

# Oral tolerance and allergic responses to food proteins

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## Purpose of review

The default response to protein antigens in the intestine is the induction of systemic and local hyporesponsiveness, ensuring the prevention of coeliac disease and food allergies. Interest is increasing in the role of dietary manipulation and probiotics in treating allergic and other diseases, but less is known about how these regimens might influence systemic and local immune responses. This paper addresses the mechanisms at the interface of innate and adaptive immunity that determine how the body responds to orally administered proteins and how local bacteria modify these.

## Recent findings

This paper discusses evidence that dendritic cells in the intestinal mucosa are the critical cells that take up dietary proteins and migrate to the draining mesenteric lymph node, where they induce regulatory CD4<sup>+</sup> T-cell differentiation. The properties of tolerated T cells are discussed and it is proposed that the gut microenvironment maintains homeostasis by conditioning dendritic cells to remain in a quiescent state. Inhibitory signalling by commensal bacteria possibly contributes to this process.

## Summary

A regulatory network controls how dietary antigens are taken up and presented to T lymphocytes by specialized antigen-presenting cells. Elucidating their nature and how they are influenced by external factors such as probiotics may help develop novel therapies for allergy and help understand diseases such as coeliac disease.

## Keywords

dendritic cell, food allergy, intestinal inflammation, oral tolerance, probiotics, regulatory T cell, Toll-like receptor

## Abbreviations

|                                |                                                     |
|--------------------------------|-----------------------------------------------------|
| <b>APC</b>                     | antigen-presenting cell                             |
| <b>GALT</b>                    | gut-associated lymphoid tissue                      |
| <b>ICOS</b>                    | inducible costimulatory molecule                    |
| <b>MHC</b>                     | major histocompatibility complex                    |
| <b>MLN</b>                     | mesenteric lymph node                               |
| <b>PPAR<math>\gamma</math></b> | peroxisome proliferator-activated receptor $\gamma$ |
| <b>TGF<math>\beta</math></b>   | transforming growth factor $\beta$                  |
| <b>TLR</b>                     | Toll-like receptor                                  |
| <b>Tollip</b>                  | Toll-inhibitory protein                             |
| <b>T<sub>reg</sub></b>         | T-regulatory cell                                   |

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## Introduction

Administration of antigen by the oral route induces hyporesponsiveness to subsequent challenge with the antigen given in an immunogenic form, a phenomenon termed *oral tolerance* (see Mowat *et al.* [1], Faria and Weiner [2], and Smith and Nagler-Anderson [3] for reviews). Described at the beginning of the 20th century by Wells and Osborne [4], it usually refers to how a prior feed of antigen can modify systemic immune responses to antigenic challenge given by a parenteral route. There is ample evidence, however, that oral tolerance also affects the responsiveness of the local immune system in the intestinal mucosa itself, thus preventing hypersensitivity reactions to food proteins that could otherwise lead to disorders such as coeliac disease or food allergies [5,6].

The state of tolerance induced by feeding antigens contrasts with the active immune responses needed to protect the gut against continual bombardment by invasive pathogens or their products. Here we discuss the most recent advances in our understanding of oral tolerance to protein antigens, the interactions between the innate and adaptive immune system, and the way in which intestinal microbiota may affect the outcome of intestinal antigen exposure.

## Sensing friend from foe

The 'milieu interieur' (C. Bernard, 1865) of the gastrointestinal tract contains around 10<sup>12–14</sup>/ml of mostly harmless commensal bacteria and a broad diversity of food and other antigens. The intestinal epithelial cells and GALT must therefore be able to distinguish and decipher a large number of discrete signals to which they must respond in an appropriate way [3,7,8]. These processes take place in a complex environment where constant bacterial signalling processes at the interface

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between the innate and adaptive immune system are likely to affect the outcome of antigen administration [9–11], a fact that is increasingly exploited by the administration of probiotics (reviewed by Saxelin *et al.* [12]). The endocytic pathway of luminal antigens in intestinal epithelial cells in rodents and in colonic epithelial cells of patients with Crohn's colitis is interferon- $\gamma$  sensitive and intersects with major histocompatibility complex (MHC) class II–enriched late endosomes, suggesting a possible role of intestinal epithelial cells in MHC class II–associated antigen presentation [13,14] (see also below [15]).

