CMLS, Cell. Mol. Life Sci. 62 (2005) 1322–1332
1420-682X/05/121322-11
DOI 10.1007/s00018-005-5036-0

CMLS Cellular and Molecular Life Sciences

Oral tolerance and regulation of mucosal immunity

B. Dubois*, A. Goubier, G. Joubert and D. Kaiserlian

INSERM U404, Immunité et Vaccination, IFR-128 Biosciences Lyon Gerland, 21 avenue Tony Garnier, 69365 Lyon, Cedex 07 (France), Fax: +33 4 37 28 23 91, e-mail: dubois@cervi-lyon.inserm.fr

Abstract. Regulated mechanisms sustain the ability of the gut immune system to discriminate harmless food antigens (Ag) and commensal bacteria from pathogenic microorganisms, resulting in tolerance versus protective immunity, respectively. Antigens of the gut commensals are not simply ignored, but rather trigger an active immunosuppressive process, more commonly known as oral tolerance, which prevents the outcome of immunopathology. Both intrinsic properties of the gut microenvironment and cellular actors, as well as peripheral events induced by systemic dissemination of oral Ag, promote the induction of regulatory mechanisms that ensure maintenance of gut homeostasis. The aim of this review is to provide a synthetic update on the mechanisms of oral tolerance, with particular emphasis on the complex interplay between regulatory $CD4^+$ T cells, dendritic cells and the gut microenvironment.

Key words. Tolerance; intestine; regulatory $CD4^+$ T cells; dendritic cells; commensal flora.

Introduction

The intestinal epithelium represents the largest surface of the body in contact with the external milieu and throughout life faces tremendous amounts of exogenous antigens (Ags) that are either resident or ingested. Mounting protective immune responses against harmful intestinal pathogens while preventing excessive responses to harmless intestinal Ags is the difficult task achieved by the intestinal immune system to maintain homeostasis (a disease-free state). Oral tolerance has long been recognized as a physiological mechanism of immune unresponsiveness to dietary Ags and indigenous bacterial Ags, that maintains tissue integrity by preventing harmful delayed-type hypersensitivity responses in the intestine. Indeed, various experimental settings in rodents have demonstrated that Ag encounters in the intestine trigger an active inhibitory process prevents the onset of both $CD4^+$ and $CD8^+$ T cell-mediated Ag-specific immune responses upon subsequent systemic immunization with the same Ag [1]. Perturbations of this process, i.e. oral tolerance breakdown, can lead to inflammatory pathologies exemplified in humans by celiac and Crohn's diseases, which result from excessive immune responses to wheat gluten and indigenous flora, respectively [2].

* Corresponding author.

The physiological response to intestinal Ag is most often characterized by immunoglobulin A (IgA) production [3] and induction of T cells producing interleukin 4 (IL-4), IL-10 and transforming growth factor β (TGF-β), rather than interferon γ (IFNγ) [4]. Both local events in the gut-associated lymphoid tissues (GALT) and peripheral events account for the induction of hyporesponsiveness after oral Ag encounters [1]. This review will focus on the various mechanisms involved in oral tolerance, with a special emphasis on the complex interplay between the gut microenvironment, regulatory T cells (T_{regs}) and dendritic cells (DCs).

Antigen penetration and presentation in the intestine

Constitutive sites of Ag penetration

The gut is chronically exposed to a tremendous amount of dietary and environmental Ags. How and where luminal Ags come into contact with immunocompetent cells is most likely instrumental in dictating the type of response generated following Ag penetration through the gut. Ag sampling can occur both in Peyer's patches (PPs) and through the villus epithelium of the mucosa. PPs, which are present along the small intestine, contain lymphoid aggregates separated from the lumen by a monostratified epithelium called follicle-associated epithelium (FAE) and

a subepithelial dome (SED) containing numerous DCs. The FAE is characterized by the presence of microfold (M) cells specialized in the capture and translocation of microorganisms and particulate Ags from the lumen to the underlying SED, where they can be captured and processed by Ag-presenting cells (APCs), such as DCs, and subsequently presented locally in the T cell-rich area and/or in the mesenteric lymph nodes (MLNs). Ag penetration through M cells and subsequent cellular activation in PPs are thought to be instrumental in initiating immune responses to intestinal Ag, should they result in immunity or tolerance. However, whether PPs represent the site of oral tolerance induction is still debated. Studies using PP-deficient mice, obtained by treatment with LTβR-Ig fusion protein, indicated that oral tolerance to proteins, but not haptens, required M cells and PPs [5], whereas others reported normal oral tolerance induction [6]. B cell-deficient mice that harbor rudimentary PPs [7] and rats in which PPs were surgically removed [8] are fully susceptible to oral tolerance induction. Treatment of pregnant mice with LTβR-Ig either alone or combined with tumor necrosis factor receptor I (TNFRI)-Ig, which abrogates the formation of PPs or PPs and MLNs, respectively, argues for a dominant role of MLNs in oral tolerance induction [9]. Finally, following oral Ag delivery, neither peptide presentation nor initial T cell activation was affected in PP-deficient mice [10]. It is more likely that the small intestinal mucosa represent the major site of oral tolerance induction.

Intestinal epithelial cells and oral tolerance

Mucosal villi represent 99% of the small intestine and are covered by a simple columnar epithelium [i.e. enterocytes or intestinal epithelial cells (IECs)] specialized in absorption of dietary proteins. IECs are capable of translocating Ags in native or partially degraded forms (see [11] for review) and represent the major site of entry of luminal Ags. IECs can capture and process apical Ag both in vitro [12] and in vivo [13], suggesting that IECs might serve as APCs for surrounding T cells, e.g. intraepithelial lymphocytes and lamina propria $CD4^+$ and $CD8^+$ T cells. Several observations indicate that IECs are nonconventional APCs that may play a tolerogenic role in vivo. IECs harbor major histocompatibility complex (MHC) class I and class II molecules but express few co-stimulatory molecules [14], suggesting that anergy rather than successful T cell activation may result from IEC/T cell interactions. Isolated IECs lack invariant chain expression and express surface MHC class II molecules with an atypical conformation [15]. Furthermore, while able to present superantigen, IECs appear inefficient in presenting nominal Ag to specific MHC-class-II-restricted T cell hybridoma and in stimulating allogeneic T cells. Whether IECs present a particular set of peptides associated with their atypical MHC class-II molecules or whether lack of an invariant chain hampers loading of self or exogenous class II peptides is still not clear [11, 15]. In agreement with their poor $CD4^+$ T cell stimulatory ability, IECs were reported to preferentially expand $CD8^+$ T cells [14] with some evidence of suppressive function in vitro [16]. Therefore, IECs might contribute to intestinal tolerance by maintaining the survival and/or function of regulatory T cells in the lamina propria. That the lamina propria essentially contains effector/activated and memory T cells but few naïve T cells would suggest a rather limited role of IECs acting as APCs in the inductive phase of oral tolerance. Nevertheless, IECs can suppress already primed $CD4^+$ [17] and $CD8^+$ T cells [18] in in vitro co-culture experiments, suggesting that they might locally control effector cells and tissue damage. In particular, we have shown that IECs isolated from hapten-fed mice mediate bystander suppression of hapten-specific $CD8^+$ T cell effectors through the release of IL-10 and TGF-β [18].

Besides cognate interaction with Ag-specific T cells, IECs can affect the immune response to intestinal Ag in several other ways. They can translocate native luminal Ag into the basolateral compartment in the form of apoptotic bodies [19] or exosomes [20] that professional APCs near the epithelium might present as tolerogenic material at a distance from the mucosal surfaces. IECs also produce numerous cytokines and chemokines that could play a role in the type of immune response generated against luminal Ag, such as recruiting certain APCs and modulating their function. Toll-like receptor (TLR)-mediated activation of IECs by pathogens stimulates the production of DC-attracting chemokines, typified by CCL20 [21], and inflammatory cytokines that can recruit and activate DCs for efficient T cell priming. Importantly, commensal bacteria suppress production of proinflammatory mediators by IECs by limiting nuclear factor-kappaB (NF-κB) activation [22, 23], demonstrating that IECs can adapt their function to the gut luminal content. Thus, IECs, which are the first cells in contact with fed Ags, likely sense Ags from the gut lumen and transmit appropriate signals to the immune system. In the case of innocuous Ags, part of the tolerogenic signals might include IL-10 and TGF-β, whose production is enhanced following Ag feeding [24]. These immunosuppressive cytokines, together with prostaglandin E2 produced by mesenchymal cells and macrophages, might condition surrounding DCs to induce T helper 2 (Th2) and/or T_{reg} differentiation [25] and thus favor induction or maintenance of oral tolerance.

