create a hybrid tool that might have enhanced power and a broader ability to reverse harmful neuronal activity in pathological conditions.

Creed and colleagues set out to investigate this possibility using a mouse model of cocaine addiction. In these mice, cocaine is injected directly into different parts of a brain region called the nucleus accumbens (NAc), inducing long-term neuronal depression or potentiation (a decrease or increase, respectively, in the responsiveness of neurons to incoming neural impulses). This alters transmission across the synaptic junctions of NAc neurons and thus specifically alters motor behaviour — a well-known effect of cocaine addiction. Previous work shows that DBS in the NAc has only a transient effect on addictive behaviour in this mouse model, but there is evidence that optogenetic stimulation of the metabotropic glutamate receptor protein (mGluR) has a longer-lasting effect. Optogenetic activation of mGluR restores normal synaptic transmission and erases addiction behaviours by depressing the activity of a population of NAc neurons that express the D1 dopamine-receptor protein and show increased activity in response to cocaine addiction.

The authors confirmed this optogenetic effect, and used the biological basis of the technique to try to determine how DBS could be modified, refined or combined with drugs to improve its effectiveness. They manipulated the parameters of DBS, using HFS or LFS, in the core or in the shell of the NAc (its two subregions), and with or without injection of D1-receptor antagonists into the NAc. They discovered that when acute LFS was refined by inhibiting D1 receptors, the response mimicked optogenetic mGluR-dependent restoration of synaptic transmission, and had a long-lasting ability to abolish addictive behaviours. The authors conclude that approaches such as this, which combine two treatments, might open up new therapeutic avenues.

But such combination studies are somewhat difficult to interpret, in part because of the different spatial scales over which each technique acts. Optogenetics is focal and specifically acts only on chosen neurons, but DBS, at whatever frequency, acts on larger regions and activates both excitatory and inhibitory neurons indiscriminately[8]. The authors predict that tailoring DBS by taking inspiration from optogenetics might lead to long-lasting, if not permanent, treatments. This would be a major improvement, and would increase its range of applications. Furthermore, the study's results point to other ways of treating patients with cocaine addiction or other pathological conditions — systemic administration of D1-receptor antagonists, for instance, or the use of 'double-channel' strategies that involve targeted delivery of both DBS and pharmacological agents (or optogenetically activated proteins) to the same brain region or to two different sites.

Achieving these improvements will not be straightforward. Sophisticated techniques will be required to achieve optogenetic or pharmacological modification of human synapses. If the effects are not permanent, strategies must be developed to enable the repeated introduction of drugs or genetic constructs to the appropriate brain region. Furthermore, treatment of some behaviours might require DBS or combination therapies at more than one site, or over large regions. Such advances might become possible through the development of nanotechnologies[9].

It is difficult to analyse different complex effects in a tiny region of the mouse brain and extend those findings to humans. This is why optogenetics, for the time being, remains at the periphery of human therapeutic applications. The possibilities opened up by Creed and colleagues' study might help us to cross this frontier. ■

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CANCER IMMUNOTHERAPY

# Dendritic-cell vaccines on the move

**Vaccines that induce an antitumour immune response are disappointingly ineffective in treating patients with cancer. Pre-conditioning the vaccination site to induce inflammation might provide a way to improve this therapy.** SEE LETTER P.366

RACHEL LUBONG SABADO & NINA BHARDWAJ

Dendritic cells (DCs) are often called nature's adjuvants because of the way in which they help to initiate an immune response. Found throughout the body, the cells acquire and process antigens (the molecules recognized and bound by antibodies) from pathogens and tumours. They then migrate to lymph nodes and activate T cells, which in turn induce protective immune responses. These properties have driven attempts to develop vaccines containing DCs loaded with tumour antigens, with the aim of inducing antitumour immune responses in patients with cancer[1]. But this strategy has fallen short of expectations. In this issue, Mitchell *et al.*[2] (page 366) show how simply improving DC migration to lymph nodes dramatically enhances antitumour responses in humans and mice, pointing to a way to optimize the use of DC vaccines.