### **Tolerance, Toll-like receptors, and the link between innate and adaptive immunity**

Pattern recognition receptors such as Toll-like receptors (TLRs) are critical for sensing bacterial infection. TLRs recognize common microbial structures and signal via a range of specific and shared adaptor molecules [16<sup>•</sup>,17,18]. Currently, 11 TLRs have been identified in human cells and 13 have been identified through searches of the murine genome (reviewed by Dunne and O'Neill [18]). TLRs are generally found on professional immune cells where they are either associated with the plasma membrane (TLRs 2, 4, 5, and 6) or are located in the cytosol (TLRs 3, 7, 8, and 9). The nature of the TLR ligand seems to determine their pattern of expression [16<sup>•</sup>,18]. TLRs 3, 7, 8, and 9 recognize nucleic acid structures whereas TLRs 1, 2, 4, 5, and 6 generally recognize bacterial cell wall components (reviewed by Kawai and Akira [16<sup>•</sup>] and Dunne and O'Neill [18]). The exact expression and role of TLRs at mucosal surfaces are still debated. For example, intestinal epithelial TLR4 expression seems to be variable and differs in human intestinal epithelial cell lines [19], although TLR4 is clearly upregulated during intestinal inflammation even in histologically normal areas [20]. It is also unclear whether the observed effects of TLR activation or down-regulation in the gut are exerted via epithelial TLRs, or via expression and activation on dendritic cells and other antigen-presenting cells (APCs). In addition, a second family of signalling molecules, the nucleotide-binding oligomerization domain family proteins (NOD1 and NOD2), plays a role in regulating the TLR signalling pathway by checking nuclear factor- $\kappa$ B activation (reviewed by Cario [21<sup>•</sup>]). Interestingly, stimulation of TLR2 and TLR4 with lipid and peptidoglycans in mice led to a decrease of allergic inflammation in the lung [22,23] and TLR9 activation is essential in mediating the anti-inflammatory responses of probiotics in experimental colitis and other intestinal disease states [24,25].

Observations that bacterial endotoxin (lipopolysaccharide) exposure may be linked to a reduction in allergic sensitization and disease [26] have supported the hygiene hypothesis, which has been expanded by shifting the emphasis from the effects of early childhood infections to

the effects of microbial exposure and intestinal colonization [3,27,28]. A complex picture is developing in which the amount, mode, and timing of the endotoxin and antigen exposure are likely to play a major role in down-regulation or enhancement of allergic immune responses via TLR involvement [29–32].

Using TLR4<sup>+</sup> and TLR4<sup>−</sup> mice, research groups demonstrated that TLR4<sup>−</sup> strains develop a T-helper 2 (TH2)-biased pattern of cytokine responses [7,33,34<sup>•</sup>]. Berin and colleagues [34<sup>•</sup>], however, reported that these findings could not be generalized to other mouse strains and that the TH2 skewing did not correlate with clinical severity after a challenge, supporting the notion that effects of TLR signalling on allergic responses are affected by the genetic background and bacterial colonization patterns [22,35].

### **Probiotics, their Tolls, and their Tollips**

The concept that intestinal microbiota can be positively modulated through administration of bacteria was put forward by Metchnikoff in 1901. This idea is now clinically exploited through the use of prebiotics, defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of bacterial species already resident in the colon [36]; probiotics, defined as live bacterial preparations with clinically documented health effects in humans [37]; and synbiotics, a combination of both [9,12,24].

In view of their potential proinflammatory activity, expression of TLRs on mucosal surfaces needs to be tightly controlled [38]. Factors implicated in its control are Toll-inhibitory protein (Tollip) and peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ), negative regulators for nuclear factor- $\kappa$ B in response to interleukin-1 $\beta$  and lipopolysaccharide [11,39,40<sup>•</sup>]. The number of bacterial (probiotic) strains is ever increasing and the effects on mucosal immunity are not necessarily interchangeable between strains or different disease states [41<sup>•</sup>]. The greatest difference in microbiota composition between breast-fed and non-breast-fed infants is the number and types of lactic acid fermenting bacteria that are commonly used as probiotic species (bifidobacteria and lactobacilli) [42], an observation that has been linked to different rates of atopic sensitization in the population (reviewed by Bjørksten [35]).

