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REGULATORY T CELLS

MARCHing for tolerance

> This finding challenges the prevailing hypothesis that higher avidities ... favour negative selection over T_{Reg} cell development

Thymus-derived regulatory T (T_{Reg}) cells are essential for the establishment of immune tolerance. Now, Oh *et al.* report that the development of T_{Reg} cells in the thymus requires the expression of the E3 ubiquitin ligase MARCH1 (membrane-associated RING-CH protein 1) in dendritic cells (DCs).

MARCH1 contributes to the control of T cell activation by ubiquitylating and promoting the endocytosis and degradation of MHC class II and CD86 molecules in DCs. In this study, the authors investigated the role of MARCH1 in T cell development. Among thymic antigen-presenting cells (APCs), MARCH1 was expressed by conventional DCs, plasmacytoid DCs and B cells, but not by thymic epithelial cells (TECs). Consistent with this finding, the deletion of MARCH1 resulted in increased cell surface expression of MHC class II and CD86 molecules on the thymic APCs that were of haematopoietic origin, but not on TECs.

Total thymocyte numbers, the percentages of thymocyte subsets and the T cell receptor (TCR) repertoire of single positive $CD4^+$ and $CD8^+$ thymocytes were normal in *March1*$^{-/-}$ mice. However, T_{Reg} cells were reduced by 50% in *March1*$^{-/-}$ mice compared with wild-type mice and this reduction could not be attributed to defects in T_{Reg} cell proliferation or survival. Experiments using bone marrow chimaeras showed that MARCH1 expression in haematopoietic cells is required for the thymic development of T_{Reg} cells, and that the role of MARCH1 in this process is T_{Reg} cell extrinsic, which suggests that MARCH1-expressing DCs might be required.

OT-II thymocytes are specific for an MHC class II-bound ovalbumin (OVA) peptide, and in OT-II transgenic mice that express membrane-bound OVA in medullary TECs (mTECs) under the control of the rat insulin promoter (RIP–mOVA OT-II mice), most OT-II thymocytes undergo negative selection and many of the remaining thymocytes develop into T_{Reg} cells. Deletion of MARCH1 did not affect negative selection in RIP–mOVA OT-II mice, but it reduced T_{Reg} cell numbers in the thymus. This indicates that uptake and presentation of mTEC-derived antigens by MARCH1-expressing APCs is important for T_{Reg} cell development.

Similarly, a lack of MARCH1 or defective MHC class II ubiquitylation impeded the differentiation of OT-II thymocytes to T_{Reg} cells without affecting negative selection following the uptake of intravenously injected OVA by $SIRP\alpha^+$ thymic DCs. Moreover, *March1*$^{-/-}$ thymus- or bone marrow-derived DCs were less efficient than wild-type DCs at promoting the generation of T_{Reg} cells from thymocytes *in vitro*. Taken together, these findings suggest that the presentation of endogenous and exogenous antigens by MARCH1-expressing DCs in the thymus is involved in T_{Reg} cell development.

Intriguingly, DCs from *March1*$^{-/-}$ mice, as well as from knock-in mice expressing MHC class II molecules that could not be ubiquitylated by MARCH1, presented higher levels of endogenous and exogenous antigens compared with DCs from wild-type mice. This finding challenges the prevailing hypothesis that higher avidities in the interactions between self-reactive thymocytes and APCs favour negative selection over T_{Reg} cell development. Future studies will help to clarify the exact mechanism through which MARCH1 expression in DCs promotes thymic T_{Reg} cell development.

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ORIGINAL RESEARCH PAPER Oh, J. *et al.* MARCH1-mediated MHCII ubiquitination promotes dendritic cell selection of natural regulatory T cells. *J. Exp. Med.* **210**, 1069–1077 (2013)

FURTHER READING Hsieh, C.-S., Lee, H.M. & Lio, C.W. Selection of regulatory T cells in the thymus. *Nature Rev. Immunol.* **12**, 157–167 (2012)