Tregs strip dendritic cells of CD70 to regulate Th1 differentiation

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When the immune system encounters an antigen, the response can result in the mobilization of effector cells or in tolerance. The outcome is largely dependent on immunosuppressive CD4 T cells that express the transcription factor Foxp3 (Tregs). Yet, how Tregs control different immune effector cells remains elusive. In this issue of *The EMBO Journal*, Dhainaut *et al* report on a novel mechanism used by Tregs to prevent differentiation of naïve CD4 T cells to proinflammatory Th1CD4 (Th1) effectors.

See also: **M Dhainaut *et al*** (May 2015)

Various subsets of CD4 T cells have different immune functions. For example, CD4 Th1 or Th17 effectors are proinflammatory and support innate immune responses, Th2 or follicular (T_{FH}) effectors help B cells make antibodies, and Tregs inhibit recognition of tissue-specific self-antigens. Tregs are the key component of the machinery responsible for peripheral tolerance. To adjust regulatory mechanisms to a specific target, Tregs express transcription factors that are typically expressed by pathogenic T effectors that are being silenced. For example, Th1 effectors and Tregs that control them share expression of Tbet transcription factor, whereas Tregs that control Th2 CD4 differentiation often express IRF4 (Koch *et al*, 2009; Zheng *et al*, 2009). Transient expression of these transcription factors may program Tregs to promote optimal suppression like secretion of inhibitory cytokines, IL-2 deprivation, metabolic disruption of effectors or modulation of co-stimulatory molecules on APCs (von Boehmer, 2005). Other mechanisms involve stripping DCs of CD80 following

ligation to CTLA-4 (Qureshi *et al*, 2011), or as reported by Dhainaut *et al*, internalization of CD70 by DCs upon binding to CD27 on Tregs. The significance of CD70 as a decision maker for Th1 differentiation was first noted when exposure to *Leishmania* major derived antigen drove naïve CD4 T-cell differentiation to Th1 subset independently of IL-12 (Soares *et al*, 2007). It was also found that binding of CD27 on precursors of thymus-derived Tregs (tTregs) to CD70 expressed on medullary epithelial cells (mTECs) rescues the former cells from apoptosis, demonstrating that early in development, tTregs use the CD27/CD70 interactions to enhance their survival (Coquet *et al*, 2013b). Whether these interactions provide similar homeostatic advantage to mature, peripheral Tregs is currently unclear.

To investigate the mechanisms that endow Tregs with ability to suppress the priming of Th1 precursors in an antigen-specific manner, Moser and colleagues studied the magnitude of Th1 responses in mice challenged with KLH-pulsed DCs, where some recipients had Tregs depleted by co-injection of the anti-CD25 monoclonal antibody (MoAb). Comparison of Th1 responses between the immunized groups treated or not with anti-CD25 MoAb revealed that far more IFN- γ was produced by peripheral CD4 cells in mice depleted of Tregs. Because IL-12 signaling pathway is known to play an important role in Th1 differentiation, the authors used the same immunization protocol to prime control and IL-12p40-deficient mice. The latter strain lacks the p40 subunit common for IL-12 and IL-23. In spite of lack of IL-12, CD4 cells harvested from these mice after priming and *in vitro* re-stimulation produced similar quantities of IFN- γ as similarly treated cells

from control mice. This result implied that IL-12 signaling is dispensable for Treg control of antigen-specific Th1 priming.