There is a general consensus that DC vaccines can safely induce long-lasting antitumour immune responses. These vaccinations have produced encouraging, if modest, clinical results in some patients with advanced cancers[3]. For instance, the vaccine sipuleucel-T (the only cell-based cancer vaccine approved for use in the United States) increases median survival times by four months in patients with prostate cancer[4]. But several factors might be limiting the efficacy of DC vaccines: the source and type of DCs used; the site and frequency of injection; and the ability of DCs to migrate to lymph nodes. Moreover, the injected DCs may not themselves directly instigate an immune response, but instead might act indirectly through DCs already present in the lymph node[5].

Less than 5% of cells in a DC vaccine reach the lymph nodes[6]. In mice, DC migration can be improved either by injecting activated DCs or by pre-conditioning the vaccination site in the skin with the inflammatory molecule TNF-α (ref. 7). Mitchell and colleagues therefore investigated whether pre-conditioning the DC vaccine site to generate local inflammatory responses might enhance DC migration in humans. To do this, they used a tetanus/diphtheria (Td) toxoid vaccine. Most people have been exposed to this toxoid during childhood vaccinations, and re-exposure activates a subset of T cells called memory $CD4^+$ T cells that recognize only the Td antigen and mount a strong and rapid inflammatory immune response in its presence.

Glioblastoma multiforme (GBM) is an aggressive brain tumour in which cells specifically express pp65, an antigen from a common

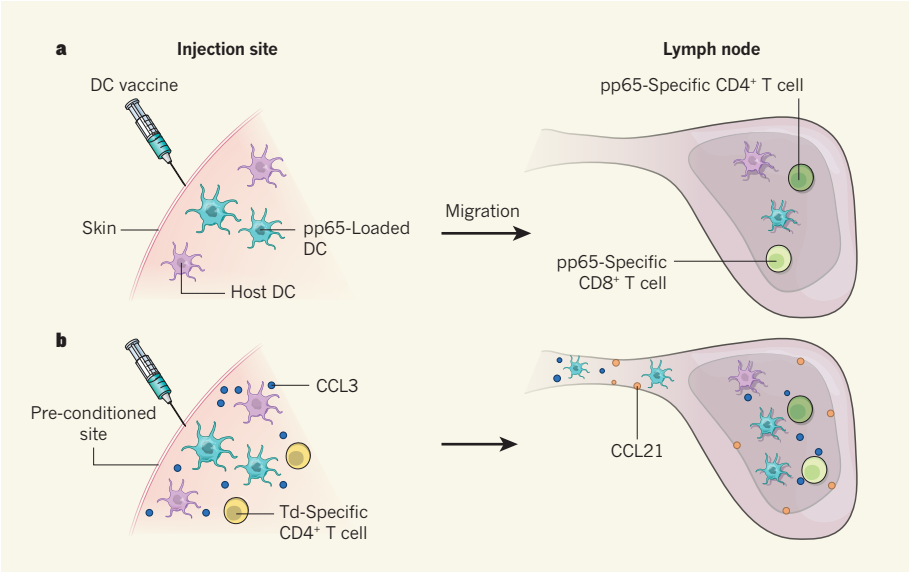


**Figure 1 | Optimizing dendritic-cell vaccines.** **a**, Dendritic cells (DCs) migrate to lymph nodes to present tumour-derived antigens to two types of immune cell: memory $CD4^+$ T cells that have previously been exposed to that antigen and so elicit strong, rapid immune responses; and $CD8^+$ T cells, which kill cells that express the antigen. This property is exploited to treat patients with cancer using vaccinations containing DCs loaded with pp65, which acts as a tumour antigen in this setting. However, migration of injected DCs to the lymph nodes is inefficient. **b**, Mitchell *et al.*[2] report that pre-conditioning the DC vaccination site with a tetanus/diphtheria (Td) toxoid vaccine improves DC migration by inducing inflammatory immune responses mediated by Td-recognizing $CD4^+$ T cells and generation of the protein CCL3. This protein upregulates production of the protein CCL21, which promotes DC and T-cell migration into lymph nodes. CCL3 may also recruit $CD8^+$ T cells to sites where DCs and $CD4^+$ T cells interact.

herpesvirus called cytomegalovirus, making pp65 an attractive target for immunotherapy. The authors treated patients who had GBM (and who had had their tumours removed and were receiving chemotherapy) by pre-conditioning them either with Td or, as a control, with DCs that had not been treated with pp65. All patients were then vaccinated with pp65-loaded DCs and given monthly DC vaccinations until their tumours regrew.