### **How do probiotics work and must they be alive while they work?**

Probiotics have many effects on intestinal function and immune responses, including repair of intestinal barrier function, restoration of tight junctions, and enhanced mucin production [43,44] (and reviewed by Saxelin *et al.* [12]); effects on dendritic cell function and skewing

of T cells toward T-helper 1 (TH1) polarization [45–47]; competitive exclusion of pathogens [48,49]; suppression of intestinal inflammation through downregulation of TLR expression [50,51] (and reviewed by Sartor [24]) and secretion of metabolites that may inhibit tumour necrosis factor- $\alpha$  secretion by blood mononuclear cells [52]; and through inhibition of nuclear factor- $\kappa$ B signaling in enterocytes [39,53]. Clinical effects are likely to be the result of a combination of these pathways. Several studies [54,55] highlight the importance of probiotic viability for beneficial effects. Methylated and unmethylated genomic DNA isolated from probiotics has been used with positive effects, however, mimicking the anti-inflammatory effects of live probiotics on TLR9 signaling [25]. Intestinal diseases in which positive effects of probiotics have been reported in randomized controlled trials include infectious diarrhoea, inflammatory bowel diseases, and irritable bowel syndrome (reviewed by Saxelin *et al.* [12] and Bischoff and Crowe [56]), although most results are preliminary and more research in larger cohorts is needed.

### **Uptake and recognition of protein antigens in the intestinal immune system**

Most aspects of oral tolerance are dependent on CD4<sup>+</sup> T lymphocytes and so require fed antigens to be presented by APCs that express class II MHC [1,2]. Although a number of such APCs have been implicated in oral tolerance, dendritic cells seem to be crucial APCs for inducing tolerance, and there is enhanced susceptibility to oral tolerance in mice treated with the cytokine flt3 ligand (flt3L), which expands dendritic cell numbers *in vivo* [57].

#### **Site(s) of antigen uptake and presentation to T lymphocytes**

It is unresolved where the initial events in oral tolerance take place and this has important implications for our understanding of how feeding antigen can have both systemic and local effects [58]. Selective uptake by M cells in Peyer's patches and transfer to neighbouring APCs appears to be the major route of entry of pathogenic organisms to the immune system [59], but the consensus is that Peyer's patches and conventional M cells may not be essential for oral tolerance. In contrast, oral tolerance of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells is absent in lymphotoxin  $\alpha$  knockout mice, which lack mesenteric lymph nodes (MLNs) [60–62] (and reviewed by Mowat [58]).

Studies that have tracked antigen uptake *in vivo* support the view that MLNs are the major sites of antigen loading and T-cell recognition in antigen-fed mice [60] (reviewed by Mowat *et al.* [1]), and several studies have identified dendritic cells with regulatory activity in the MLN [63,64] (and reviewed by Billsborough and Viney [65]). Furthermore MLN dendritic cells from antigen-fed mice

have been found to induce tolerance in naïve mice *in vivo* [66]. The exact subset of the dendritic cells responsible for these effects in MLN remains to be clarified, as it has been reported to be both CD11b<sup>+</sup>CD8 $\alpha$ <sup>−</sup> [61] or CD11b<sup>−</sup>CD8 $\alpha$ <sup>lo</sup> [65], which are the major phenotypic groups of dendritic cells in mice.

#### **How does antigen reach the dendritic cells in the mesenteric lymph nodes?**

Although antigens could reach the dendritic cells in MLN due to direct dissemination of antigen in the bloodstream or draining lymph, current evidence suggests that it requires migration of dendritic cells that have taken up antigen in the mucosal lamina propria to the MLN. It has long been known that antigen-loaded dendritic cells are present in lymph draining the intestine, but it was not known whether these were derived from Peyer's patches or conventional villous mucosa [67]. Recent work suggests that dendritic cells in villous lamina propria account for the majority of protein uptake from the intestine [68<sup>•</sup>] and other studies indicate that MLN dendritic cells share many more phenotypic markers with lamina propria-derived dendritic cells than with Peyer's patch dendritic cells [69,70]. Recently it has been shown directly that CCR7-dependent migration of dendritic cells from the lamina propria to MLN is required for the induction of oral tolerance [70,71<sup>••</sup>].