Ag spreading and systemic tolerance

One important consideration that may explain the efficacy of systemic tolerance to fed proteins is that fed Ags can be captured by APCs both in the GALT and systemic lymphoid organs. DCs, which are numerous both in the subepithelial dome of PPs and in the lamina propria of the in-

testine, are the most likely candidates for capture of fed Ags after translocation through the epithelium. In addition, lamina propria DCs can directly sample luminal Ags by extending their dendrites between IECs [26, 27]. DCs can present the Ags to T cells locally or after migration via efferent lymphatics to MLNs. Liu and MacPherson showed that intestinal DCs acquire orally delivered Ags and migrate through the mesenteric lymph to draining MLNs [28]. In addition to cell- or exosome-mediated Ag transport, free Ags can circulate via the bloodstream and rapidly spread to systemic lymphoid organs [29]. However, the contribution of free versus cell-transported Ags depends on the dose and type of Ag, and their significance in oral tolerance is unclear. Most studies aimed at tracking fed Ags and defining the fate of Ag-specific T cells have identified PPs and MLNs as major sites of Ag presentation and T cell activation [10, 30–33]. However, fed Ags have also been detected in APCs from the spleen and peripheral lymph nodes [34, 35] and induce activation of $CD4^+$ T cells as early as 3–6 h post-feeding [34, 36], supporting the conclusion that oral feeding results in concomitant Ags presentation in the GALT and peripheral lymphoid organs. Presentation of fed Ags in specific peripheral tissues might also contribute to oral tolerance. In particular, the liver collects large number of Ags from the intestine through portal veins, and fed Ags (i.e. haptens) have been detected near portal veins, in bile ducts and in sinusoidal cells [37]. Presentation of fed Ags to T cells is more efficient and long-lasting in MLNs than in peripheral lymphoid organs [10, 32, 34, 36]. Along these lines, fed hapten can be detected in MLNs for at least 24 h, whereas it rapidly disappears from the spleen and liver [37]. It is thus likely that the functional consequences of T cell stimulation are different in MLNs, which collect fed Ags, either free or loaded on migrating intestinal APC, and in peripheral LNs, which receive only circulating Ags. The nature of the bone marrow-derived APCs [32] responsible for presentation of fed Ags in MLNs remains largely unknown but may include myeloid DCs [35].

Various mechanisms contribute to oral tolerance

Two mechanisms, differing in sensitivity to cyclophosphamide (CPA) treatment, contribute to oral tolerance induction [38]. Tolerance induced by feeding low doses of Ag favors active immune suppression and is mediated by CPA-sensitive T_{regs} that can transfer tolerance to naïve hosts, whereas tolerance after feeding high Ag doses is insensitive to CPA treatment and proceeds through induction of T cell hyporesponsiveness via anergy and/or deletion of Ag-specific T cells [39].

Clonal deletion by apoptosis has been demonstrated in $CD4^+$ T lymphocytes of T cell receptor (TCR) transgenic mice using various model Ags, including ovalbumin (OVA) [40], myelin basic protein (MBP) [33] and cytochrome C [34]. In conditions that promote systemic spreading of fed Ags (i.e. after feeding either a single high dose of Ag [33, 40] or multiple low doses [34]), T cell apoptosis preferentially occurs in systemic lymphoid organs and probably also in the thymus [41]. Deletion of Ag-specific T cells is preceded by rapid activation [34] and transient downregulation of TCRs and anergy [33] and associated with upregulation of the active form of caspase 3 [42]. The deletion mechanisms of Ag-specific T cells and their contribution in oral tolerance in conventional animals harboring physiological numbers of Ag-specific T cells remains to be determined.

Although deletion might contribute to oral tolerance in certain situations, other mechanisms, such as clonal anergy or active suppression, are necessary to maintain efficient tolerance, since the thymus continuously releases naïve lymphocytes that could be activated after feeding. That clonal anergy participates in oral tolerance was first suggested in MBP-fed rats [43] and then demonstrated in OVA-fed mice in which Ag-specific T cell hyporesponsiveness could be reverted in vitro by exogenous IL-2 [44, 45]. Adoptive transfer of limited numbers of TCR transgenic T cells into normal mice further revealed that Ag-specific cells can persist after oral feeding but remain hyporesponsive after in vitro or in vivo re-stimulation with the same Ag [46]. Induction of anergy or T cell-mediated suppression following oral Ag delivery seems to depend on the Ag dose and feeding regimen [39], but based on the fact that anergic cells have been found to exert some level of suppression [47], the two mechanisms might be interrelated.

Active suppression has been demonstrated in various model of oral tolerance by the ability of T cells from various lymphoid organs of fed donors to transfer tolerance to naïve recipients. Although initial studies indicated a role for $CD8^+$ T cells [48] and $\gamma\delta$ T cells [49], there is now compelling evidence that $CD4^+$ regulatory T cells are of paramount importance for orally induced tolerance. In particular, oral tolerance of B cell- or $CD8^+$ T cell-mediated responses cannot be induced in $CD4^+$ T cell-deficient mice [50–53]. Moreover, the observation that a single oral administration of hapten in mice depleted of $CD4^+$ T cells or in MHC class-II-deficient mice induces oral priming of functional $CD8^+$ cytotoxic T cells but not tolerance [52] strongly argues for a pivotal role of regulatory $CD4^+$ T cells in driving the decision process between immunity and tolerance following Ag penetration through the gut.

$CD4^+$ regulatory T cells

Dedicated subsets of $CD4^+$ T cells involved in oral tolerance?

Studies in the experimental autoimmune encephalomyelitis (EAE) model in both rats and mice have indicated

that feeding autoantigens generate $CD4^+$ T_{regs} cells named Th3 that transfer suppression in vivo and protect animals from EAE through TGF-β production [54, 55]. Th3 cells can be cloned from PPs and MLNs as soon as 48 h after feeding low doses of Ag and produce TGF-β in association with a low to moderate amount of IL-4 and IL-10 [4]. The mucosal Th3 cells inhibit differentiation of autoimmune effector cells through TGF-β-mediated bystander suppression and likely favor mucosal IgA class switching [56].

More recently, $CD4^+CD25^+$ T cells were also proposed to play a role in oral tolerance induction. These naturally occurring T_{regs} cells, which represent 5–10% of peripheral $CD4^+$ T cells, are involved in protection from autoimmune diseases, colitis and allograft rejection [57]. Differentiation of $CD4^+CD25^+$ T_{regs} critically depends on the transcription factor Foxp3 [58]. Induction of tolerance through the oral route in $CD4^+$ OVA-specific TCR transgenic mice was found to increase the number and suppressive function of $CD4^+CD25^+$ T cells [59, 60]. These $CD4^+CD25^+$ T_{regs} rapidly divided preferentially in gut lymphoid tissues and could transfer tolerance when isolated from MLNs, but not spleen, 48 h after feeding Ag [61]. Our own results, in a pathophysiological model of skin inflammation mediated by hapten-specific $CD8^+$ T cells [53, 62], further demonstrated that constitutive $CD4^+CD25^+$ T_{regs} are instrumental for induction of oral tolerance in normal mice [63]. We showed that depletion of $CD25^+$ cells by antibody treatment impaired oral tolerance, since hapten feeding was unable to prevent the hapten-specific CD8 response. Moreover, adoptive transfer of naïve $CD4^+CD25^+$ T cells from normal mice fully restored susceptibility to oral tolerance in invariant chain-deficient mice (which have a dramatic $CD4^+$ T cell deficiency). In this model we further showed that hapten feeding enhanced the suppressive function of $CD4^+CD25^+$ T cells, allowing them to prevent the priming of specific $CD8^+$ effector cells upon skin sensitization with the hapten. A recent report indicates that $CD4^+CD25^+$ T cells might also be involved in oral tolerance in humans [64]. Cow's milk allergy in children is a disease of rather short duration that can disappear after a period of a milk-free diet. Allergic children who became tolerant to cow's milk displayed increased frequency of blood $CD4^+CD25^+$ T cells that were able to inhibit the proliferation of peripheral blood mononuclear cells stimulated with β-lactoglobulin, suggesting that milk reintroduction had activated $CD4^+CD25^+$ T_{regs}. Therefore, $CD4^+CD25^+$ T cells capable of silencing inflammatory effector T cells can be activated in the human gut mucosa. Because oral feeding enhances TGF-β production by $CD4^+CD25^+$ T cells [59], it is unclear at present whether $CD4^+CD25^+$ T_{regs} depend on TGF-β for their regulatory functions [65] and differ from Th3 T_{regs} cells.

