> CAR T cells engineered to constitutively produce IL-1 receptor antagonist prevented CRS-related mortality without compromising antitumour activity.

The transfer of T cells engineered to express chimeric antigen receptors (CARs) is a highly specific and effective therapy for a subset of patients with B cell malignancies. But broader use of this powerful approach is limited by potentially lethal toxicities, such as the induction of severe cytokine release syndrome (CRS) and neurotoxicity. Now, two new studies published in *Nature Medicine* have recapitulated these side effects in mouse models to provide a better understanding of the mechanisms involved. They reveal a key role for macrophages in driving CRS and show that the toxic effects can be mitigated by IL-1 blockade.

CAR T cell-associated CRS can occur within the first few days after T cell infusion and is characterized by high serum cytokine levels, fever, vascular leakage, hypotension and sometimes death. In some individuals, a potentially lethal form of neurotoxicity can also develop after several weeks. Severity of CRS is thought to correlate with high tumour burden and peak expansion of the CAR T cells.

Norelli et al. set out to replicate CAR T cell-induced CRS and neurotoxicity using humanized mice, in which the endogenous immune system is replaced with human immune cells, thereby avoiding the induction of graft-versus-host disease after human CAR T cell infusion. When T cells expressing CAR constructs specific for CD19 or CD44 variant isoform 6 (CD44v6) were infused into humanized mice, the T cells expanded in response to the resident human $CD19^+$ B cells or $CD44v6^+$ monocytes, causing long-lasting B cell or monocyte depletion, respectively. Moreover, in both cases the animals developed a violent systemic inflammatory syndrome, similar to human CRS, characterized by severe weight loss, high fever and increased serum levels of pro-inflammatory cytokines, such as IL-6 and tumour necrosis factor. A subset of mice experiencing CRS also developed delayed neurotoxicity. The toxic effects of CAR T cell therapy were even more severe if the humanized mice had a high leukaemia burden and correlated with kinetics of the CAR T cell response. Humanized mice with leukaemia receiving CAR T cells of an irrelevant specificity did not develop CRS but died from leukaemia.

Further analyses showing that the severity of CRS and neurotoxicity was associated with high monocyte numbers and that monocyte depletion completely abated CRS incidence and mortality implicated monocytes as key drivers of CAR T cell-associated CRS. Indeed, single-cell RNA sequencing of leukaemic humanized mice treated with CAR T cells identified monocytes as the main producers of inflammatory cytokines, including IL-1 and IL-6. Importantly, blocking either of these cytokines with the IL-1 receptor antagonist anakinra or with the IL-6 receptor-specific antibody tocilizumab prevented CRS without affecting CAR T cell expansion and leukaemia clearance. However, only mice that were given anakinra were also protected against the subsequent development of lethal neurotoxicity.

Giavridis et al. were also able to recapitulate the development of CAR T cell-associated CRS, this time in immunodeficient mice that had a high tumour burden in the intraperitoneal cavity (although this approach did not model later-onset neurotoxicity). They identified macrophages among several myeloid cell types present at the tumour site to be central producers of IL-6 and IL-1. Indeed, myeloid cells accumulated at the site of CAR T cell–tumour interaction and in the spleen; yet only those proximal to the tumour and CAR T cells produced IL-6. The authors suggest that myeloid cells are activated in the tumour site through direct contact with CAR T cells, in part mediated by CD40–CD40 ligand interactions.

In their model, Giavridis et al. also observed increased expression of inducible nitric oxide synthase (iNOS) by activated macrophages; treatment of mice with iNOS inhibitors alleviated CRS severity. iNOS is known to be induced by IL-1 and IL-6, prompting the authors to examine this pathway. Anakinra treatment inhibited CRS-related mortality and reduced iNOS expression levels. They also elegantly show that CAR T cells engineered to constitutively produce IL-1 receptor antagonist prevented CRS-related mortality without compromising antitumour activity.

So, these studies provide promise that combining IL-1 blockade with CAR T cell therapy could increase the safety and extend the application of this cancer treatment.

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ORIGINAL ARTICLES Norelli, M. et al. Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. *Nat. Med.* **24**, 739–748 (2018) | Giavridis, T. et al. CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. *Nat. Med.* **24**, 731–738 (2018)