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IMMUNOTHERAPY

Enlisting the enemy

> the gap junction mechanism of cross-presentation generates a more effective antitumour response

Effective cytotoxic T cell (CTL) responses against tumours require the cross-presentation of tumour-derived antigens to tumour-specific CTLs by dendritic cells (DCs). However, the antigen presentation machinery of tumour cells and DCs differs, and this can lead to the production of different tumour-specific peptides by the two cell types, limiting the effectiveness of CTL antitumour responses. This challenge has hindered the development of DC-based anticancer immunotherapies, but a recent study provides a novel solution by using *Salmonella typhimurium* to promote antigen cross-presentation by DCs.

Reduced expression of connexins — proteins that are required for gap junction formation — has been observed in some tumours.

SUPERSTOCK

Saccheri *et al.* examined the effect of infecting highly aggressive B16F10 (B16) mouse melanoma cells with *S. typhimurium,* a bacterium which preferentially homes towards tumours. B16 cells express low levels of the ubiquitously expressed connexin Cx43, but infection with *S. typhimurium* led to Cx43 upregulation. Is this followed by the formation of functional gap junctions? To test this, the authors co-cultured B16 cells with DCs and observed the transfer of a gap junction-diffusible dye from B16 cells to DCs only when the tumour cells were infected with *S. typhimurium*, suggesting that Cx43 upregulation induces gap junction formation between tumour cells and adjacent DCs.

To test whether antigenic peptides from bacteria-infected tumour cells can be transferred to DCs, the authors co-cultured ovalbumin (OVA)-expressing B16 cells with DCs, and found that the DCs only presented antigenic OVA peptides after *S. typhimurium* infection of B16-OVA cells. This effect was inhibited by treatment with a gap junction-uncoupling agent and did not require tumour cell phagocytosis by the DCs, suggesting that antigenic peptides are transferred between B16 cells and DCs through gap junctions.

Do these effects of bacterial infection also apply *in vivo*? Saccheri *et al.* subcutaneously injected mice with B16 cells and infected the resulting tumours with *S. typhimurium.* Cx43 expression was upregulated in the melanoma cells, DCs were activated and the treated tumours regressed. However, this regression could be mediated through elimination of infected cells by a non-adaptive immune response, so the authors investigated whether Cx43 upregulation could initiate a systemic antitumour immune response. They injected B16 cells into both flanks of mice, and then treated only the tumour on the left flank with bacteria, which also led to regression of the distal tumour. This effect was abolished when the *S. typhimurium*-treated tumour was silenced for Cx43 expression using short hairpin RNA or when CTLs were deleted using a neutralizing antibody, indicating that local activation of tumour-specific CTLs by gap junction-mediated antigen cross-presentation can affect the growth of distant metastases.

Saccheri *et al.* also showed that the gap junction mechanism of cross-presentation generates a more effective antitumour response than other mechanisms commonly used in the clinic, such as treating DCs *ex vivo* with purified tumour cell peptides, cell lysates or apoptotic material. Therefore, the elucidation of this mechanism could have implications for the development of effective immunotherapies for cancer. In addition, the authors showed that prior vaccination with bacteria-treated B16 cells could protect mice against a subsequent challenge with B16 cells, suggesting that such an approach could be used as an adjuvant therapy in patients with minimal residual disease.

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ORIGINAL RESEARCH PAPER Saccheri, F. *et al.* Bacteria-induced gap junctions in tumors favor antigen cross-presentation and antitumor immunity. *Sci. Trans. Med.* **2**, 44ra57 (2010)