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TUMOUR IMMUNOLOGY

A close-range dual hit for tumour immunity

In this study, Blander and colleagues describe a new approach to induce effective antitumour immunity in mice, by introducing the bacterial protein flagellin — which triggers a Toll-like receptor (TLR) and two NOD-like receptors (NLRs) — into tumour cells. This approach enhances the priming of $CD4^+$ and $CD8^+$ T cells and the suppression of tumour growth.

Flagellin activates TLR5 and two NLRs: namely, NAIP5 (NLR family, apoptosis inhibitory protein 5) and NLRC4 (NOD-, LRR- and CARD-containing 4), which oligomerize to mediate assembly of the inflammasome in response to flagellin. Based on previous observations, the authors proposed that the introduction of flagellin into tumour cells would allow for concomitant recognition of tumour-associated antigens in the context of TLR and NLR activation, resulting in a more potent antigen-specific immune response. Indeed, EL4 thymoma cells that were transduced to express flagellin and the model antigen ovalbumin (EL4–OVA–flagellin cells), but not EL4 cells expressing OVA only (EL4–OVA cells), were rapidly eliminated (within 12 hours) from the peritoneal cavity following injection, in a macrophage-dependent manner. Tumour clearance required TLR5 signalling but not NAIP5–NLRC4 activation, and injection of EL4–OVA cells together with recombinant flagellin was sufficient for tumour cell clearance from the peritoneum. This suggests that TLR5 activation triggers innate immune clearance of free tumour cells from the peritoneum.

But is antigen recognition in the context of TLR and NLR activation required for the induction of protective immunity against a solid tumour? Subcutaneous injection of EL4–OVA cells with or without recombinant flagellin resulted in the formation of palpable subcutaneous tumours, whereas injection of EL4–OVA–flagellin cells did not. However, injection of EL4–OVA–flagellin cells into mice that lacked adaptive immune cells resulted in tumour growth, indicating that the adaptive immune response has an important role in protection against tumour growth. Only injection of EL4–OVA–flagellin cells, and not of EL4–OVA cells, resulted in the proliferation of OVA-specific $CD8^+$ T cells in tumour-draining lymph nodes and the production of interferon-γ and granzyme B by these cells. OVA-specific $CD4^+$ T cell proliferation was also induced, and the priming of $CD4^+$ and $CD8^+$ T cells required antigen presentation by dendritic cells. In addition, flagellin did not need to be fused with the antigen in the tumour cell, but the antigen and flagellin did need to be expressed in the same tumour cell for T cell priming. Loss of either TLR5 signalling or the ability of flagellin to activate NAIP5–NLRC4 resulted in subcutaneous tumour growth following injection with EL4–OVA–flagellin cells and severely impaired T cell priming.

Finally, immunization of mice with irradiated EL4–OVA–flagellin cells, but not irradiated EL4–OVA or EL4 cells, protected against the subsequent growth of EL4 wild-type cells. Interestingly, immunization with irradiated EL4 cells expressing flagellin but not OVA also protected against parental EL4 tumour development, indicating that flagellin can enhance a protective response to one or more endogenous tumour antigens. Furthermore, the disruption of flagellin recognition by TLR5 or NAIP5–NLRC4 impaired protection against existing or subsequent tumour growth by flagellin- and antigen-expressing tumour cells.

Together, these data describe a new strategy to induce antitumour immunity in mice by enforcing the recognition of tumour antigens in the presence of dual TLR and NLR stimulation. Importantly, because protection against tumour growth was observed in the absence of enforced antigen expression, this approach may have translational potential.

Olive Leavy

DIGITAL VISION

ORIGINAL RESEARCH PAPER Garaude, J. *et al.* Simultaneous targeting of Toll- and Nod-like receptors induces effective tumor-specific immune responses. *Sci. Transl. Med.* **4**, 120ra16 (2012)
FURTHER READING Blander, J. M. & Sander, L. E. Beyond pattern recognition: five immune checkpoints for scaling the microbial threat. *Nature Rev. Immunol.* **12**, 215–225 (2012)