IMMUNOMODULATORS

# Enhancing regulation

**DOI:**
10.1038/nrd2473

Currently under clinical investigation as anticancer therapies, histone deacetylase inhibitors (HDACis) also have anti-inflammatory effects, but the underlying mechanisms of action are not clear. Hancock and colleagues now show that HDACi therapy increases the number and suppressor ability of regulatory T cells ($T_{reg}$ cells), which are key mediators of immune tolerance. They also provide evidence that HDACis have therapeutic potential for autoimmune diseases and preventing transplant rejection.

Using three different mouse models, the authors showed that HDACi treatment enhances the production of $T_{reg}$ cells by either increasing thymic output of $T_{reg}$ cells or peripheral conversion of conventional T cells (non-$T_{reg}$ cells) into $T_{reg}$ cells. HDACis are known to increase histone acetylation, resulting in chromatin remodelling and modulation of gene transcription. HDACi therapy also increases expression of $T_{reg}$-associated genes such as *Foxp3* and cytotoxic T-lymphocyte-associated protein 4 (*Ctla4*). *In vitro*, HDACi-treated $T_{reg}$ cells were two- to three-fold more effective than wild-type $T_{reg}$ cells at suppressing proliferation of T effector ($T_{eff}$) cells, which produce cytokines associated with inflammation.

Specifically investigating HDAC expression in $T_{reg}$ cells, the authors showed that HDAC9 expression was higher in these cells than in non-$T_{reg}$ cells. Following T-cell receptor stimulation, expression of HDAC9 in non-$T_{reg}$ cells decreased as they became $T_{eff}$ cells, whereas expression in $T_{reg}$ cells was 30-fold higher. Furthermore, *Hdac9*$^{-/-}$ mice had significantly more $T_{reg}$ cells compared with wild-type mice with enhanced expression of *Foxp3*, *Ctla4* and *Gitr*, and increased acetylation of Foxp3, indicating that HDAC9 is a regulator of $T_{reg}$-associated genes.

To further investigate Foxp3 acetylation, western blotting and immunoprecipitations were performed on HDACi-treated $T_{reg}$ and non-$T_{reg}$ cells, revealing increased Foxp3 acetylation as well as increased binding of Foxp3 to promoter regions of target genes in the HDACi-treated $T_{reg}$ cells. The importance of Foxp3 acetylation for the suppressor activity of $T_{reg}$ cells was also demonstrated by transduction of non-$T_{reg}$ cells with a vector encoding wild-type Foxp3, which resulted in non-$T_{reg}$ cells with suppressive ability equivalent to $T_{reg}$ cells. Mutagenesis studies showed that FOXP3 protein acetylation was required for avid DNA binding and Foxp3-dependent suppression and modulation of $T_{reg}$ gene expression. Taken together, these data show that HDACi therapy increases histone and Foxp3 acetylation, as well as FOXP3 protein expression and DNA binding.

Suppressor activity of HDACi-enhanced $T_{reg}$ cells *in vivo* was explored using three models. First, co-transfer of HDACi-treated $T_{reg}$ and non-$T_{reg}$ cells into immunodeficient mice resulted in substantially decreased T-cell proliferation compared with non-$T_{reg}$ cells alone. Second, in a model of colitis, HDACi treatment increased the number of $T_{reg}$ cells in lymphoid tissues and decreased disease severity. Last, HDACi treatment was shown to modestly prolong the survival of cardiac and islet transplants, with addition of low-dose rapamycin inducing permanent transplant survival. Overall, inhibiting HDACs has a broad therapeutic potential for immunomodulation.

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**ORIGINAL RESEARCH PAPER** Tao, R. *et al.* Deacetylase inhibition promotes the generation and function of regulatory T cells. *Nature Med.* **13**, 1299–1307 (2007)
**FURTHER READING** Bolden, J. E. *et al.* Anticancer activities of histone deacetylase inhibitors. *Nature Rev. Drug Discov.* **5**, 769–784 (2006)