The third major subset of $CD4^+$ T_{regs} is composed of Tr1 cells that can be induced in vitro by repetitive stimulation in the presence of IL-10 or immature DCs [66, 67]. Tr1 cells often express CD25 and have comparable regulatory functions to natural $CD4^+CD25^+$ T_{regs}, but they are most likely distinct from the latter subset since they do not express Foxp3 [68]. Tr1 cells display a low proliferative potential, produce high levels of IL-10 as well as low to moderate amounts of TGF-β and can suppress the proliferation and differentiation of most T cell subsets in an IL-10-dependent fashion. Their implication in oral tolerance induction has not been formally demonstrated, although mucosal (nasal) Ag delivery was shown to generate Tr1 cells able to prevent airway hyper-reactivity by a mechanism dependent on IL-10 and inducible costimulator (ICOS)-ICOS-ligand (L) interactions [69]. In addition, $CD4^+$ T cells from the intestinal lamina propria exhibit characteristics of Tr1 cells [70]. This, together with the fact that activation of adoptively transferred Tr1 cells by Ag feeding was found to prevent colitis in severe combined immunodeficiency (SCID) mice reconstituted with $CD4^+CD45RB^{high}$ cells [66], suggests that Tr1 induced by the mucosal route might contribute to gut homeostasis and maintenance of tolerance.

The gut microenvironment favors induction of T_{regs}

The GALT, particularly MLNs and PPs, provides a unique microenvironment prone to T_{regs} cell differentiation. This microenvironment is characterized by the presence of immunomodulatory factors and cytokines, a constant exposure to commensal flora and, as will be discussed later, DC subsets with particular functional properties.

The presence of cytokines during in vitro T cell activation and in the lymphoid tissue microenvironment in vivo, can induce functional polarization of T cells [71]. Modulation of the gut cytokine milieu profoundly affects the nature of T cell response to fed Ag. Indeed, while intestinal delivery of IL-18, IL-12 [72] or IL-1 [73] abrogates oral tolerance, mucosal delivery of IL-4, TGF-β [74], IL-10 or anti-IL-12 [75] favors hyporesponsiveness. At homeostasis, the PP microenvironment renders naïve T cells hyporesponsive to several stimuli [76] and favors $Th2/T_{reg}$ differentiation [77, 78]. This might be at least partly mediated by gut immunosuppressive cytokines such as IL-10 and TGF-β [24]. IL-10 is a critical cytokine that drives T cell differentiation toward Tr1 cells [66] and conditions DCs to induce anergic T cells endowed with immunosuppressive properties [79]. IL-10 also contributes to $CD4^+CD25^+$ T_{regs} induction, as their number increases dramatically after systemic overexpression of IL-10 [80]. TGF-β stimulates Th3 cell differentiation [81] and was recently found to convert naïve peripheral $CD4^+CD25^-$ cells into $CD4^+CD25^+$ regulatory cells through induction of Foxp3 [82, 83]. A combination of IL-10 and TGF-β also induces conventional $CD4^+$ T cells to acquire regulatory functions [84].

The prominent feature of the gut microenvironment is the constant contact with the commensal flora, which participates in the differentiation of the mucosal immune system. It may be postulated that bacterial components translocating through the epithelium may exert immunomodulatory effects that could contribute to induction of oral tolerance. Indeed, axenic mice and C3H/HeJ mice, which are unable to respond to lipopolysaccharide (LPS) due to a TLR4 mutation, were reported to be refractory to tolerance induction after feeding sheep red blood cells (SRBC) [85–87], and oral tolerance to OVA was either incomplete [88] or of shorter duration [89]. This deficient oral tolerance to SRBC in LPS hyporesponsive animals correlated with failure to generate suppressor cells [85, 87]. That oral tolerance could be restored in germ-free mice after reconstitution of the intestinal flora with *Bifidobacterium infantis* [88] or oral treatment with LPS from *Escherichia coli* [86] suggests that both Gram-positive and Gram-negative commensal bacteria contribute to oral tolerance induction. In addition, oral LPS delivery at the time of MBP feeding was found to ameliorate protection from EAE [90].

The mechanisms by which commensal bacteria exert their immunomodulatory functions are still not clear. As discussed earlier, commensal bacteria can interact with IECs through their apical pole and modulate their functions. Intact bacteria and bacterial compounds such as LPS [91] can translocate through IECs and might thus directly affect the key actors of oral tolerance, e.g. DCs and $CD4^+$ T cells. Feeding noncommensal bacteria, in the form of probiotics, has yielded promising results in the treatment of inflammatory diseases [92], and we recently documented that *Lactobacilli* can reduce skin inflammation in mice by reducing priming of Ag-specific $CD8^+$ T cells [93]. There is some evidence that the beneficial effect of probiotics might be mediated through induction or activation of $CD4^+$ regulatory cells [93] and is associated with enhanced IL-10 production by $CD4^+CD25^+$ T cells [D. Kaiserlian, unpublished data]. Recent studies indicated that $CD4^+$ T cells, and T_{regs} in particular, can detect and respond to conserved components of bacteria [94]. Indeed, $CD4^+CD25^+$ T_{regs} express most members of the TLR family, and TLR4 activation by LPS elicits their proliferation, enhances their survival and promotes their suppressive functions even in the absence of APCs [95]. Another study also suggests that TLR2 signaling might contribute to $CD4^+CD25^+$ homeostasis [96]. Effects of commensal bacteria on APCs, including DCs, might also indirectly affect T_{regs} survival and functions. In particular, *Lactobacilli* have been shown to differentially modulate the maturation and cytokine production of DCs depending on species, with some being able to inhibit pro-Th1 cytokine production while maintaining IL-10 secretion, a cytokine milieu that favors T_{regs} differentiation [97]. In addition, a strain of *Lactobacillus paracasei* was found to induce $CD4^+$ T cells to differentiate into IL-10 and TGF-β-producing cells with low proliferating potential [98], reminiscent of previously described regulatory cells involved in oral tolerance. Much has to be learned about how indigenous nonpathogenic bacteria contribute to gut homeostasis, and understanding their interaction with regulatory circuits would allow their use as adjuvants to induce or maintain tolerance.

Mechanisms of suppression by T_{regs}

Orally induced T_{regs} are characterized by their ability to exert bystander suppression, e.g. once properly activated, they can suppress effector function of Ag-specific surrounding T cells [54]. Adoptive transfer studies revealed that the suppressive function of Th3 and Tr1 T_{regs} essentially depends on TGF-β and IL-10. In contrast, the mechanisms by which $CD4^+CD25^+$ T_{regs} achieve their suppressive functions remains unclear. In vitro, $CD4^+CD25^+$ T_{regs} require essentially cell surface molecules [57] including membrane-bound TGF-β [65], whereas in vivo their protective effect is often mediated by IL-10 and TGF-β [99–102]. Whether IL-10 and TGFβ contribute to the suppressive role of $CD4^+$ T_{regs} remains unclear at present. Indeed, oral tolerance to OVA can be induced both in anti-IL-10-treated mice [103] and in TGF-β1-deficient mice [104]. In a model of uveitis, neutralizing anti-TGF-β monoclonal antibodies (mAbs) did not affect the outcome of oral tolerance, and IL-10 did not contribute to Ag-specific T cell anergy but was mandatory to prevent disease [105]. Likewise, our data in the model of contact sensitivity to hapten demonstrate that IL-10 is instrumental for orally induced prevention of skin inflammation, since oral tolerance was impaired both in $IL\text{-}10^{-/-}$ mice and in mice treated with neutralizing anti-IL-10 R mAbs [B. Dubois and D. Kaiserlian, unpublished data]. However, IL-10 produced by natural $CD4^+CD25^+$ T cells is not responsible for their suppressive effect on hapten-specific $CD8^+$ T cell response [63], indicating that IL-10 produced by other cell types, possibly mucosal mesenchymal or epithelial cells, might contribute to oral tolerance. This is reminiscent of another study showing that IL-10 production by Ag-specific cells is dispensable for oral tolerance induction [106]. However, this does not exclude IL-10 as being instrumental in T_{regs} induction and/or T_{regs}-mediated suppression. Indeed, Fuss et al. showed that protection from experimental trinitrobenzene sulfonic acid (TNBS) colitis induced by feeding haptenated colonic protein is mediated by TGF-β-producing (Th3) cells and IL-10, which favor T_{regs} expansion and enhance their suppressive function [107], likely by maintaining TGF-β responsiveness on Th1 target cells [108]. More recently, using a system of TGF-β production regulated by doxycycline, it was further shown that TGF-β producing cells induce IL-10 production [109], suggesting that both cytokines

may act in a coordinated fashion to induce or maintain tolerance.

