IMMUNE REGULATION

TOX for tired T cells

T cell 'exhaustion' or dysfunction is a common feature of chronic viral infections and cancer, in which persistent antigenic stimulation of T cells causes an attenuation of their effector functions and the upregulation of inhibitory receptors. The mechanisms controlling the differentiation of exhausted T cells have remained elusive, but six recent studies now describe a crucial role for the transcription factor TOX.

The studies by Alfei et al., Khan et al., Scott et al. and Yao et al. explored $CD8^+$ T cell differentiation using mouse models of infection with lymphocytic choriomeningitis virus (LCMV) strains Armstrong and clone 13, which share dominant T cell epitopes but induce acute and chronic infections, respectively. This allowed the authors to compare the gene expression programmes that are associated with the various $CD8^+$ T cell subsets that emerge following acute and chronic antigenic stimulation. Each group identified several genes that were differentially expressed between effector, memory and exhausted $CD8^+$ T cell subsets; an important common finding was the association of *Tox* expression with the exhausted T cell phenotype. TOX protein levels were increased and remained high in exhausted $CD8^+$ T cells that developed during chronic LCMV infection, whereas only low-level and transient TOX upregulation was seen in effector and memory $CD8^+$ T cells during acute LCMV infection.

In keeping with this, Alfei et al. and Khan et al. found that LCMV-specific $CD8^+$ T cells in which TOX was conditionally deleted or rendered non-functional could generate effector and memory T cell populations but not exhausted T cells. During chronic LCMV infection, TOX-deficient $CD8^+$ T cells showed decreased expression of inhibitory receptors, such as PD1, and increased expression of molecules associated with effector functions, including KLRG1, TNF and IFNγ. A similar link between TOX and PD1 expression was seen in T cells from patients with chronic hepatitis C virus infection, and several of the studies found that overexpression of TOX in mouse T cells or in healthy human T cells that had been activated ex vivo led to increased PD1 expression and decreased expression of KLRG1, TNF and IFNγ.

Yao et al., Alfei et al. and Khan et al. identified a key role for TOX in promoting the survival of the progenitor-like $TCF1^+CD8^+$ T cells that arise during chronic LCMV infection and can replace terminally exhausted $CD8^+$ T cells. TOX deficiency led to a loss of these progenitor-like T cells during chronic LCMV infection, and overexpression of *Tox* enhanced the persistence of progenitor-like $CD8^+$ T cells and exhausted $CD8^+$ T cells.

Alfei et al. also showed that adoptive transfer of LCMV-specific TOX-deficient T cells led to a greater reduction in viral titres at early stages of infection, but this was associated with increased immunopathology. This suggests that the exhausted phenotype that is mediated by TOX in T cells is important for mitigating tissue damage during chronic infection.

A similar link between TOX and T cell exhaustion was seen in the setting of cancer. Scott et al., Khan et al. and Wang et al. detected high TOX expression in tumour-infiltrating lymphocytes (TILs) isolated from patients with cancer and they showed that TOX expression positively correlated with the expression of PD1 and other markers of exhaustion. In mouse tumour models, Scott et al. found that TOX-deficient tumour-specific $CD8^+$ T cells failed to upregulate inhibitory receptors and ultimately became overstimulated and underwent activation-induced cell death. Although notably, and in contrast to the findings made by several other groups, Scott et al. did not see upregulation of effector molecules in TOX-deficient tumour-specific $CD8^+$ T cells, and these cells were still dysfunctional in their cancer models. They therefore suggest that the regulation of inhibitory receptor expression is uncoupled from effector $CD8^+$ T cell functions. By contrast, in a distinct model, Seo et al. showed that tumour-specific chimeric antigen receptor (CAR) T cells lacking TOX and TOX2 showed both decreased expression of inhibitory receptors and increased antitumour effector T cell function; likewise, Khan et al. and Wang et al. found that TOX-deficiency in $CD8^+$ T cells could improve tumour control. Furthermore, Seo et al. observed reciprocal regulation between TOX, TOX2 and members of the NR4A family, which they had previously shown to contribute to T cell exhaustion in tumours.

> “TOX promotes the acquisition of an exhausted phenotype in $CD8^+$ T cells following chronic antigenic stimulation”

Most of the groups showed that NFAT transcription factors are necessary for inducing TOX in chronically stimulated $CD8^+$ T cells, with the authors reporting that TOX promotes an exhausted or dysfunctional phenotype in $CD8^+$ T cells by driving epigenomic remodelling events at key gene loci.

These studies collectively suggest that TOX promotes the acquisition of an exhausted phenotype in $CD8^+$ T cells following chronic antigenic stimulation that is important for preventing T cell overstimulation and activation-induced cell death, as well as for limiting tissue pathology.

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ORIGINAL ARTICLE Scott, A. C. et al. TOX is a critical regulator of tumour-specific T cell differentiation. *Nature* https://doi.org/10.1038/s41586-019-1324-y (2019) | Khan, O. et al. TOX transcriptionally and epigenetically programs $CD8^+$ T cell exhaustion. *Nature* https://doi.org/10.1038/s41586-019-1325-x (2019) | Alfei, F. et al. TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. *Nature* https://doi.org/10.1038/s41586-019-1326-9 (2019) | Yao, C. et al. Single-cell RNA-seq reveals TOX as a key regulator of $CD8^+$ T cell persistence in chronic infection. *Nat. Immunol.* **20**, 890–901 (2019) | Seo, H. et al. TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose $CD8^+$ T cell exhaustion. *Proc. Natl Acad. Sci. USA* **116**, 12410–12415 (2019) | Wang, X. et al. TOX promotes the exhaustion of antitumor $CD8^+$ T cells by preventing PD1 degradation in hepatocellular carcinoma. *J. Hepatol.* https://doi.org/10.1016/j.jhep.2019.05.015 (2019)