#### **How do lamina propria dendritic cells acquire proteins from the intestinal lumen?**

Lamina propria dendritic cells could acquire proteins from the intestinal lumen in several ways. For instance, it has been suggested that the villus epithelium may contain M cells similar to those in Peyer's patches [72]. Mucosal dendritic cells may also send processes through the epithelium to capture antigens in the lumen [73<sup>•</sup>], or the entire cell may itself enter the epithelium, forming tight junctions with enterocytes to preserve barrier integrity before retreating into the lamina propria with the antigen [74]. Alternatively, it has been shown that dendritic cells in lamina propria may acquire antigens associated with apoptosing epithelial cells and carry them in lymph to the MLN, and such dendritic cells have been shown to prime T cells preferentially for anti-inflammatory cytokine production [70,75]. Finally, uptake and transcytosis of antigen itself by epithelial cells may deliver soluble antigen to underlying dendritic cells [76<sup>••</sup>].

#### **The intestinal mucosa: a specialized immunologic niche for the generation of tolerogenic dendritic cells**

The gut mucosa appears to favour the differentiation or accumulation of tolerogenic dendritic cells under physiological conditions. Recent studies by Rescigno and colleagues [74] suggest that this may reflect a 'conditioning'

effect of intestinal epithelial cell production of the cytokine thymic stromal lymphopoietin (TSLP). These experiments show that even brief exposure to normal enterocytes or TSLP itself renders immature dendritic cells unable to differentiate into interleukin-12-producing, TH1-polarizing APCs when subsequently stimulated by appropriate TLR stimuli, and dendritic cells acquire the ability to polarize naïve T cells to interleukin-4 and interleukin-10 production. Other local factors, which could drive intestinal dendritic cells to this tolerogenic phenotype, may include transforming growth factor  $\beta$  (TGF $\beta$ ) released by enterocytes or prostaglandin E<sub>2</sub> produced by mucosal stromal cells [77].

#### **Molecular basis of dendritic cell–T-cell interactions in oral tolerance**

Other experimental models indicate that tolerance in CD4<sup>+</sup> T cells occurs when presentation of antigen in the context of a lack of conventional costimulatory molecules on dendritic cells such as CD80, CD86, or CD40 or because of the involvement of alternative costimulatory molecules with inhibitory functions such as CTLA-4 or PD-1 [78]. Freshly isolated mucosal dendritic cells express relatively low levels of costimulatory molecules and oral tolerance can be prevented by agents that activate dendritic cells *in vivo*, such as  $\alpha$  galactosyl ceramide, interleukin-12, and interleukin-18 [79,80]. Surprisingly, however, activating dendritic cells by cross-linking CD40 does not prevent the induction of oral tolerance [81], whereas cross-linking of the related costimulatory molecule on dendritic cells, RANK (receptor activator for nuclear factor- $\kappa$ B), enhances oral tolerance [82]. These effects are opposite to what is found in immune responses in other tissues, reinforcing the view that dendritic cells in the intestine may be a distinct functional subset. One costimulatory molecule that may be involved in oral tolerance is the inducible costimulatory molecule (ICOS), although this appears to reflect a need for ICOS on T-regulatory cells (T<sub>reg</sub>) to interact with its ligand during the expression of regulation, rather than ICOS-mediated costimulation during the induction phase [83].

The CTLA-4 molecule on T cells is believed to play a crucial role in the induction of tolerance and is expressed by most populations of T<sub>reg</sub> cells [78,84]. CTLA-4 expression is increased on antigen-specific T cells after the induction of oral tolerance [85], but its role in oral tolerance is unclear (reviewed by Mowat *et al.* [1]).

#### **Mechanisms of oral tolerance**

Many mechanisms have been implicated to explain the defects in T-cell-dependent immune responses that occur in oral tolerance. These include clonal anergy, clonal deletion, and active regulatory processes. Recent studies have extended these ideas by showing that orally

tolerized T cells have defects in locomotor activity, which restricts their ability to cross blood vessel endothelium [86] or to interact with dendritic cells and B cells *in vivo* [87–89].