One mechanism that likely accounts for T_{regs} efficiency during oral tolerance is their ability to convey their suppressive activity to the T cells they suppress, a process know as infectious tolerance. Thus, $CD4^+CD25^+$ T_{regs} induce anergy of co-cultured $CD4^+$ conventional T cells and confer to them a suppressive ability mediated in part by IL-10 and TGF-β [110, 111]. Several findings support the view that APCs might be instrumental in these regulatory amplification loops. In a model of oral tolerance to nickel, T_{reg}, and also spleen APCs, can transfer tolerance to naïve recipients and spread their suppressive ability to the other cell type following immunization [112]. In a diabetes model, orally induced insulin-specific T_{regs} can suppress auto-reactive destructive T cells of a different Ag specificity, likely via a cytokine-mediated modulation of APCs [113]. A recent study by Matzinger et al. demonstrates that DCs act as messengers to convey tolerance from orally induced T_{regs} to naïve $CD4^+$ T cells [114]. The relay of information requires that the same DCs interacts sequentially with two types of T cells. This education of DC is dependent on IL-4, IL-10 and cell-cell contacts and is favored when CD40 signaling on DCs is low or absent.

DCs and oral tolerance induction

That DCs are involved in both induction of immunity and control of peripheral tolerance is now clearly established in several models. The role played by mucosal DCs in the regulation of adaptive immunity is the subject of a review in this issue, and we will thus concentrate only on the most silent points regarding their role in oral tolerance. The first experimental evidence that DCs are involved in oral tolerance was provided by Viney et al. who demonstrated that Flt3L-driven enhancement of DC numbers resulted in increased oral tolerance, especially with low doses of fed Ag [115]. In addition, DCs recovered from the MLNs of orally tolerized mice were able to induce in vitro differentiation of T cells into cells producing IL-4, IL-10 and TGF-β [7, 116], a cytokine profile reminiscent of Th2/Th3/Tr1 cells that differentiate in the GALT of tolerant mice.

Tolerogenic DCs of the GALT

Phenotypic characterization of DCs has revealed a high number of different subsets in lymphoid organs that may perform specialized functions. The GALT does not escape this rule, and both MLNs and PPs contain at least four populations of DCs, including $CD11c^{high}CD11b^+CD8\alpha^-$ myeloid DCs, $CD11c^{high}CD11b^-$ $CD8\alpha^+$ DCs, $CD11c^{high}$-$CD11b^-$ $CD8\alpha^-$ DCs and $CD11c^{low}Ly6C^+B220^+$ plasmacytoid DC [117–121]. To date, analysis of DC subsets failed to identify a particular lineage unique to – or at least hyper-represented in – the GALT that may account for the propensity of these intestinal tissues to generate tolerogenic responses. However, with regard to their tolerogenic potential, two populations of DCs have recently gained attention. Although present in all lymphoid organs, myeloid DCs from PPs have the unique ability to produce high levels of IL-10 in response to CD40 ligation, a property that gives them the ability to induce differentiation of Tr1-like cells in vitro [118, 122]. That intestinal DCs might be predisposed to IL-10 production was confirmed by the finding that RANK stimulation induced PP DCs to produce IL-10, whereas spleen DCs essentially produced IL-12 [123]. Interestingly, treatment with RANK-L in vivo at the time of Ag feeding resulted in enhanced induction of T cell hyporesponsiveness, suggesting that activation of GALT DCs to produce IL-10 might contribute to oral tolerance [123]. Similarly, Wakkach et al. described a population of IL-10-producing $CD11c^{low}$-$CD45RB^+B220^-$ DCs characterized by an immature-like phenotype and the ability to induce T_{regs} in vitro and in vivo [124]. The finding that IL-10 is a key factor in the differentiation of these tolerogenic DCs in vitro and in vivo suggests that this DC subset might be enriched in the GALT.

The GALT hosts a population of plasmacytoid DCs (pDCs) that have tolerogenic potential. In the mouse, pDCs are defined as $CD11c^{low}B220^+Ly6C^+$ and are the main producers of type I IFN in response to virus [125]. One striking feature of these DCs is their extraordinary functional plasticity; they can induce differentiation of Th1 and cytotoxic $CD8^+$ T lymphocytes cells when infected by virus or stimulated via TLR9 and differentiation Th2 cells when stimulated with IL-3 and/or CD40L [126, 127]. Recent findings also indicate that pDCs can induce differentiation of IL-10-producing T_{regs} in both human [128] and mouse [121, 129]. That pDCs from MLNs are far more efficient than spleen pDCs in supporting Tr1-like cell differentiation from naïve $CD4^+$ T cells [121] suggests their potential contribution in oral tolerance induction. In the steady state, pDCs display a low T cell stimulatory potential that can result in anergy [130]. These tolerogenic functions of pDCs might be linked to their ability to produce α-IFN and IL-10. In addition, pDCs express a high amount of indoleamine 2,3-dioxygenase (IDO) [131, 132], a tryptophan-catabolizing enzyme that has been proposed to contribute to several forms of tolerance [133]. In particular, IDO activity induces tryptophan deprivation that results in suppression of T cell proliferation and generates kynurenines that can induce apoptosis through processes mediated by oxygen-free radicals [134]. It is interesting to note that IL-10 and TGF-β, two characteristic cytokines of the gut microenvironment, have been demonstrated to favor stable IDO expression in DCs [135]. Thus, pDCs display intrinsically all the func-

tional characteristics needed to induce T cell tolerance to ingested Ag and could be involved in several mechanisms that sustain oral tolerance.

A role for the liver DCs?

The liver is believed to play a critical role in peripheral tolerance induction, as evidenced in clinical transplantation and many experimental systems [136]. The mechanisms underlying immunological tolerance in the liver may involve hepatocytes and sinusoidal endothelial cells, which have been demonstrated to tolerize $CD8^+$ T cells [136]. In addition to the fact that Ag injection in the portal vein results in a state of T cell hyporesponsiveness comparable to that induced by Ag feeding, a porto-caval shunt in rats and dogs provided some direct evidence of the critical role of the liver in oral tolerance induction to haptens and proteins [137, 138]. In addition, oral administration of Ag was found to result in massive apoptosis of Ag-specific $CD4^+$ T cells in the liver and the emergence of T_{regs} producing IL-4, IL-10 and TGF-β [139]. The finding that hepatic $CD11c^+$ cells that have captured ingested Ag can induce T cell apoptosis and $Th2/T_{regs}$ differentiation both in vitro and upon adoptive transfer suggests a critical role of liver DCs in oral tolerance induction [140]. At least four DC subsets were recently identified within the liver, including myeloid $CD11c^+CD8\alpha^-$ DCs and $CD11c^+CD8\alpha^+$ lymphoid DCs that displayed potent T cell stimulatory abilities similar to their splenic counterparts [141]. Strikingly, the liver also contains a high proportion of $CD11c^{low}NK1.1^+$ DCs, accounting for 40–50% of the total liver DCs [142] [A. Goubier and D. Kaiserlian unpublished observations], that have been shown to display a regulatory potential in a diabetes model [143]. The last population is composed of $CD11c^{low}B220^+$ pDCs, which are over-represented compared with their number in other lymphoid organs and which display poor T cell stimulatory ability [141, 142]. Confirming their poor immunogenic potential [141], we recently demonstrated that liver $CD11c^+$ DCs cannot prime hapten-specific $CD8^+$ cytotoxic T cells upon adoptive transfer, but instead could tolerize mice to subsequent skin sensitization. This latter suppressive property is confined to pDCs and their in vivo depletion using anti-Gr1 mAb abrogated oral tolerance [A. Goubier and D. Kaiserlian, unpublished observations]. We therefore propose that liver pDCs, which likely encounter ingested Ags, might play a critical role in the induction of T cell hyporesponsiveness during oral tolerance induction.