More DCs accumulated in the lymph nodes of patients who received Td treatment than of those who did not. Furthermore, these patients survived longer on average than those treated with DCs — three out of six survived past the end of the trial, almost twice as long as expected with standard treatment. Mitchell and co-workers report that Td-treated patients had longer-lasting, higher levels of pp65-specific T-cell responses than controls, a possible reason for their improved outcomes. However, this is only a small study, and so the relationship between elevated T-cell responses and clinical outcome remains unclear and warrants investigation in a larger patient cohort.

The authors then validated their results in mice that had been previously exposed to Td, using ovalbumin as a tumour antigen. They found that, as in humans, increased trafficking of antigen-loaded DCs to the lymph nodes depended on Td-specific memory $CD4^+$ T cells, because migration was less efficient in mice not previously exposed to Td. Furthermore, the efficiency with which DCs migrated to lymph nodes increased on both sides of the body following pre-conditioning, even though the animals were pre-conditioned on only one side. This systemic effect seems to be mediated in both humans and mice by CCL3, a chemokine found in blood serum that was rapidly induced following Td pre-conditioning (chemokines are signalling proteins that guide cell migration).

The researchers demonstrated that both CCL3 expression and Td-specific memory $CD4^+$ T-cell responses are required for DC migration. They propose that CCL3 functions by upregulating another chemokine, CCL21, in the skin and lymph nodes. CCL21 binds to and activates the chemokine-receptor protein CCR7, an interaction that is key for the efficient migration of activated DCs and T cells[8,9]. In support of their hypothesis, Mitchell and colleagues demonstrated that the efficacy of ovalbumin-loaded vaccines in a mouse model of melanoma depends on Td pre-conditioning and production of CCL3 and CCL21 (Fig. 1).

These results expand our understanding of the mechanisms underlying successful DC vaccines in several ways. Because both DCs and $CD4^+$ T cells produce CCL3 following antigen recognition[10], one could speculate that the interaction between these cell types explains the fact that pre-conditioning depends on host CCL3 and activation of Td-specific memory $CD4^+$ T cells. Moreover, CCL3 has other roles in the immune response that may further enhance DC vaccines: mobilizing DC precursors, regulating DC maturation and migration, and recruiting $CD8^+$ T cells, which kill cancer cells, to sites of interaction between DCs and $CD4^+$ T cells[11]. Finally, regulatory T cells, which inhibit the induction of tumour-specific immune responses, can decrease production of CCL3 (ref. 12), and pre-conditioning might elevate CCL3 to sufficient levels to overcome this effect.

DC vaccines loaded with patient-derived tumour preparations are being tested in large-scale phase III trials for renal cancer and GBM. Regardless of the outcome of these trials, Mitchell *et al.* provide a tantalizing approach to improving suboptimal DC vaccines. However, the increased efficiency that the authors observe may also be partially attributable to other factors: the use of a tumour antigen that provokes a stronger immune response than those used previously[13]; the fact that the authors vaccinated patients whose tumours had already been removed; or the fact that the patients also received chemotherapy, which may deplete regulatory T cells[14]. Furthermore, it will be key to identify which patients' tumours actually expressed the pp65 protein targeted by the vaccine. Pre-conditioning and activating DC vaccines more potently[15], combining vaccines with agents to counteract factors that inhibit tumour-specific immune responses[16], and targeting DC vaccines directly to the tumour[17] are all approaches with the potential to further enhance vaccine efficacy. ■

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