It was originally believed that the mechanisms responsible for oral tolerance depended on the dose regimen used to induce tolerance, with single high doses of antigen thought to produce anergy or deletion of T cells, whereas multiple low-dose feeds were associated with the generation of T<sub>reg</sub> [90]. This dichotomy has become less clear in recent years, as work in other fields of immunology indicates that anergy and active regulation may not be distinct aspects of T-cell function [84]. As a result, the focus in oral tolerance has returned to T<sub>reg</sub> in recent years. It was thought that these cells belonged to the CD8<sup>+</sup> subset of suppressor T cells (reviewed by Mowat *et al.* [1]) and some recent studies have continued to implicate cells of this phenotype [91]. Most current work, however, suggests that the T<sub>reg</sub>s are likely to be CD4<sup>+</sup>. Several such subsets have been identified, including TGF $\beta$ -producing ‘TH3’ cells, interleukin-10-producing Tr1 cells, and CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> [84].

Evidence shows that both interleukin-10 and TGF $\beta$  play important roles in regulating inflammatory responses to bacteria in the large intestine [92], and the levels of both cytokines and of T cells producing them are increased in the GALT after induction of oral tolerance in normal mice [93] (and see Mowat *et al.* [1] and Smith and Nagler-Anderson [3] for review). Although studies depleting these cytokines from mice have provided inconsistent findings [94], food-allergic children have decreased numbers of TGF $\beta$ -secreting cells in the duodenal mucosa compared with normal control subjects [95], suggesting this cytokine may also be important for regulating intestinal immunity in humans.

#### **CD4<sup>+</sup>CD25<sup>+</sup>T-regulatory cells**

Originally described as a naturally occurring population of T<sub>reg</sub> that arise in the thymus and that help prevent autoimmunity, CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> have been implicated in a wide range of different immune responses [84]. Several groups have shown that there is expansion of antigen-specific CD4<sup>+</sup>CD25<sup>+</sup> T cells in Peyer's patches, the MLN, and peripheral lymphoid tissues of antigen-fed mice [85,93]. In most cases, these cells have been shown to have regulatory activity *in vitro* or *in vivo* and they express the FoxP3 transcription factor, generally considered to be the hallmark marker of T<sub>reg</sub> of this kind [94]. They may also express other markers associated with CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub>, including CTLA-4, glucocorticoid inducible tumor necrosis factor receptor family molecule (GITR), and CD103 [85,96]. The suppressor functions of orally induced CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> appear to be at least partly dependent on TGF $\beta$ , with a minor role

for interleukin-10 [93,94,96]. These findings are consistent with the evidence that CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub>, which produce TGFβ or express it on their surface, may be responsible for preventing several forms of experimental inflammatory disease, including colitis [84,92].

It is controversial whether naïve T cells can differentiate into CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> after exposure to antigen in the peripheral immune system, as many immunologists consider these cells to be derived entirely from aberrant selection by self-antigens in the thymus [84]. CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub>, however, have been cloned from the Peyer's patches of normal mice tolerized by feeding β-lactoglobulin [97], and increased numbers of β-lactoglobulin-specific CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> can be found in the peripheral blood of children recovering from cow's milk allergy [98]. Together these findings suggest that induction and restoration of oral tolerance in the normal immune system may be associated with the presence of CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub>. As human and mouse lamina propria have been found to contain large numbers of these cells with regulatory activity [99,100], we predict this is the major site of accumulation of T<sub>reg</sub> that have been 'primed' in MLN and migrate back to the gut mucosa under CCR9 control to maintain local homeostasis.

## Conclusion

The intestinal mucosa is characterized by a downregulatory microenvironment whose aim is to ensure that active immune responses are not generated against food proteins and commensal bacteria. Lamina propria dendritic cells appear to be the cells that translate these signals into immunologic tolerance, by virtue of their quiescent properties and ability to migrate to the MLN, where we propose they stimulate differentiation of regulatory CD4<sup>+</sup> T cells. The role of the intestinal epithelial cell in these processes is unresolved.

## References and recommended reading

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 245–246).

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