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TUMOUR IMMUNOLOGY

Intestinal bacteria are in command

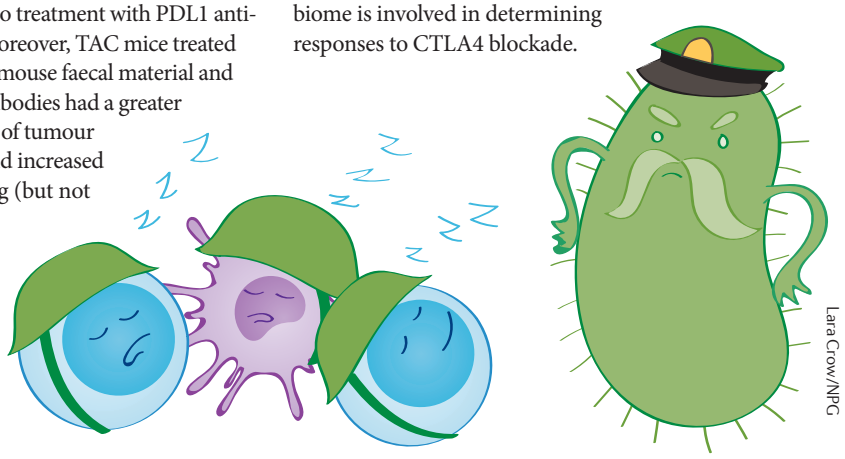
Two papers in *Science* present evidence in mice that certain species of intestinal bacteria can drive antitumour immune responses and modulate responses to immune checkpoint blockade.

Sivan *et al.* investigated the effects of the microbiome on responses to programmed cell death protein 1 (PD1) pathway blockade. B16 mouse melanoma cells engineered to express the SIY antigen (B16.SIY cells) were subcutaneously injected into syngeneic C57BL/6 mice from either Jackson Laboratories (JAX) or Taconic Farms (TAC). Curiously, B16.SIY-tumour growth was higher in TAC mice than in JAX mice. The tumours in JAX mice had significantly higher levels of infiltrating SIY-specific $CD8^+$ T cells and interferon-γ (IFNγ). These differences were ablated when the JAX and TAC mice were cohoused, which results in the microbiomes of the mice becoming similar. Indeed, JAX mouse faecal transplant into TAC mice (by oral gavage) reduced B16.SIY tumour growth and increased tumour-infiltrating SIY-specific $CD8^+$ T cells. TAC mouse faecal material did not significantly increase B16.SIY tumour growth in JAX mice, indicating that the JAX mouse microbiome somehow facilitates $CD8^+$ T cell-mediated antitumour immune responses.

Next, these authors investigated whether the JAX-derived microbiome could modulate responses to PD1 ligand 1 (PDL1) blockade. JAX mice had greater reductions in B16.SIY tumour growth and antitumour immune responses than TAC mice in response to treatment with PDL1 antibodies. Moreover, TAC mice treated with JAX mouse faecal material and PDL1 antibodies had a greater reduction of tumour growth and increased circulating (but not tumour-infiltrating) SIY-specific $CD8^+$ T cells than treatment with either modality alone. Further investigations revealed that certain *Bifidobacterium* species were significantly associated with tumour-infiltrating SIY-specific $CD8^+$ T cell-mediated antitumour immune responses and were increased by >400-fold in TAC mice receiving JAX mouse faecal material. Oral administration of a mixture of *Bifidobacterium* species to TAC mice reduced tumour growth and increased tumour-infiltrating SIY-specific $CD8^+$ T cells. Further investigation revealed that signals from *Bifidobacterium* species improve dendritic cell (DC) activation, which leads to improved antigen presentation to, and activation of, $CD8^+$ T cells.

Vétizou *et al.* investigated the effects of the microbiome on responses to cytotoxic T lymphocyte-associated antigen 4 (CTLA4) blockade. They implanted MCA205 sarcoma cells expressing the antigen ovalbumin (MCA205.OVA cells) subcutaneously in syngeneic mice that were housed in either specific-pathogen free (SPF) or germ-free (GF) conditions. Treatment of these mice bearing established MCA205.OVA tumours with CTLA4 antibodies resulted in reduced tumour growth in SPF mice but had no effect on tumour growth in GF mice. The reduced tumour growth in SPF mice treated with CTLA4 antibodies was prevented by treatment with broad-spectrum antibiotics (ampicillin, colistin and streptomycin; ACS), demonstrating that the microbiome is involved in determining responses to CTLA4 blockade.

> “intestinal bacteria can drive antitumour immune responses and modulate responses to immune checkpoint blockade”



Lara Crow/NPG

Analyses of faecal samples revealed that CTLA4 blockade induced reduced levels of Bacteroidales and Burkholderiales and increased levels of Clostridiales populations. Certain species of *Bacteroides* were shown to increase in the small intestine mucosa of mice treated with CTLA4 antibodies, indicating that these species flourish on CTLA4 blockade (hence their reduction in stools). The growth of MCA205.OVA tumours was restored in GF and ACS-treated mice when they were first fed with *Bacteroides fragilis* and *Burkholderia cepacia*. The authors went on to show that *B. fragilis*-derived polysaccharides induced mobilization and activation of $CD11b^+$ DCs in the lamina propria, which were required for improved T helper 1 (T_H1) immune responses in tumour-draining lymph nodes.

To clarify whether these bacterial species affect therapeutic responses in patients, Vétizou *et al.* analysed the bacterial populations in stool samples from 25 patients with metastatic melanoma that were taken before and after treatment with ipilimumab. They identified three clusters (A, B and C); clusters B and C were largely populated by different *Bacteroides* species and treatment with ipilimumab increased the number of patients with cluster C stools and decreased those with cluster B stools. Tumour-bearing GF mice receiving cluster C faecal transplants had marked antitumour responses after treatment with CTLA4 antibodies and showed mucosal colonization by *B. fragilis* (among other *Bacteroides* species).

These data are surprising in many ways, not least because of the prospect that providing a probiotic may improve antitumour immune responses induced by immune checkpoint blockade. However, further work is needed to demonstrate that the same is true in patients and, if true, it will be interesting to see whether the same bacteria elicit antitumour immune responses in all patients.

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ORIGINAL ARTICLES Sivan, A. *et al.* Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti–PD-L1 efficacy. *Science* **350**, 1084–1089 (2015) | Vétizou, M. *et al.* Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* **350**, 1079–1084 (2015)

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