Conclusions and perspectives

The efficiency of the oral route to induce tolerance in experimental models of autoimmune and inflammatory diseases has prompted the design of numerous clinical trials, which unfortunately have led to variable results and sometimes even adverse effects (see [144]). The major pitfalls in these approaches are (i) that the nature of the Ag responsible for the disease is not precisely defined in most instances and (ii) that optimal regimen and antigen dose is most often empirically determined. Progress in understanding the mechanisms of oral tolerance and, in particular, the role of the naturally occurring subset of regulatory $CD4^+CD25^+$ T cells may by-pass the need to design antigen-specific approaches. In this respect, recent advances in the regulatory/tolerogenic function of commensal bacteria and the potential of selected probiotics to alleviate allergic and inflammatory diseases, possibly by acting as selective adjuvants for regulatory T cells, may open new therapeutic avenues for inducing oral tolerance.

1 Mowat A. M. (2003) Anatomical basis of tolerance and immunity to intestinal antigens. Nat. Rev. Immunol. **3:** 331–341
2 Garside P., Mowat A. M. and Khoruts A. (1999) Oral tolerance in disease. Gut **44:** 137–142
3 Richman L. K., Graeff A. S., Yarchoan R. and Strober W. (1981) Simultaneous induction of antigen-specific IgA helper T cells and IgG suppressor T cells in the murine Peyer's patch after protein feeding. J. Immunol. **126:** 2079–2083
4 Chen Y., Kuchroo V. K., Inobe J., Hafler D. A. and Weiner H. L. (1994) Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science **265:** 1237–1240
5 Fujihashi K., Dohi T., Rennert P. D., Yamamoto M., Koga T., Kiyono H. et al. (2001) Peyer's patches are required for oral tolerance to proteins. Proc. Natl. Acad. Sci. USA **98:** 3310–3315
6 Spahn T. W., Fontana A., Faria A. M., Slavin A. J., Eugster H. P., Zhang X. et al. (2001) Induction of oral tolerance to cellular immune responses in the absence of Peyer's patches. Eur. J. Immunol. **31:** 1278–1287
7 Alpan O., Rudomen G. and Matzinger P. (2001) The role of dendritic cells, B cells, and M cells in gut-oriented immune responses. J. Immunol. **166:** 4843–4852
8 Enders G., Gottwald T. and Brendel W. (1986) Induction of oral tolerance in rats without Peyer's patches. Immunology **58:** 311–314
9 Spahn T. W., Weiner H. L., Rennert P. D., Lugering N., Fontana A., Domschke W. et al. (2002) Mesenteric lymph nodes are critical for the induction of high-dose oral tolerance in the absence of Peyer's patches. Eur. J. Immunol. **32:** 1109–1113
10 Kunkel D., Kirchhoff D., Nishikawa S., Radbruch A. and Scheffold A. (2003) Visualization of peptide presentation following oral application of antigen in normal and Peyer's patches-deficient mice. Eur. J. Immunol. **33:** 1292–1301
11 Kaiserlian D. (1999) Epithelial cells in antigen. Sampling and presentation in mucosal tissues. Curr. Top. Microbiol. Immunol. **236:** 55–78
12 Hershberg R. M., Cho D. H., Youakim A., Bradley M. B., Lee J. S., Framson P. E. et al. (1998) Highly polarized HLA class II antigen processing and presentation by human intestinal epithelial cells. J. Clin. Invest. **102:** 792–803
13 Zimmer K. P., Buning J., Weber P., Kaiserlian D. and Strobel S. (2000) Modulation of antigen trafficking to MHC class II-positive late endosomes of enterocytes. Gastroenterology **118:** 128–137
14 Hershberg R. M. and Mayer L. F. (2000) Antigen processing and presentation by intestinal epithelial cells – polarity and complexity. Immunol. Today **21:** 123–128

15 Vidal K., Samarut C., Magaud J. P., Revillard J. P. and Kaiserlian D. (1993) Unexpected lack of reactivity of allogeneic anti-Ia monoclonal antibodies with MHC class II molecules expressed by mouse intestinal epithelial cells. J. Immunol. **151:** 4642–4650

16 Allez M., Brimnes J., Dotan I. and Mayer L. (2002) Expansion of CD8+ T cells with regulatory function after interaction with intestinal epithelial cells. Gastroenterology **123:** 1516–1526

17 Cruickshank S. M., McVay L. D., Baumgart D. C., Felsburg P. J. and Carding S. R. (2004) Colonic epithelial cell-mediated suppression of CD4 T cell activation. Gut **53:** 678–684

18 Galliaerde V., Desvignes C., Peyron E. and Kaiserlian D. (1995) Oral tolerance to haptens: intestinal epithelial cells from 2,4-dinitrochlorobenzene-fed mice inhibit hapten-specific T cell activation in vitro. Eur. J. Immunol. **25:** 1385–1390

19 Huang F. P,. Platt N., Wykes M., Major J. R., Powell T. J., Jenkins C. D. et al. (2000) A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. J. Exp. Med. **191:** 435–443

20 Karlsson M., Lundin S., Dahlgren U., Kahu H., Pettersson I. and E. Telemo. (2001) 'Tolerosomes' are produced by intestinal epithelial cells. Eur. J. Immunol. **31:** 2892–2900

21 Sierro F., Dubois B., Coste A., Kaiserlian D., Kraehenbuhl J. P. and Sirard J. C. (2001) Flagellin stimulation of intestinal epithelial cells triggers CCL20- mediated migration of dendritic cells. Proc. Natl. Acad. Sci. USA **98:** 13722–13727

22 Neish A. S., Gewirtz A. T., Zeng H., Young A. N., Hobert M. E., Karmali V. et al. (2000) Prokaryotic regulation of epithelial responses by inhibition of IκB-α ubiquitination. Science **289:** 1560–1563

23 Kelly D., Campbell J. I., King T. P., Grant G., Jansson E. A., Coutts A. G. et al. (2004) Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat. Immunol. **5:** 104–112

24 Gonnella P. A., Chen Y., Inobe J., Komagata Y., Quartulli M. and Weiner H. L. (1998) In situ immune response in gut-associated lymphoid tissue (GALT) following oral antigen in TCR-transgenic mice. J. Immunol. **160:** 4708–4718

25 Kalinski P., Hilkens C. M., Snijders A., Snijdewint F. G. and Kapsenberg M. L. (1997) IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naive T helper cells. J Immunol **159:** 28–35

26 Maric I., Holt P. G., Perdue M. H. and Bienenstock J. (1996) Class II MHC antigen (Ia)-bearing dendritic cells in the epithelium of the rat intestine. J. Immunol. **156:** 1408–1414

27 Rescigno M., Urbano M., Valzasina B., Francolini M., Rotta G., Bonasio R. et al. (2001) Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat. Immunol. **2:** 361–367

28 Liu L. M. and MacPherson G. G. (1993) Antigen acquisition by dendritic cells: intestinal dendritic cells acquire antigen administered orally and can prime naive T cells in vivo. J. Exp. Med. **177:** 1299–1307

29 Peng H. J., Turner M. W. and Strobel S. (1990) The generation of a 'tolerogen' after the ingestion of ovalbumin is time-dependent and unrelated to serum levels of immunoreactive antigen. Clin. Exp. Immunol. **81:** 510–515

30 Williamson E., O'Malley J. M. and Viney J. L. (1999) Visualizing the T-cell response elicited by oral administration of soluble protein antigen. Immunology **97:** 565–572

31 Sun J., Dirden-Kramer B., Ito K., Ernst P. B. and Van Houten N. (1999) Antigen-specific T cell activation and proliferation during oral tolerance induction. J. Immunol. **162:** 5868–5875

32 Blanas E., Davey G. M., Carbone F. R. and Heath W. R. (2000) A bone marrow-derived APC in the gut-associated lymphoid tissue captures oral antigens and presents them to both $CD4^+$ and $CD8^+$ T cells. J. Immunol. **164:** 2890–2896

33 Benson J. M., Campbell K. A., Guan Z., Gienapp I. E., Stuckman S. S., Forsthuber T. et al. (2000) T-cell activation and receptor downmodulation precede deletion induced by mucosally administered antigen. J. Clin. Invest. **106:** 1031–1038.

