission may assist in the further development of these therapies. Dolton and colleagues studied T cells from MM909.24, a patient with stage IV melanoma who had successful TIL immunotherapy treatment and remains cancer free for a decade and counting.[7] 50% of expanded TIL cells from this patient were able to destroy the patient's melanoma cell line in vitro. These T cells were also able to recognize and kill other melanoma cell lines in a TCR-HLA-dependent manner. One T cell clone was isolated from the TIL product of MM909.24, labeled as MEL8. MEL8 T cells could kill melanoma and non-melanoma cancer cells in a TCR-HLA-dependent manner. Interestingly, MEL8 TCR recognizes a peptide that comes from Melan A protein which is broadly expressed in cutaneous melanomas.[7] The breadth of antitumor activity was surprising because non-melanoma cell lines do not express Melan A protein. When the Melan A gene was knocked out in melanoma cell line, MEL8 T cells were still able to kill tumor cells. Together, these experiments suggest that MEL8 TCR also recognizes additional TAAs.

To identify what other peptides MEL8 TCR can recognize, the team built a database for human TAA peptides. Using this database, 3 of the top 10 predicted peptides were recognized by the MEL8 TCR, and these came from 3 distinct TAAs: Melan A, Bone marrow stromal cell antigen 2 (BST2), and insulin-like growth factor 2 mRNA-binding protein 2 (IMP2) (Fig. 1d). While TCR cross reactivity has been demonstrated previously, it has never been shown across different TAAs. When all three TAA peptides were presented, they had an additive effect on MEL8 T cell activation. Most importantly, when Melan A and BST2 genes were knocked out, leaving only IMP2, MEL8 T cells were still able to kill tumor cells. This is particularly important because antigen loss is a common immune evasion strategy used by cancer cells.[8]

To test whether the general population has T cell clones that can recognize multiple TAAs, immune cells were isolated from healthy individuals. Then the T cells were stimulated with HLA loaded with only one TAA peptide at a time for 4 weeks. These stimulated T cells were able to bind to all three TAAs, although stimulated with one only. This shows that most people have the capacity to mount an immune response targeting multiple TAAs.

TAAs have a normal protein sequence but abnormal expression levels. This means that TAAs can be highly expressed in tumor cells but also expressed at lower levels in normal cells. This raises the question of whether targeting TAAs is a good strategy because there is a potential off-target effect where normal healthy cells can be killed by CTL cells. MM909.24 patient was infused with billions of TIL cells which contained MEL8 T cells. Even after complete remission, MEL8 T cells remained in the blood, yet no pathological signs of tissue damage were reported. This might suggest that targeting multiple TAAs is safe, but more studies and larger patient samples are needed. The work of Dolton and colleagues shows for the first time that a single TCR can recognize multiple TAAs. This is particularly useful because being able to

