

# Oral tolerance: is it all retinoic acid?

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**Oral tolerance has been argued to depend on "special" presentation of antigen in the gut. New studies support this idea by showing that the catalysis of vitamin A into retinoic acid (RA) in gut-associated dendritic cells (DCs) enhances the transforming growth factor (TGF)- $\beta$ -dependent conversion of naive T cells into regulatory T (T reg) cells and also directs T reg cell homing to the gut. These results reveal new tolerance mechanisms that will aid the use of T reg cells in the clinic.**

## The mystery of oral tolerance

Oral tolerance has been on the minds of people even before rational approaches to immunity. Every now and then, someone realized that eating certain substances could reduce their otherwise "toxic" impact on the body. As an example, the swallowing of hair dyes by aging men was claimed to interfere with their allergizing properties, thereby permitting less painful "rejuvenation." In the immunology world, oral tolerance after enteral uptake of antigen became known as "immunologic unresponsiveness." Special antigen-presenting cells in the gut were held responsible for inducing oral tolerance, and local generation of "suppressor" cells was raised as a possible mechanism (1). Such extrapolations, however, were not free of controversy, which may have to do with different guts of different individuals. It seems, however, that the times of "gut feelings" are over, as scientific evidence now sheds new light on the mystery of oral tolerance. Three new papers demystify oral tolerance by showing that RA made by gut-associated DCs enhances the conversion of T cells into T reg cells (see Coombes et al. [2] on p. 1757 of this issue; Sun et al. [3] on p. 1775 of this issue; and Benson et al. [4] on p. 1765 of this issue).

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## Gut-associated lymphoid tissue (GALT)

The GALT consists of lymphoid cells in Peyer's patches, mesenteric lymph nodes, lamina propria, and gut epithelium. In GALT, DCs in both the Peyer's patches and mesenteric lymph nodes exhibit a unique CD103<sup>+</sup>, CD8<sup>-</sup>, CD11b<sup>+</sup> phenotype. It was initially demonstrated that recognition of antigen on the surface of these DCs by lymphocytes made the lymphocytes return to the gut (5). This process is mediated by the DC-induced expression of the  $\alpha 4\beta 7$  integrin on lymphocytes, which then binds to the mucosal addressin cell adhesion molecule on blood vessels in the gut (6). These lymphocytes also express CC chemokine receptor (CCR) 9, which binds to the thymus-expressed chemokine ligand on epithelial cells in the crypts of the small intestine (7). Subsequently, it was discovered that the vitamin A metabolite RA was responsible for the upregulation of the  $\alpha 4\beta 7$  integrin and CCR9. The CD103<sup>+</sup>, gut-associated DCs express relatively high levels of retinal dehydrogenases, the enzymes required for the irreversible generation of RA from vitamin A (retinal). Inhibiting these enzymes reduced the expression of the  $\alpha 4\beta 7$  integrin on T cells and resulted in their depletion from the intestinal lamina propria (8). These results explained why T cells stimulated by antigen on gut-associated DCs return to GALT.

## T reg cells

T reg cells have been postulated to play an important role in oral tolerance.

Research on this cell type has been on the upswing since specific markers became available that allow these cells to be distinguished from other T cells; among these markers are the  $\alpha$  chain of the high affinity interleukin (IL) 2 receptor (CD25) on the cell surface and the transcription factor FoxP3. FoxP3 is essential for the generation and function of certain T reg cells, which prevent deadly autoimmune disease by suppressing effector T cells (9). T reg cells can be generated intrathymically upon confrontation with T cell receptor agonist ligands expressed by thymic epithelial cells (10). This process does not require TGF- $\beta$  or intact TGF- $\beta$  signaling in developing T cells but is dependent on co-stimulation by the CD28 receptor. The generation of Foxp3-expressing T reg cells is not restricted to the thymus, however, as formation of these cells can be induced in peripheral lymphoid tissue (11, 12). In this case, the modalities of T reg cell induction are different from those in the thymus. It was found that generation of T reg cells in peripheral lymphoid organs required antigen presentation in the absence of co-stimulation (i.e., antigen presentation under subimmunogenic conditions) (11, 12). Furthermore, the conversion of naive T cells into T reg cells was shown to require intact TGF- $\beta$  signaling in the T cells, indicating an obligatory role for TGF- $\beta$  in this process (12). These results contrast somewhat with experimental conditions that induce T reg cell conversion in vitro (13)—stimulation with CD3 and CD28 antibodies in the presence of TGF- $\beta$ . This particular protocol of inducing conversion in the presence of CD28 antibodies may be responsible for the apparent instability of Foxp3 expression by in vitro-converted cells (14), whereas Foxp3 expression of in vivo-converted cells is more stable (11, 12). The results from Benson et al.

actually show that strong co-stimulation *in vitro* is, in fact, also counterproductive to TGF- $\beta$ -dependent T reg cell conversion (4).

