EXPERIMENTAL MODEL

Considering the non-specific effects of diphtheria toxin in cell-depletion studies

Peng, J. et al. *Nat. Commun.* **13**, 3874 (2022)

Microglia are resident immune cells of the central nervous system (CNS) that have a critical role in CNS homeostasis and functions. Impaired microglia can contribute to neuroinflammation and neurodegeneration, making these cells potential candidates for targeted therapeutics. Microglial depletion is a novel approach that can be used to understand microglial biology and modulate neuroinflammation in the context of CNS diseases.

There are several ways of depleting microglia, including pharmacological inhibition and gene targeting strategies. In genetically modified *DTR* mice used for diphtheria toxin (DT)-induced microglia depletion, the expression of the suicide gene encoding diphtheria toxin receptor (DTR) is regulated by the activity of a microglia-specific promoter, such as the fractalkine receptor (CX3CR1); and microglia death is triggered by DT administration. In 2018, Rubino et al. reported that DT-induced microglia depletion in adult *DTR* mice initiates an inflammatory cascade that causes neurodegeneration and ataxia-like behavior. Rubino et al. was the first study to report a severe motor deficit after microglia ablation and subsequent repopulation. A new paper calls for caution when interpreting the phenotype of these mice.

In *Nature Communications*, Peng et al. publish a Matters Arising article as a response to Rubino et al. They report that when repeating the experiments using the same DT dose and injection scheme treatment (three daily doses of 1.0 µg DT intraperitoneally (i.p.)), they did observe motor deficits and microglial activation in *DTR* mice, but noticed the same phenomenon in wild-type (WT) mice in which they had injected the same DT treatment. "We argue that the motor deficits seen by Rubino et al. may be due to a non-specific high dose DT-induced damage but not microglial repopulation itself," write Peng et al. in their report.

Peng et al. also used a low dose DT paradigm with two doses of 0.5 µg DT injected i.p. at a 48 h interval. Low dose DT treatment in *DTR* mice was sufficient to induce microglial ablation, similar to high DT dose, but with milder activation of repopulated microglia and without causing severe motor deficits. Peng et al. argue that it is unlikely that DT-induced microglia ablation and repopulation cause ataxia-like behaviors and motor deficits in *DTR* mice, and that including a control group (such as WT mice) is critical for all experiments using DT-mediated cell-depleting approaches. "Experimenters should be aware of possible side effects when using DT on mice," they conclude.

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