

EDITORIAL

Clinical & Experimental Allergy

Induction of regulatory T cells by probiotics: potential for treatment of allergy?

This editorial discusses the findings in the paper by de Roock et al. [23] pp. 103–10.

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Induction of regulatory T cells (Tregs) is an attractive goal in treatment of diseases characterized by an overreaction of the immune system. Such diseases include autoimmune diseases, inflammatory metabolic diseases, asthma and allergy, all of which are becoming more prevalent in most parts of the world. Certain bacteria exploit regulatory mechanisms of the immune system to escape elimination, whereas other bacteria induce regulatory mechanisms as a part of maintaining an immunologically quiescent environment in mucosal tissues. These findings have prompted the investigation of specific bacteria as inducers of regulatory T cells, especially focusing on the gut-derived lactic acid bacteria termed 'probiotic' bacteria, which possess potential health-promoting properties. If a regulatory function can be proven, these non-pathogenic bacteria, which are easily produced and consumed, can be an extremely valuable means to prevent and treat inflammatory diseases.

Regulatory CD4-positive T cells (Tregs) were first described in 1994, as key players in the establishment of oral tolerance to soluble antigen [1], and this incited a renewed interest in the field of suppressor T cells abandoned since the late 1980s. Multiple Treg subsets have been identified; most of them considered belonging to either 'naturally occurring' Tregs or 'inducible' Tregs. Naturally occurring Tregs are imprinted with regulatory properties in the thymus, and exit from there with the ability to down-regulate the proliferation of other T cells [2]. In the periphery, Tregs are induced from naïve T cells through the action of the anti-inflammatory cytokines IL-10 and TGF- β and/or through cell-to-cell contact with naturally

occurring Tregs [1, 3]. Besides acting on T cell proliferation, Tregs can control the activity of B cells and other antigen-presenting cells (APCs). The regulatory action of Tregs can be antigen dependent or independent and is (like the generation of induced Tregs) mediated by secretion of IL-10 and TGF- β and cell-to-cell contact. The study of Tregs is hampered by the fact that true markers of regulatory capacity are lacking, as the preferred extracellular Treg markers (CD25, CTLA-4, GITR, LAG-3 and low CD127 receptor expression) can also designate activated T cells. Although not expressed in all types of induced Tregs, the transcription factor forkhead box P3 (Foxp3) is considered the best functional marker of Tregs, as its absence leads to severe immunopathology and its expression restores regulatory properties [4]. However, Foxp3 is also transiently expressed in activated non-Treg CD4-positive T cells. It is therefore continuously discussed, whether Tregs are true cell lineages or whether they possess regulatory properties as a part of an activation process and can revert to effector cells [5, 6].

Probiotics are bacteria which, when eaten with food or in capsules, reach the intestine either intact or as fragments and exert actions beneficial to the host, such as exclusion of pathogens from access to the mucosa, production of short chain fatty acids, and modulation of the immune response [7]. The profound interest in the immune modulating properties of probiotic bacteria stems from observations that an intestinal microbiota is essential for development and regulation of the immune system [8]. Thus also changing the microbiota by the means of probiotics may have potent effects on immune regulation.

In the early studies of probiotic bacteria (especially lactic acid bacteria of the genera *Lactobacillus* and *Bifidobacterium*) in cultured monocytes and human peripheral blood mononuclear cells (PBMC) *in vitro*, it became clear that certain strains of these bacteria were strong inducers of IL-12 and TNF- α [9, 10]. Stimulation of dendritic cells, the type of APC capable of activating and polarizing T

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Cite this as: L. N. Fink, *Clinical & Experimental Allergy*, 2010 (40) 5–8.

cells to become committed T cell clones (Th1, Th2, Th17, Tregs, etc.), with probiotic bacteria yielded a more complicated picture. Certain probiotic strains induced IL-12 production in dendritic cells, other induced high amounts of IL-10, and as some strains were able to limit the IL-12 production induced by other strains, the resulting dendritic cell cytokine secretion pattern of these combinations would favour Treg induction [11, 12]. However, it did not prove trivial to induce Tregs with probiotics-primed dendritic cells. Dendritic cells cultured with probiotics, although displaying different cytokine profiles, induced Th1 cells differing only in levels of IFN- γ production, and not Tregs [13]. Induction of Tregs by *Lactobacillus reuteri* and *Lactobacillus casei* was shown not to require IL-10, but stimulation of dendritic cells with TNF- α and binding of bacteria to DC-SIGN on dendritic cells [14]. The difficulties encountered may be due to the fact that non-pathogenic microbes are recognized by receptors on dendritic cells eliciting a state of alertness, which may not be comparable to the dendritic cell and downstream Treg responses to 'naked' soluble antigens. Despite the few reports of induction of Tregs by probiotics *in vitro*, it is increasingly recognized that commensal intestinal bacteria (including bacteria that, if isolated, could be termed probiotics), are required for induction of gut-homing Tregs [15]. Commensal bacteria may even play a poorly described role in balancing the development of Tregs and Th17 cells in the intestine [16], underscoring the potential for probiotic therapy in inflammatory diseases.