34 Gutgemann I., Fahrer A. M., Altman J. D., Davis M. M. and Chien Y. H. (1998) Induction of rapid T cell activation and tolerance by systemic presentation of an orally administered antigen. Immunity **8:** 667–673

35 Kobets N., Kennedy K. and Garside P. (2003) An investigation of the distribution of antigen fed in tolerogenic or immunogenic forms. Immunol. Lett. **88:** 147–155

36 Smith K. M., Davidson J. M. and P. Garside (2002) T-cell activation occurs simultaneously in local and peripheral lymphoid tissue following oral administration of a range of doses of immunogenic or tolerogenic antigen, although tolerized T cells display a defect in cell division. Immunology **106:** 144–158

37 Ju C. and Pohl L. R. (2001) Immunohistochemical detection of protein adducts of 2,4-dinitrochlorobenzene in antigen-presenting cells and lymphocytes after oral administration to mice: lack of a role of Kupffer cells in oral tolerance. Chem. Res. Toxicol. **14:** 1209–1217

38 Hanson D. G. and Miller S. D. (1982) Inhibition of specific immune responses by feeding protein antigens. V. Induction of the tolerant state in the absence of specific suppressor T cells. J. Immunol. **128:** 2378–2381

39 Friedman A. and Weiner H. L. (1994) Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. Proc. Natl. Acad. Sci. **91:** 6688–6692

40 Chen Y., Inobe J., Marks R., Gonnella P., Kuchroo V. K. and Weiner H. L. (1995) Peripheral deletion of antigen-reactive T cells in oral tolerance. Nature **376:** 177–180

41 Ueda Y., Hachimura S., Somaya T., Hisatsune T. and Kaminogawa S. (2001) Apoptosis of antigen-specific T cells induced by oral administration of antigen: comparison of intestinal and non-intestinal immune organs. Biosci. Biotechnol. Biochem. **65:** 1170–1174

42 Kaji T., Hachimura S., Ise W. and Kaminogawa S. (2003) Proteome analysis reveals caspase activation in hyporesponsive CD4 T lymphocytes induced in vivo by the oral administration of antigen. J. Biol. Chem. **278:** 27836–27843

43 Whitacre C. C., Gienapp I. E., Orosz C. G. and Bitar D. M. (1991) Oral tolerance in experimental autoimmune encephalomyelitis. III. Evidence for clonal anergy. J. Immunol. **147:** 2155–2163

44 Melamed D. and A. Friedman (1993) Direct evidence for anergy in T lymphocytes tolerized by oral administration of ovalbumin. Eur. J. Immunol. **23:** 935–942

45 Melamed D. and A. Friedman (1994) In vivo tolerization of Th1 lymphocytes following a single feeding with ovalbumin: anergy in the absence of suppression. Eur. J. Immunol. **24:** 1974–1981

46 Van Houten N. and Blake S. F. (1996) Direct measurement of anergy of antigen-specific T cells following oral tolerance induction. J. Immunol. **157:** 1337–1341

47 Lombardi G., Sidhu S., Batchelor R. and Lechler R. (1994) Anergic T cells as suppressor cells in vitro. Science **264:** 1587–1589

48 Lider O., Santos L. M., Lee C. S., Higgins P. J. and Weiner H. L. (1989) Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein. II. Suppression of disease and in vitro immune responses is mediated by antigen-specific CD8+ T lymphocytes. J. Immunol. **142:** 748–752

49 Wildner G., Hunig T. and Thurau S. R. (1996) Orally induced, peptide-specific gamma/delta TCR^+ cells suppress experimental autoimmune uveitis. Eur. J. Immunol. **26:** 2140–2148

50 Garside P., Steel M., Liew F. Y. and Mowat A. M. (1995) $CD4^+$ but not $CD8^+$ T cells are required for the induction of oral tolerance. Int. Immunol. **7:** 501–504

51 Barone K. S., Jain S. L. and Michael J. G. (1995) Effect of in vivo depletion of $CD4^+$ and $CD8^+$ cells on the induction and maintenance of oral tolerance. Cell. Immunol. **163:** 19–29

52 Desvignes C., Bour H., Nicolas J. F. and Kaiserlian D. (1996) Lack of oral tolerance but oral priming for contact sensitivity to dinitrofluorobenzene in major histocompatibility complex class II- deficient mice and in $CD4^+$ T cell-depleted mice. Eur. J. Immunol. **26:** 1756–1761

53 Desvignes C., Etchart N., Kehren J., Akiba I., Nicolas J. F. and Kaiserlian D. (2000) Oral administration of hapten inhibits in vivo induction of specific cytotoxic $CD8^+$ T cells mediating tissue inflammation: a role for regulatory $CD4^+$ T cells. J. Immunol. **164:** 2515–2522

54 Miller A., Lider O., Roberts A. B., Sporn M. B. and Weiner H. L. (1992) Suppressor T cells generated by oral tolerization to myelin basic protein suppress both in vitro and in vivo immune responses by the release of transforming growth factor beta after antigen-specific triggering. Proc. Natl. Acad. Sci. USA **89:** 421–425

55 Santos L. M., al-Sabbagh A., Londono A. and Weiner H. L. (1994) Oral tolerance to myelin basic protein induces regulatory TGF-beta-secreting T cells in Peyer's patches of SJL mice. Cell. Immunol. **157:** 439–447

56 Weiner H. L. (2001) Oral tolerance: immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. Microbes Infect. **3:** 947–954

57 Shevach E. M. (2002) $CD4^+$ $CD25^+$ suppressor T cells: more questions than answers. Nat. Rev. Immunol. **2:** 389–400

58 O'Garra A. and Vieira P. (2003) Twenty-first century Foxp3. Nat. Immunol. **4:** 304–306

59 Zhang X., Izikson L., Liu L. and Weiner H. L. (2001) Activation of $CD25(^+)CD4(^+)$ regulatory T cells by oral antigen administration. J. Immunol. **167:** 4245–4253

60 Thorstenson K. M. and A. Khoruts (2001) Generation of anergic and potentially immunoregulatory $CD25^+CD4$ T cells in vivo after induction of peripheral tolerance with intravenous or oral antigen. J. Immunol. **167:** 188–195

61 Hauet-Broere F., Unger W. W., Garssen J., Hoijer M. A., Kraal G. and Samsom J. N. (2003) Functional $CD25^-$ and $CD25^+$ mucosal regulatory T cells are induced in gut-draining lymphoid tissue within 48 h after oral antigen application. Eur. J. Immunol. **33:** 2801–2810

62 Kehren J., Desvignes C., Krasteva M., Ducluzeau M. T., Assossou O., Horand F. et al. (1999) Cytotoxicity is mandatory for $CD8(^+)$ T cell-mediated contact hypersensitivity. J. Exp. Med. **189:** 779–786

63 Dubois B., Chapat L., Goubier A., Papiernik M., Nicolas J. F. and Kaiserlian D. (2003) Innate $CD4^+CD25^+$ regulatory T cells are required for oral tolerance and inhibition of $CD8^+$ T cells mediating skin inflammation. Blood **102:** 3295–3301

64 Karlsson M. R., Rugtveit J. and Brandtzaeg P. (2004) Allergen-responsive $CD4^+CD25^+$ regulatory T cells in children who have outgrown cow's milk allergy. J. Exp. Med. **199:** 1679–1688

65 Nakamura K., Kitani A. and Strober W. (2001) Cell contact-dependent immunosuppression by $CD4(^+)CD25(^+)$ regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J. Exp. Med. **194:** 629–644

66 Groux H., O'Garra A., Bigler M., Rouleau M., Antonenko S., de Vries J. E. et al. (1997) A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature **389:** 737–742

67 Jonuleit H., Schmitt E., Schuler G., Knop J. and Enk A. H. (2000) Induction of interleukin 10-producing, nonproliferating $CD4(^+)$ T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. J. Exp. Med. **192:** 1213–1222

68 Vieira P. L., Christensen J. R., Minaee S., O'Neill E. J., Barrat F. J., Boonstra A. et al. (2004) IL-10-secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring $CD4^+CD25^+$ regulatory T cells. J. Immunol. **172:** 5986–5993

