IMMUNOTHERAPY

Two hits are better than one

Studying the mechanisms of tumour immune evasion has led to the identification of several targets for immunotherapy to activate anti-tumour immune responses. However, multiple immunosuppressive mechanisms may occur at once in a tumour, indicating that combinatorial therapeutic approaches may be beneficial. Highfill *et al.* report the possibility of improving responses to immune checkpoint blockade by inhibiting the recruitment of myeloid-derived suppressor cells (MDSCs) into the tumour microenvironment.

Highfill *et al.* used a syngeneic mouse model of embryonal rhabdomyosarcoma (ERMS), a type of paediatric sarcoma, in which M3-9-M mouse ERMS cells are inoculated into female mice. M3-9-M tumours develop despite activation of peripheral T cells and the authors found evidence of tumour-induced signalling by the T cell inhibitory receptor programmed cell death protein 1 (PD1), indicating that the tumours can progress by inducing T cell unresponsiveness through PD1 signalling. Indeed, treating mice with a PD1-specific blocking antibody at the same time as tumour inoculation prevented tumour development. However, mice with established tumours that were treated with a PD1-specific blocking antibody exhibited only a modest (although significant) reduction in tumour growth even though activated CD8⁺ T cells were present in the tumour. Further analyses revealed that there were increased numbers of MDSCs in the spleen and within established M3-9-M tumours, indicating that the MDSCs may suppress the activity of T cells in response to PD1 blockade.

To identify the tumour-derived factors responsible for MDSC recruitment, the authors analysed

conditioned media from M3-9-M cell culture and found that the levels of several cytokines that are known to cause MDSC expansion were significantly increased compared with controls. The conditioned media also had high levels of the CXC-chemokine receptor 2 (CXCR2) ligands, CXCL1 and CXCL2. CXCR2 was expressed by circulating CD11b⁺LY6G^{hi} granulocytic MDSCs from mice bearing M3-9-M tumours. In wild-type mice bearing M3-9-M tumours, haematopoietic cell reconstitution with *Cxcr2*^{-/-} bone marrow resulted in a significant reduction in the number of CD11b⁺LY6G^{hi} cells within the tumours, whereas the spleens of these mice had significantly increased numbers of CD11b⁺LY6G^{hi} cells. This indicates that although MDSC expansion occurs, these cells are no longer recruited to the tumour owing to the lack of CXCR2 expression.

Can CXCR2 be targeted therapeutically to limit MDSC-mediated immunosuppression? Mice bearing established M3-9-M tumours that were reconstituted with *Cxcr2*^{-/-} bone marrow and treated with a PD1-specific blocking antibody had reduced tumour growth and increased survival. These tumours also had significantly increased T cell/MDSC ratios, indicating that failure to recruit MDSCs allows T cell-mediated antitumour immune responses following treatment with PD1-targeted therapy. The treatment of mice bearing established M3-9-M tumours with a CXCR2-specific antibody also significantly reduced the numbers of CD11b⁺LY6G^{hi} cells within the tumours (but not in the

spleen). Furthermore, combining CXCR2-specific antibody with PD1-specific antibody significantly reduced tumour growth and significantly improved the survival of mice bearing established M3-9-M tumours, more so than either treatment alone.

Analysis of the serum from 53 patients with metastatic paediatric sarcoma revealed that levels of CXCL1 and CXCL8 — the major ligands for CXCR2 in humans — were significantly higher compared with controls, and levels of CXCL8 inversely correlated with survival. This indicates that circulating CXCR2 ligands may also recruit CXCR2⁺ MDSCs in patients with sarcoma, which suggests that combining therapies that target CXCR2 and PD1 could be beneficial in these patients. However, it is important to note that MDSCs are not well characterized in humans and so further work is required to clarify the phenotype of these cells before they can be therapeutically targeted in patients.

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