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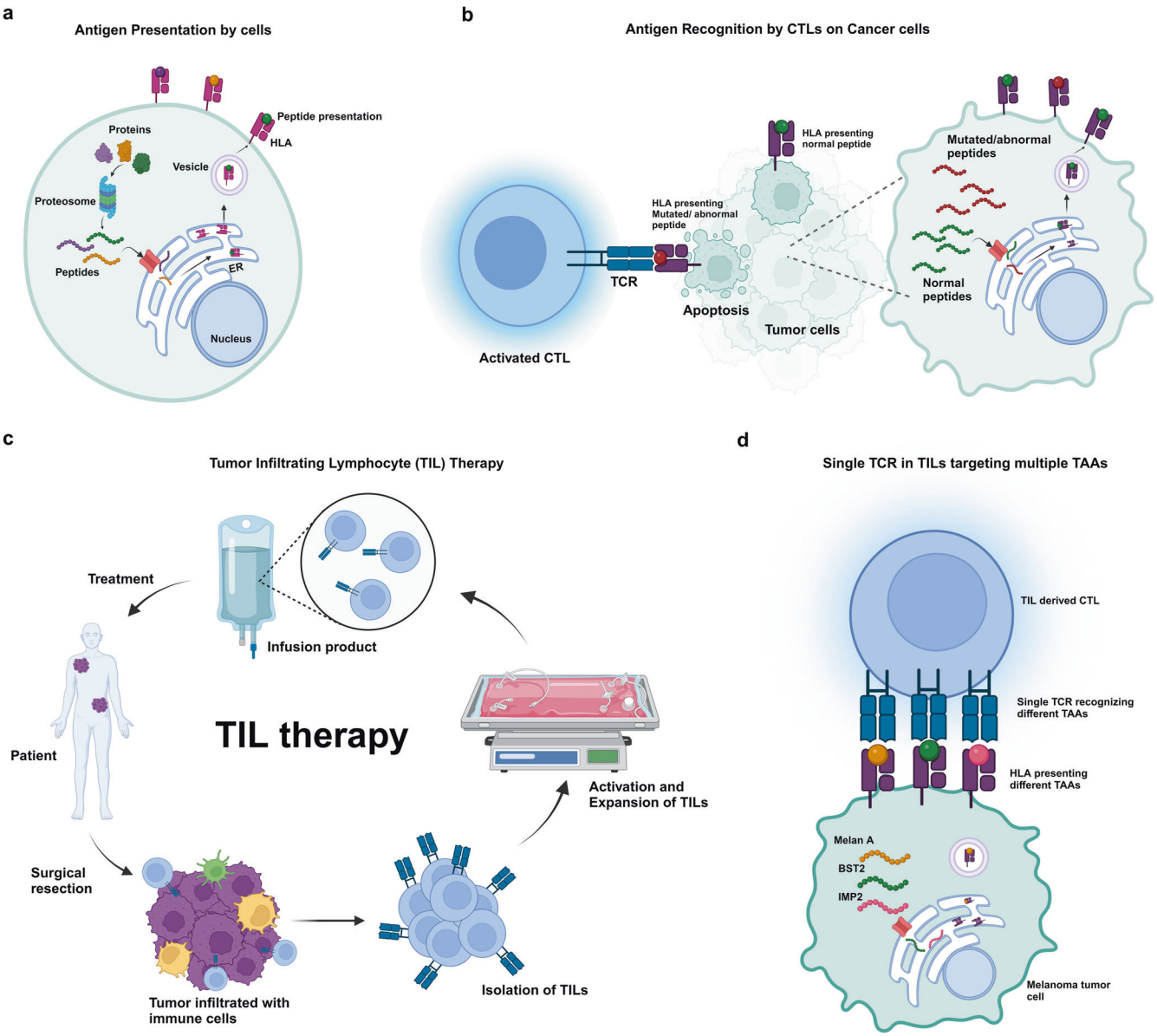

Fig. 1 Schematic diagram showing the main findings from Dolton et al.[7] a Overview of antigen presentation. Proteins get degraded by proteasome into small peptides, and then get loaded into HLA proteins. Once loaded, HLA can leave the ER and get presented on the cell surface. **b** A CTL cell recognizes a peptide from an abnormal protein through its TCR leading to killing of the target cell. **c** Overview of the TIL therapy procedure. Tumors are surgically resected, and T cells are isolated, activated and expanded ex vivo prior to infusion into the patient, typically following lymphodepleting chemotherapy, and in combination with subcutaneous IL-2. **d** A T cell clone such as MEL8 expresses a single TCR that can recognize 3 peptides from 3 different TAAs enhancing T cell recognition and tumor cell destruction.

recognize multiple TAAs had an addictive effect and may limit tumor escape. The findings thus have important translational implications such as in the development of TCR-transgenic T cells.

REFERENCES

1. Xie, N. et al. *Signal Transduct. Target. Ther.* **8**, 9 (2023).
2. Haen, S. P., Loffler, M. W., Rammensee, H. G. & Brossart, P. *Nat. Rev. Clin. Oncol.* **17**, 595–610 (2020).
3. Hewitt, E. W. *Immunology* **110**, 163–169 (2003).
4. Bridgeman, J. S., Sewell, A. K., Miles, J. J., Price, D. A. & Cole, D. K. *Immunology* **135**, 9–18 (2012).
5. Qi, Q. et al. *Proc. Natl. Acad. Sci. USA* **111**, 13139–13144 (2014).
6. Zhao, Y. et al. *Cancers* **14**, 4160 (2022).
7. Dolton, G. et al. *Cell* **186**, 3333–3349.e27 (2023).
8. Olson, B. M. & McNeel, D. G. *Expert Rev. Vaccines* **11**, 1315–1317 (2012).

ADDITIONAL INFORMATION

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