69 Akbari O., Freeman G. J., Meyer E. H., Greenfield E. A., Chang T. T., Sharpe A. H. et al. (2002) Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. Nat. Med. **8:** 1024–1032

70 Cong Y., Weaver C. T., Lazenby A. and Elson C. O. (2002) Bacterial-reactive T regulatory cells inhibit pathogenic immune responses to the enteric flora. J. Immunol. **169:** 6112–6119

71 Daynes R. A., Araneo B. A., Dowell T. A., Huang K. and D. Dudley (1990) Regulation of murine lymphokine production in vivo. III. The lymphoid tissue microenvironment exerts regulatory influences over T helper cell function. J. Exp. Med. **171:** 979–996

72 Eaton A. D., Xu D. and Garside P. (2003) Administration of exogenous interleukin-18 and interleukin-12 prevents the induction of oral tolerance. Immunology **108:** 196–203

73 Williamson E., Westrich G. M. and Viney J. L. (1999) Modulating dendritic cells to optimize mucosal immunization protocols. J. Immunol. **163:** 3668–3675

74 Kitani A., Fuss I. J., Nakamura K., Schwartz O. M., Usui T. and Strober W. (2000) Treatment of experimental (trinitrobenzene sulfonic acid) colitis by intranasal administration of transforming growth factor (TGF)-beta1 plasmid: TGF-beta1-mediated suppression of T helper cell type 1 response occurs by interleukin (IL)-10 induction and IL-12 receptor beta2 chain downregulation. J. Exp. Med. **192:** 41–52

75 Slavin A. J., Maron R. and Weiner H. L. (2001) Mucosal administration of IL-10 enhances oral tolerance in autoimmune encephalomyelitis and diabetes. Int. Immunol. **13:** 825–833

76 Kellermann S. A. and McEvoyv L. M. (2001) The Peyer's patch microenvironment suppresses T cell responses to chemokines and other stimuli. J. Immunol. **167:** 682–690

77 Xu-Amano J., Aicher W. K., Taguchi T., Kiyono H. and McGhee J. R. (1992) Selective induction of Th2 cells in murine Peyer's patches by oral immunization. Int. Immunol. **4:** 433–445

78 Jump R. L. and Levine A. D. (2002) Murine Peyer's patches favor development of an IL-10-secreting, regulatory T cell population. J. Immunol. **168:** 6113–6119

79 Steinbrink K., Graulich E., Kubsch S., Knop J. and Enk A. H. (2002) $CD4(^+)$ and $CD8(^+)$ anergic T cells induced by interleukin-10-treated human dendritic cells display antigen-specific suppressor activity. Blood **99:** 2468–2476

80 Goudy K. S., Burkhardt B. R., Wasserfall C., Song S., Campbell-Thompson M. L., Brusko T. et al. (2003) Systemic overexpression of IL-10 induces $CD4^+CD25^+$ cell populations in vivo and ameliorates type 1 diabetes in nonobese diabetic mice in a dose-dependent fashion. J. Immunol. **171:** 2270–2278

81 Seder R. A., Marth T,. Sieve M. C., Strober W., Letterio J. J., Roberts A. B. et al. (1998) Factors involved in the differentiation of TGF-beta-producing cells from naive $CD4^+$ T cells: IL-4 and IFN-gamma have opposing effects, while TGF-beta positively regulates its own production. J. Immunol. **160:** 5719–5728

82 Chen W., Jin W., Hardegen N., Lei K. J., Li L., Marinos N. et al. (2003) Conversion of peripheral $CD4^+CD25^-$ naive T cells to $CD4^+CD25^+$ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J. Exp. Med. **198:** 1875–1886

83 Fantini M. C., Becker C., Monteleone G., Pallone F., Galle P. R. and M. F. Neurath. (2004) Cutting edge: TGF-beta induces a regulatory phenotype in $CD4^+CD25^-$ T cells through Foxp3 induction and down-regulation of Smad7. J Immunol **172:** 5149–5153

84 Chen Z. M., O'Shaughnessy M. J., Gramaglia I., Panoskaltsis-Mortari A., Murphy W. J., Narula S. et al. (2003) IL-10 and TGF-beta induce alloreactive $CD4^+CD25^-$ T cells to acquire regulatory cell function. Blood **101:** 5076–5083

85 Kiyono H., McGhee J. R., Wannemuehler M. J. and Michalek S. M. (1982) Lack of oral tolerance in C3H/HeJ mice. J. Exp. Med. **155:** 605–610

86 Wannemuehler M. J., Kiyono H., Babb J. L., Michalek S. M. and McGhee J. R. (1982) Lipopolysaccharide (LPS) regulation of the immune response: LPS converts germfree mice to sensitivity to oral tolerance induction. J. Immunol. **129:** 959–965

87 Michalek S. M., Kiyono H., Wannemuehler M. J., Mosteller L. M. and McGhee J. R. (1982) Lipopolysaccharide (LPS) regulation of the immune response: LPS influence on oral tolerance induction. J. Immunol. **128:** 1992–1998

88 Sudo N., Sawamura S., Tanaka K., Aiba Y., Kubo C. and Koga Y. (1997) The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J. Immunol. **159:** 1739–1745

89 Moreau M. C. and Corthier G. (1988) Effect of the gastrointestinal microflora on induction and maintenance of oral tolerance to ovalbumin in C3H/HeJ mice. Infect. Immun. **56:** 2766–2768

90 Khoury S. J., Lider O., al-Sabbagh A. and Weiner H. L. (1990) Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein. III. Synergistic effect of lipopolysaccharide. Cell. Immunol. **131:** 302–310

91 Beatty W. L. and Sansonetti P. J. (1997) Role of lipopolysaccharide in signaling to subepithelial polymorphonuclear leukocytes. Infect. Immun. **65:** 4395–4404

92 Gionchetti P., Rizzello F., Helwig U., Venturi A., Lammers K. M., Brigidi P. et al. (2003) Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. Gastroenterology **124:** 1202–1209

93 Chapat L., Chemin K., Dubois B., Bourdet-Sicard R. and Kaiserlian D. (2004) Lactobacillus casei reduces CD8(+) T cell-mediated skin inflammation. Eur J Immunol **34:** 2520–2528

94 Sakaguchi S. (2003) Control of immune responses by naturally arising CD4+ regulatory T cells that express toll-like receptors. J. Exp. Med. **197:** 397–401

95 Caramalho I., Lopes-Carvalho T., Ostler D., Zelenay S., Haury M. and Demengeot J. (2003) Regulatory T cells selectively express Toll-like receptors and are activated by lipopolysaccharide. J. Exp. Med. **197:** 403–411

96 Netea M. G., Sutmuller R., Hermann C., Van der Graaf C. A., Van der Meer J. W., van Krieken J. H. et al. (2004) Toll-like receptor 2 suppresses immunity against *Candida albicans* through induction of IL-10 and regulatory T cells. J. Immunol. **172:** 3712–3718

97 Christensen H. R., Frokiaer H. and Pestka J. J. (2002) Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. J. Immunol. **168:** 171–178

98 von der Weid T., Bulliard C. and Schiffrin E. J. (2001) Induction by a lactic acid bacterium of a population of CD4(+) T cells with low proliferative capacity that produce transforming growth factor beta and interleukin-10. Clin. Diagn. Lab. Immunol. **8:** 695–701

99 Asseman C., Mauze S., Leach M. W., Coffmanv and Powrie F. (1999) An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J. Exp. Med. **190:** 995–1004

100 Suri-Payer E. and Cantor H. (2001) Differential cytokine requirements for regulation of autoimmune gastritis and colitis by CD4(+)CD25(+) T cells. J Autoimmun **16:** 115–123

101 Liu H., Hu B., Xu D. and Liew F. Y. (2003) $CD4^+CD25^+$ regulatory T cells cure murine colitis: the role of IL-10, TGF-beta and CTLA4. J. Immunol. **171:** 5012–5017

102 Maloy K. J., Salaun L., Cahill R., Dougan G., Saunders N. J. and Powrie F. (2003) $CD4^+CD25^+$ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms. J. Exp. Med. **197:** 111–119

103 Aroeira L. S., Cardillo F., De Albuquerque D. A., Vaz N. M. and Mengel J. (1995) Anti-IL-10 treatment does not block either the induction or the maintenance of orally induced tolerance to OVA. Scand. J. Immunol. **41:** 319–323