### RA and T reg cell conversion *in vivo* and *in vitro*

The new papers by Coombes et al. and Sun et al. now show that conversion takes place more effectively in GALT than in other lymphoid tissues (2, 3) and that  $\sim 1.3\%$  of ovalbumin-specific T cells converted into T reg cells when mice were given ovalbumin in their drinking water (3). These *in vivo* conversion rates are rather low compared with those seen when antigen is delivered systemically via the parenteral route under subimmunogenic conditions (i.e., when co-stimulation is lacking). Under these conditions, 10–20% of antigen-specific cells convert into T reg cells (11, 12). Both Coombes et al. and Sun et al. go on to show that antigen-presenting CD103<sup>+</sup>, but not CD103<sup>-</sup>, DCs purified from GALT induced the generation of Foxp3<sup>+</sup> cells *in vitro*. The conversion induced by CD103<sup>+</sup> cells could be diminished by adding inhibitors of retinal dehydrogenases, indicating that RA produced in CD103<sup>+</sup> DCs contributed to the conversion process (Fig. 1) (2, 3). According to Coombes et al., this conversion process also required TGF- $\beta$ , as antibody-mediated inhibition of TGF- $\beta$  blocked the CD103<sup>+</sup> DC-induced conversion (2). Indeed, RA alone could not induce conversion but strongly enhanced the TGF- $\beta$ -dependent conversion of naive T cells into Foxp3<sup>+</sup> T reg cells *in vitro* (2–4). As previously noted for conventional T cells, RA was also responsible for the up-regulation of the  $\alpha 4\beta 7$  integrin on the converted T reg cells (2–4), enabling them to home efficiently to GALT (4).

With regard to the negative role of co-stimulation in the conversion process that was previously shown in *in vivo* studies (12), it is of interest that Benson et al. now show that the TGF- $\beta$ -dependent conversion *in vitro* becomes much less efficient when the T cells are strongly co-stimulated by increasing doses of CD28 antibodies (4). The new experiments by Benson et al.

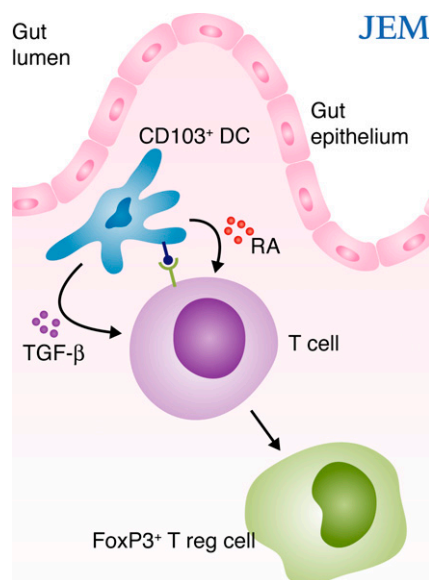
also show that RA interferes, at least partially, with the negative impact of co-stimulation in the conversion process (4).

Finally, Foxp3 expression induced in T reg cells by RA and TGF- $\beta$  appeared stable *in vitro* (2–4) but less stable after antigenic stimulation *in vivo* (4). However, it is somewhat difficult to determine whether some of the results are caused by outgrowth of contaminating Foxp3<sup>-</sup> cells rather than the instability of Foxp3 expression.

In summary, the results of these three papers (2–4) show that CD103<sup>+</sup> DCs in GALT are specially equipped for converting antigen-specific T cells into Foxp3<sup>+</sup> T reg cells in an RA- and TGF- $\beta$ -dependent manner. The RA-enhanced conversion process also leads to the up-regulation of  $\alpha 4\beta 7$  integrin and CCR9 permitting the newly formed T reg cells to accumulate preferentially in GALT. Finally, RA can reduce the negative impact of co-stimulation on the TGF- $\beta$ -dependent conversion of T cells into Foxp3<sup>+</sup> T reg cells.

