IMMUNOTHERAPY

Programmable bacteria as cancer therapy

Quorum-sensing bacteria can deliver a nanobody targeting the 'don't eat me' ligand CD47 to tumors that results in systemic anti-tumor immunity-induced regression in mice.

Michael Dougan and Stephanie K. Dougan

The first injection of heat-killed mixtures of bacteria into patients with cancer was performed in the 1880s by William Coley, who observed anecdotal regression of solid tumors[1]. Although Coley's toxins fell out of medical practice, introducing bacteria into tumors to generate an immune response remains a powerful idea. Bacteria provide adjuvants— substances such as the cell-wall components lipopolysaccharide and peptidoglycan— that activate innate immune receptors recognizing common molecular patterns found in microbes. Simple in approach and execution, intratumoral delivery of adjuvant creates an in situ vaccine. Antigens released from dying tumor cells are taken up by activated dendritic cells; these cells then migrate to draining lymph nodes and prime adaptive immunity in the form of cytotoxic T cells that seek out and destroy tumor cells. In this issue of *Nature Medicine*, Chowdhury et al.[2] report a strategy using quorum-sensing bacteria that allows the delivery of a nanobody—a single-chain antibody fragment—that targets the phagocyte inhibitory ligand CD47 (CD47nb). This strategy was found to lead to systemic anti-tumor immunity that induced the regression of both injected and non-injected lesions in mice.

For practical reasons, Chowdhury et al. chose a therapeutic payload that could be genetically encoded and easily produced by bacteria, and that lacked the complicated disulfide bonds and glycans found in most therapeutic antibodies. Nanobodies, which are truncated forms of camelid heavy-chain-only antibodies, have stable conformations, are not glycosylated and do not require pairing with light chains, thus making them ideal candidates for this system[3]. A high-affinity nanobody that targets mouse CD47 and blocks its binding to its ligand had already been developed[4]. CD47 associates with integrins and is abundant on hematopoietic cells and in many cancers. Binding of CD47 to Sirpα on phagocytes triggers an inhibitory 'don't eat me' signal that blocks phagocytosis. In mouse xenograft models of human
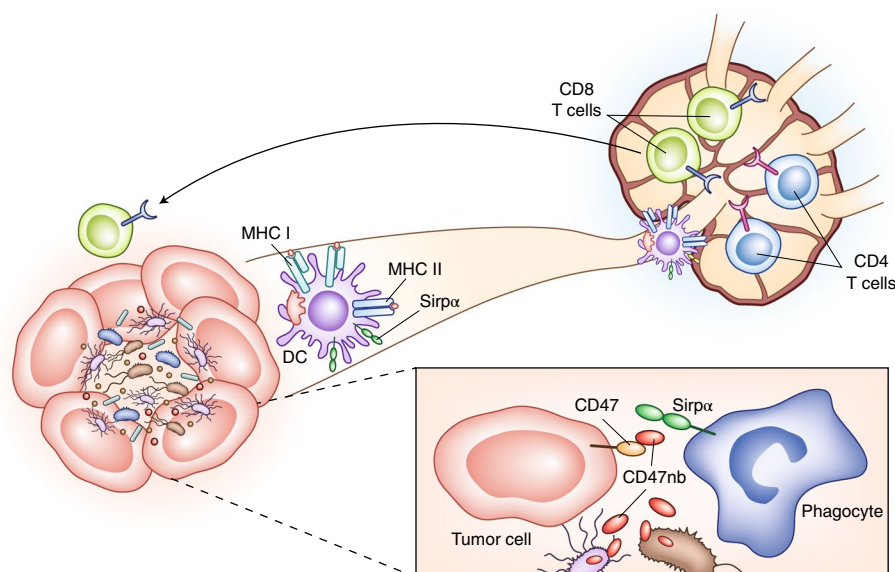

Fig. 1 | Inducing systemic anti-tumor immunity with quorum-sensing bacteria carrying nanobodies. Chowdhury et al. used bacteria that lyse when a quorum is reached and subsequently deliver an anti-CD47 nanobody to tumors in mice. The nanobody prevents tumor cell engulfment by phagocytes; however, the authors' data suggest that it also enhances systemic anti-tumor immunity. MHC, major histocompatibility complex; DC, dendritic cell.

cancer, blockade of human CD47 induces clearance of a wide range of tumors by mouse Sirpα+ macrophages[5–7].

