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REGULATORY T CELLS

Going over to the dark side

> bona fide T_{Reg} cells can lose FOXP3 expression and become exFOXP3 cells in inflammatory settings

Reporting in *Immunity*, Bluestone and colleagues show that a subset of fully committed forkhead box P3 $(FOXP3)^+$ regulatory T (T_{Reg}) cells can become unstable in response to antigen activation during an inflammatory autoimmune response and participate in disease pathogenesis.

The concept of T_{Reg} cell instability is not new — previous studies have shown that T_{Reg} cells can lose FOXP3 expression (turning into 'exFOXP3' cells) and become pathogenic. However, it has been argued that exFOXP3 cells derive from an early, 'aborted' T_{Reg} cell differentiation pathway rather than from fully differentiated, thymus-derived T_{Reg} cells, which have been suggested to be stable. Determining the source of exFOXP3 cells has important clinical implications, as T_{Reg} cell-based therapies are currently in clinical trials and T_{Reg} cell instability could lead to unwarranted effects in patients.

In this study, the authors used C57BL/6 dual-reporter mice that allow for the identification of $FOXP3^+$ T_{Reg} cells and exFOXP3 cells in the same animal. Experimental autoimmune encephalomyelitis (EAE; a mouse model of multiple sclerosis) was then induced in these mice using a myelin oligodendrocyte glycoprotein (MOG) antigen. Analysis of these mice showed that a subset of MOG-specific $CD4^+$ T_{Reg} cells preferentially downregulated FOXP3 expression compared with polyclonal T_{Reg} cells during the induction and peak phases of the disease. However, FOXP3 expression was restored at a population level during the resolution of the inflammatory response, either because of reversion of the exFOXP3 cells or replacement by a more stable $FOXP3^+$ T_{Reg} cell population.

To determine whether these exFOXP3 cells derive from bona fide T_{Reg} cells, the authors transferred purified T_{Reg} cells to mice during the onset of EAE. Most of the transferred polyclonal T_{Reg} cells maintained stable FOXP3 expression, whereas FOXP3 expression was significantly lower in transferred MOG-specific T_{Reg} cells, with FOXP3 being low or undetectable in 39% of these cells. Next, the authors examined the methylation status of CpG motifs in the T_{Reg} cell-specific demethylated region (TSDR) of the *Foxp3* locus. Previous studies have shown that these motifs are fully methylated in unstable T_{Reg} cells and conventional T cells but are demethylated in T_{Reg} cells. Interestingly, the CpG motifs in the TSDR of MOG-specific exFOXP3 cells isolated from the central nervous system (CNS) during the peak of EAE were predominantly demethylated, which supports the hypothesis that bona fide T_{Reg} cells can lose FOXP3 expression and become exFOXP3 cells in inflammatory settings.

But do these cells contribute to disease pathogenesis? CNS-derived exFOXP3 cells that were stimulated *in vitro* produced interferon-γ at levels comparable with those produced by pathogenic effector T cells. Furthermore, transferred MOG-specific exFOXP3 cells induced EAE in T cell-deficient mice with similar incidence and severity to that induced by pathogenic effector T cells.

Interleukin-2 (IL-2) is crucial for the expression of FOXP3 in T_{Reg} cells, and the authors showed that treatment of mice with complexes of IL-2 and non-neutralizing IL-2-specific antibodies at the initiation of EAE reduced the proportion and number of MOG-specific exFOXP3 cells. The treated mice were shown to be protected from disease.

These data show that bona fide self-antigen-specific T_{Reg} cells can become unstable and contribute to a pathogenic immune response in a mouse model of autoimmunity, but this instability can be rescued by treatment with IL-2–IL-2-specific antibody complexes.

Olive Leavy

ORIGINAL RESEARCH PAPER Bailey-Bucktrout, S. L. *et al.* Self-antigen-driven activation induces instability of regulatory T cells during an inflammatory autoimmune response. *Immunity* **39,** 949–962 (2013)
FURTHER READING Sakaguchi, S. *et al.* The plasticity and stability of regulatory T cells. *Nature Rev. Immunol.* **13**, 461–467 (2013)