The strongest evidence for efficacy of probiotic bacteria for combating inflammatory diseases through regulatory action is within childhood allergy. *Lactobacillus rhamnosus* GG has been shown to reduce the incidence and severity of atopic dermatitis if given to pregnant mothers or infants although allergic sensitization was similar between treatment and placebo groups [17]. However, in other controlled clinical trials this and other strains have failed to show an effect [18, 19]. Taylor et al. [18] investigated the effect of probiotic supplementation on the expression of Foxp3, but found no correlation, although Foxp3 expression, surprisingly, correlated with development of atopic dermatitis. In a mouse study of skin allergy, where the severity of skin lesions was decreased after supplementation with *Lactobacillus* GG, no correlation between CD25-positive cells in the spleen and severity was found [20]. This does not, however, rule out an effect of Tregs, as IL-10 expression was increased in lymph nodes. In a separate study, Foxp3-positive T cells were increased locally in the skin after supplementation of mice with *L. casei* [21]. Similarly, an increase in Foxp3-positive cells was found in the spleen after *L. reuteri* supplementation in a mouse model of asthma [22].

In this issue of the journal, de Roock et al. [23] present data on induction of Foxp3-positive Tregs from human

PBMCs by probiotic stimulation *in vitro*. One strain of each of the species *Bifidobacterium lactis*, *Lactobacillus acidophilus*, and *Lactobacillus plantarum* were tested, and *L. acidophilus* proved to be the most potent Treg-inducing bacterium, although all strains induced significant numbers of Tregs. This study is thorough, as not only the induction of Foxp3, CD25, GITR, CTLA-4, and the reduction of CD127 expression in T cells is reported, but also the ability of the sorted Tregs to reduce proliferation of effector T cells. The expression of Foxp3 was increasing for up to 10 days in cell culture, perhaps indicating that Tregs induced by probiotics in this case are not merely a transient activation phenomenon. The authors furthermore demonstrate that the increase in Foxp3-positive Tregs caused by probiotic bacteria is a *de novo* induction of Foxp3, and not an effect caused by proliferation of naturally occurring Tregs. In this study, the presence of naturally occurring Tregs in PBMCs limited the conversion of T cells to induced Tregs, underlining the schism between regulation and activation and showing that even induction of Tregs may be governed by naturally occurring Tregs.

No intracellular IL-10 was detected in the induced Tregs, indicating that suppression is not mediated by this cytokine. Moreover, although the amount of IL-10 in the PBMC culture supernatant correlated with the number of induced Tregs, IL-10 was shown to be dispensable for Treg induction. This is in accordance with the study of Smits et al. [14], and confirms that, although screening for probiotics-induced anti-inflammatory cytokines in PBMCs and dendritic cells can be useful for selecting strains for clinical studies, the induction of these cytokines does not necessarily correlate with the potential to induce Tregs. Other features acquired by APCs following probiotic stimulation may direct the induction of Tregs or the bacteria may interact directly with T cells. T cells express Toll-like receptor 2 (TLR2), which recognizes components of Gram-positive bacteria, in particular in the activation phase that precedes the acquisition of the regulatory phenotype [24]. TLR2 stimulation of naturally occurring Tregs was shown to increase proliferation of these cells [25], a mechanism that might, however, not be relevant in this case.

Induction of Foxp3-positive Tregs is not likely to take place in the blood, as in the reported *in vitro* assay, because commensal bacteria as well as ingested probiotic bacteria under normal circumstances only penetrate as far as the mesenteric lymph nodes [26]. However, as a proof of principle, the findings of de Roock and colleagues indicate that probiotic bacteria can imprint regulatory T cells and this process may even be favoured in the tolerogenic intestinal environment. The authors emphasize that the Treg-inducing properties of probiotics are – like most other probiotic properties – highly strain dependent. Unfortunately, little information is available regarding the specific bacterial components that induce

the expression of cytokines or, as here, transcription factors in immune cells, partly because the cell wall components of these bacteria are very difficult to isolate to purity. Advances can be made in this area through the sequencing of more and more bacterial genomes and transcriptomes that can be correlated to effect measures (such as Foxp3 expression in the de Roock and colleagues study) and enable testing of which bacterial genes and thereby components are essential to specific immunomodulatory properties. Furthermore, such an approach will attribute immunomodulatory properties to the species and strain levels, a distinction that is often not made with the type of data typically available today.

The study by de Roock et al. [23] firmly establishes that probiotic bacteria have a strain-dependent capability to endow T cells with regulatory properties. Such induction of Tregs by probiotics may involve APCs (monocytes, dendritic cells), or a direct action on T cells, and may take place in the intestine, where these cells encounter commensal bacteria. The study supports the evidence of induction of Foxp3-positive T cells by probiotic bacteria in mouse models of allergy and asthma, findings that have not been confirmed in human trials. The system developed by de Roock and colleagues will be useful for screening for Treg-inducing probiotics preceding the necessary expensive and, especially when involving children, ethically complex clinical trials. For truly targeted interventions, there is a need for an increased understanding of the role of probiotic bacteria in allergy and autoimmunity, where they may contribute to balancing the Treg and Th17 cell compartments, both in the intestine and locally in inflamed tissues.

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