The first results from a phase I trial to treat B cell lymphoma with anti-CD47 blockade in combination with rituximab, an IgG1 antibody against the B cell marker CD20, showed objective responses in 11 of 22 patients, thus greatly bolstering enthusiasm for blocking CD47 to generate an anti-tumor response[8]. The major treatment-limiting toxicity is anemia due to phagocytosis of erythrocytes. In mice treated with CD47nb-Fc, the resulting anemia is fatal[9]. Humans experience severe but temporary anemia and return to homeostasis within days, despite persistent occlusion of CD47 (ref. [8]). This species difference in the severity of toxicity may reflect differences in the mechanisms of erythrocyte recycling between mice and humans, but is more likely to be

explained by differences in the binding epitopes of the drugs.

Sirpα is expressed by multiple phagocytic cell types. Whether the primary effect of CD47 blockade occurs via macrophage-mediated tumor clearance, as originally supposed, is unclear. Subsequent studies targeting mouse CD47 in syngeneic tumor models have suggested that most of the therapeutic effect of CD47 blockade occurs through facilitating dendritic cell phagocytosis of tumor cell antigens and the resulting activation of anti-tumor cytotoxic T cell responses[4,10,11].

The effect of CD47 blockade on tumor clearance is greatly enhanced by the addition of pro-phagocytic signals, such as activating Fc receptors with tumor-specific antibodies. Current clinical trials blocking CD47 are largely based on a macrophage-mediated mechanism in which both anti-CD47 and the anti-tumor antibody would need to

be delivered systemically to reach each metastatic lesion at sufficiently high concentrations to observe benefit. CD47 is an abundant protein, and whether antibody levels in tissues provide saturation coverage remains unclear. In mouse models, >80% occupancy of CD47 in tumors has been found to be required to achieve therapeutic benefit[9]. If T cell priming contributes to therapeutic efficacy, however, CD47 blockade could be delivered transiently to a single lesion to generate an in situ vaccine and systemic regression mediated by tumor-specific T cells.

The approach by Chowdhury et al. uses quorum-lysis-sensing bacteria, which grow to a certain level, lyse and release their contents, and then grow back again. In the current study, the authors used *Escherichia coli*, which grow extracellularly, are non-pathogenic and persist for at least 6 days after intratumoral injection. After the bacteria are delivered into the tumors, several rounds of bacterial lysis ensure a constant, high local concentration of CD47nb. The authors were able to show robust tumor-antigen-specific CD8 T cell responses that targeted non-injected tumors and established immunologic memory (Fig. 1). These results again strongly suggest the induction of adaptive immunity as a major therapeutic mechanism by which CD47 blockade operates in immune-competent animals, although whether tumor-specific T cell responses are enhanced in humans remains to be seen.

Successful induction of T cell responses requires both antigen and adjuvant to be present at the same time. Microbes offer a convenient means of inducing anti-tumor immunity. Oncolytic viruses were among the first successful attempts to use microbes as cancer therapy, and intratumoral injection of the herpes virus–based T-VEC (talimogene laherparepvec) received approval from the US Food and Drug Administration for metastatic melanoma in 2015. Bacteria offer an orthogonal approach, and the quorum-sensing bacteria described here achieve three major engineering accomplishments. First, the bacteria provide an abundance of adjuvant and persist at high levels for as long as one week in tumors. Second, local delivery and quorum lysis prevent sepsis and leakage of therapeutic cargo into circulation, thereby decreasing systemic exposure of the drug, which could lead to treatment-limiting toxicity and the generation of anti-drug antibodies. Finally, bacteria have an enormous capacity for genetic cargo and could be modified to encode many more therapeutics than the single CD47nb used here. Other protein-based therapies that would benefit from local production include cytokines and chemokines, which act locally and have per-molecule higher potencies than blocking antibodies. Synthetic cytokines have also been reported, which would be amenable to prokaryotic production and have themselves already been engineered for enhanced stability

and function[12]. Molecular biology tools of the twenty-first century have transformed Coley's toxins into programmable drug-delivery platforms. ❐

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Competing interests

The authors declare no competing interests.