Since binding of CD27 to CD70 downregulates CD70 expression (Kuka *et al*, 2013), and as CD70/CD27 interactions inhibit the responses of Th17 CD4 T cells (Coquet *et al*, 2013a), the authors also examined whether engagement of CD27 on Tregs inhibits CD70 co-stimulation. When CD70 was partially blocked, Treg depletion only marginally enhanced antigen-specific IFN- γ response. In addition, mice that lacked CD27 on T cells or CD70 on DCs showed no enhanced IFN- γ production in response to antigen priming when their Tregs were depleted. In sum, these results suggested that CD27/CD70 interactions influence Th1 responses and that differentiation of these effectors is influenced by Tregs. To directly investigate whether Tregs regulate Th1 activation by modulating the surface levels of CD70 on DCs, the expression of this co-stimulatory molecule and CD86 was examined on DCs re-stimulated in co-cultures with Tregs isolated from CD27-deficient or sufficient mice. First, they observed that loss of Tregs resulted in elevated number of CD70⁺ DCs and increased CD70 expression on these cells. In contrast, lack of Tregs had no effect on the expression of class II MHC or CD86 on DCs, but increased the amount of IFN- γ produced by Th1 cells. The authors excluded that soluble factors downregulate CD70 expression, and showed that this phenomenon requires direct cell-cell contact. They found that only CD27-expressing CD4 T cells could reduce CD70 expression on DCs in co-cultures, and that reduced CD70 levels correlates with concomitant lower expression of CD27 on co-cultured Tregs and activated CD4 T cells. These results implied that upon

Figure 1. Interactions between CD27 on Tregs and CD70 on DCs prevent priming of Th1CD4 T cells.

Within immunological synapse formed between naïve conventional CD4 T cells and DCs, TCRs engage MHC/peptide complexes while CD27 and CD28 co-receptors bind their specific ligands on DCs: CD70 and CD80/86, respectively. These stimulatory interactions induce differentiation of naïve, conventional (non-Treg) CD4 T cells to interferon gamma-producing Th1CD4 T cells. On the other hand, interaction of CD27 on Tregs with CD70 on DCs results in internalization and degradation of CD27/CD70 complex by DCs. As a consequence, DCs become deprived of co-stimulatory molecules required for priming of conventional CD4 T cells, thus preventing their differentiation to Th1CD4 effectors.

engagement, both molecules are partially eliminated from the surface of interacting cells. To examine where CD70/CD27 complexes allocate from the cell surface, investigators used tracking experiments that involved Tregs expressing CD27GFP and DCs expressing CD70 labeled with different fluorescent protein. These experiments determined that CD70/CD27 complexes clustered within the immunological synapse and shortly afterwards moved from the contact site into intracellular compartment of DCs. How exactly the fragments of membrane were exchanged is not yet known, but observation of nanotubules extending from DCs suggested formation of transient intercellular communication channels through which cell

surface proteins can traffic between immune cells (Davis & Sowinski, 2008).

Reportedly, mice that overexpress CD70 on APCs are prone to autoimmunity, whereas mice that lack CD70 had reduced antigen-specific CD8 but not CD4 T-cell response (Munitic *et al.*, 2013). Notably, genetic ablation of CD27 or CD70 reduced Treg numbers in the thymus and peripheral lymphoid tissues, and CD70-deficient Tregs were less efficient in controlling Th1 differentiation as compared to Tregs from wild-type mice (Coquet *et al.*, 2013b; Allam *et al.*, 2014). This could be in part due to the aforementioned anti-apoptotic signals provided by CD27/CD70 interaction to thymic precursors of Tregs. On the other

hand, DCs that had CD70 overexpressed induced *ex vivo* effectors that were resistant to Treg suppression and induced plasticity in Treg lineage commitment (Pen *et al.*, 2013). This finding suggests that the CD70-CD27 pathway may regulate Treg functionality and phenotype, although the physiological significance of this phenomenon remains to be determined.

The results of Dhainaut *et al.* give rise to a number of new questions. Do thymus and peripherally derived Tregs target the same or different co-stimulatory receptors on the surface of DCs? Does intrathymic CD27/CD70 interaction during tTreg development pre-disposes tTregs to utilize internalization of CD70/CD27 as regulatory mechanism in the periphery? Why does the internalization of CD70 not occur within the immunological synapse formed between naïve and dendritic cells? What are the potential therapeutic implications of the anti-inflammatory actions of Tregs via CD27/CD70 for different autoimmune diseases? Regardless of all the questions that remain to be answered, the work of Dhainaut *et al.* not only describes a new mechanism of Treg suppression, but also suggests that therapeutic interference with co-stimulatory CD70 molecules can be applied for the treatment of auto-aggressive disorders.

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