### Where to go from here?

The new experiments lend credibility to the notion of oral tolerance by elabo-

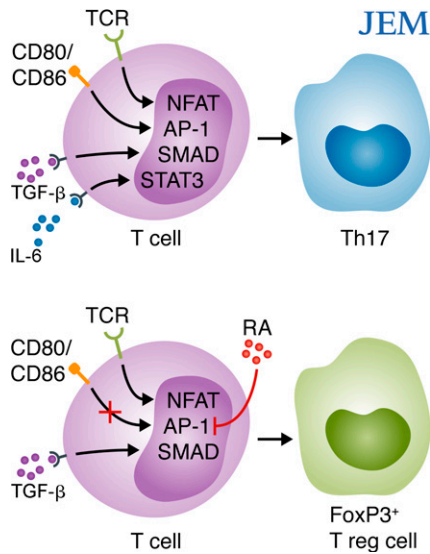


**Figure 1. RA promotes the differentiation of Foxp3<sup>+</sup> T reg cells in the gut.** Antigen from the gut lumen gains access to CD103<sup>+</sup> gut-associated DCs. T cells that recognize antigen in the context of DC-produced RA and TGF- $\beta$  up-regulate Foxp3 and become T reg cells.

rating a gut-specific mechanism of RA-enhanced, TGF- $\beta$ -dependent conversion of T cells into T reg cells (Fig. 1). It remains to be seen whether this type of conversion induced by CD103<sup>+</sup> DCs in GALT represents the sole mechanism involved in oral tolerance.

RA binds to nuclear RA receptors (RARs) that regulate genes both directly and indirectly. RAR $\beta$  contains an RA-responsive element in its promoter, which upon RA binding induces more RAR $\beta$  expression (15). Interestingly, RAR ligation can inhibit the transcriptional activity of activating protein-1 (AP-1), a dimeric transcription factor that, in mammals, consists mostly of Jun and Fos proteins, perhaps by a direct protein–protein interaction with c-Jun (16). AP-1 is induced through co-stimulation of T cells and regulates gene expression of, for instance, the IL-2 gene in a DNA binding complex with nuclear factor of activated T cells (NFAT; Fig. 2). It has recently been shown that Foxp3 also regulates gene expression in a DNA binding complex with NFAT (17, 18) and that AP-1 can interfere with the formation of the Foxp3–NFAT complex (17). How co-stimulation interferes with conversion of T cells into T reg cells is presently unknown. AP-1 may interfere with stable Foxp3 expression that is induced by TCR signals and by TGF- $\beta$  and may involve a Foxp3-dependent autoregulatory loop. RA will in turn interfere with the negative effect of co-stimulation in this process by inhibiting the action of AP-1 (Fig. 2). In fact, this might be the sole mechanism by which RA enhances TGF- $\beta$ -dependent conversion of T cells into T reg cells.

It is of interest to note that Ito cells in the liver are a major storage place for vitamin A in the body. It is, however, not known whether these cells can metabolize vitamin A into RA. If so, these cells could also be involved in the conversion of naive T cells into T reg cells, even though a recent study suggested more of an immunogenic than a suppressive role for these cells (19). Yet, this study does not strictly rule out a role for Ito cells in T reg cell conversion.



**Figure 2. Model of gene regulation that leads to the differentiation of T helper type 17 or T reg cells.** TCR stimulation and co-stimulation of T cells results in NFAT and AP-1 activation, which form a DNA binding complex that regulates gene expression. TGF- $\beta$  signaling results in phosphorylation and nuclear translocation of TGF- $\beta$ -associated Smad proteins, and IL-6 signaling activates Stat3. These signals combine to promote differentiation into T helper type 17 (Th17) effector cells. In the absence of co-stimulation and/or the presence of RA, AP-1 is not produced. In the absence of IL-6, Stat3 is not activated. Without AP-1 and Stat3, the cell converts into a Foxp3-expressing T reg cell in a TCR and TGF- $\beta$  signaling-dependent manner.

It certainly appears useful for the clinical application of T reg cells that RA, which is readily available, enhances the antigen-induced and TGF- $\beta$ -dependent conversion of T cells into T reg cells in vitro and in vivo. However, because of the side effects of RA, other RAR agonists may be better suited for in vivo experiments. It will be important to determine whether RA is suitable only for the conversion of

naive T cells into T reg cells or whether it can also be used to convert already activated and polarized T cells and, thereby, to interfere with unwanted, ongoing immune responses.

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