104 Barone K. S., Tolarova D. D., Ormsby I., Doetschman T. and Michael J. G. (1998) Induction of oral tolerance in TGF-beta 1 null mice. J. Immunol. **161:** 154–160

105 Rizzo L. V., Morawetz R. A., Miller-Rivero N. E., Choi R., Wiggert B., Chan C. C. et al. (1999) IL-4 and IL-10 are both required for the induction of oral tolerance. J. Immunol. **162:** 2613–2622

106 Fowler S. and Powrie F. (2002) CTLA-4 expression on antigen-specific cells but not IL-10 secretion is required for oral tolerance. Eur. J. Immunol. **32:** 2997–3006

107 Fuss I. J., Boirivant M., Lacy B. and Strober W. (2002) The interrelated roles of TGF-beta and IL-10 in the regulation of experimental colitis. J. Immunol. **168:** 900–908

108 Cottrez F. and Groux H. (2001) Regulation of TGF-beta response during T cell activation is modulated by IL-10. J. Immunol. **167:** 773–778

109 Kitani A., Fuss I., Nakamura K., Kumaki F., Usui T. and Strober W. (2003) Transforming growth factor (TGF)-beta1-producing regulatory T cells induce Smad-mediated interleukin 10 secretion that facilitates coordinated immunoregulatory activity and amelioration of TGF-beta1-mediated fibrosis. J. Exp. Med. **198:** 1179–1188

110 Dieckmann D., Bruett C. H., Ploettner H., Lutz M. B. and Schuler G. (2002) Human CD4(+)CD25(+) regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells. J. Exp. Med. **196:** 247–253

111 Jonuleit H., Schmitt E., Kakirman H., Stassen M., Knop J. and Enk A. H. (2002) Infectious tolerance: human CD25(+) regulatory T cells convey suppressor activity to conventional CD4(+) T helper cells. J. Exp. Med. **196:** 255–260

112 Roelofs-Haarhuis K., Wu X., Nowak M., Fang M., Artik S. and Gleichmann E. (2003) Infectious nickel tolerance: a reciprocal interplay of tolerogenic APCs and T suppressor cells that is driven by immunization. J. Immunol. **171:** 2863–2872

113 Homann D., Dyrberg T., Petersen J., Oldstone M. B. and von Herrath M. G. (1999) Insulin in oral immune 'tolerance': a one-amino acid change in the B chain makes the difference. J. Immunol. **163:** 1833–1838

114 Alpan O., Bachelder E., Isil E., Arnheiter H. and Matzinger P. (2004) 'Educated' dendritic cells act as messengers from memory to naive T helper cells. Nat. Immunol. **5:** 615–622

115 Viney J. L., Mowat A. M., O'Malley J. M., Williamson E. and Fanger N. A. (1998) Expanding dendritic cells in vivo enhances the induction of oral tolerance. J. Immunol. **160:** 5815–5825

116 Akbari O., DeKruyff R. H. and Umetsu D. T. (2001) Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. Nat. Immunol. **2:** 725–731

117 Kelsall B. L. and Strober W. (1996) Distinct populations of dendritic cells are present in the subepithelial dome and T cell regions of the murine Peyer's patch. J. Exp. Med. **183:** 237–247

118 Iwasaki A. and Kelsall B. L. (2001) Unique functions of $CD11b^+$, CD8 $alpha^+$, and double-negative Peyer's patch dendritic cells. J. Immunol. **166:** 4884–4890

119 Iwasaki A. and Kelsall B. L. (2000) Localization of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines macrophage inflammatory protein (MIP)-3alpha, MIP-3beta, and secondary lymphoid organ chemokine. J. Exp. Med. **191:** 1381–1394

120 Asselin-Paturel C., Brizard G., Pin J. J., Briere F. and Trinchieri G. (2003) Mouse strain differences in plasmacytoid dendritic cell frequency and function revealed by a novel monoclonal antibody. J. Immunol. **171:** 6466–6477

121 Bilsborough J., George T. C., Norment A. and Viney J. L. (2003) Mucosal CD8alpha$^+$ DC, with a plasmacytoid phenotype, induce differentiation and support function of T cells with regulatory properties. Immunology **108:** 481–492
122 Iwasaki A. and Kelsall B. L. (1999) Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. J. Exp. Med. **190:** 229–239
123 Williamson E., Bilsborough J. M. and Viney J. L. (2002) Regulation of mucosal dendritic cell function by receptor activator of NF-κB (RANK)/RANK ligand interactions: impact on tolerance induction. J. Immunol. **169:** 3606–3612
124 Wakkach A., Fournier N., Brun V., Breittmayer J. P., Cottrez F. and Groux H. (2003) Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. Immunity **18:** 605–617
125 Asselin-Paturel C., Boonstra A., Dalod M., Durand I., Yessaad N., Dezutter-Dambuyant C. et al. (2001) Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. Nat. Immunol. **2:** 1144–1150
126 Rissoan M. C., Soumelis V., Kadowaki N., Grouard G., Briere F., de Waal R., Malefyt et al. (1999) Reciprocal control of T helper cell and dendritic cell differentiation. Science **283:** 1183–1186
127 Boonstra A., Asselin-Paturel C., Gilliet M., Crain C., Trinchieri G., Liu Y. J. et al. (2003) Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential toll-like receptor ligation. J. Exp. Med. **197:** 101–109
128 Gilliet M. and Liu Y. J. (2002) Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. J. Exp. Med. **195:** 695–704
129 Martin P., Del Hoyo G. M., Anjuere F., Arias C. F., Vargas H. H., Fernandez L. A. et al. (2002) Characterization of a new subpopulation of mouse CD8alpha$^+$ B220$^+$ dendritic cells endowed with type 1 interferon production capacity and tolerogenic potential. Blood **100:** 383–390
130 Kuwana M., Kaburaki J., Wright T. M., Kawakami Y. and Ikeda Y. (2001) Induction of antigen-specific human CD4(+) T cell anergy by peripheral blood DC2 precursors. Eur. J. Immunol. **31:** 2547–2557
131 Mellor A. L., Keskin D. B., Johnson T., Chandler P. and Munn D. H. (2002) Cells expressing indoleamine 2,3-dioxygenase inhibit T cell responses. J. Immunol. **168:** 3771–3776
132 Fallarino F., Vacca C., Orabona C., Belladonna M. L., Bianchi R., Marshall B. et al. (2002) Functional expression of indoleamine 2,3-dioxygenase by murine CD8 alpha(+) dendritic cells. Int. Immunol. **14:** 65–68
133 Grohmann U., Fallarino F. and Puccetti P. (2003) Tolerance, DCs and tryptophan: much ado about IDO. Trends Immunol. **24:** 242–248
134 Fallarino F., Grohmann U., Vacca C., Bianchi R., Orabona C., Spreca A. et al. (2002) T cell apoptosis by tryptophan catabolism. Cell Death Differ. **9:** 1069–1077
135 Munn D. H., Sharma M. D., Lee J. R., Jhaver K. G., Johnson T. S., Keskin D. B. et al. (2002) Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science **297:** 1867–1870
136 Crispe I. N. (2003) Hepatic T cells and liver tolerance. Nat. Rev. Immunol. **3:** 51–62
137 Cantor H. M. and Dumont A. E. (1967) Hepatic suppression of sensitization to antigen absorbed into the portal system. Nature **215:** 744–745
138 Yang R., Liu Q., Grosfeld J. L. and Pescovitz M. D. (1994) Intestinal venous drainage through the liver is a prerequisite for oral tolerance induction. J. Pediatr. Surg. **29:** 1145–1148
139 Watanabe T., Yoshida M., Shirai Y., Yamori M., Yagita H., Itoh T. et al. (2002) Administration of an antigen at a high dose generates regulatory CD4$^+$ T cells expressing CD95 ligand and secreting IL-4 in the liver. J. Immunol. **168:** 2188–2199
140 Watanabe T., Katsukura H., Shirai Y., Yamori M., Nishi T., Chiba T. et al. (2003) A liver tolerates a portal antigen by generating CD11c$^+$ cells, which select Fas ligand$^+$ Th2 cells via apoptosis. Hepatology **38:** 403–412
141 Pillarisetty V. G., Shah A. B., Miller G., Bleier J. I. and DeMatteo R. P. (2004) Liver dendritic cells are less immunogenic than spleen dendritic cells because of differences in subtype composition. J. Immunol. **172:** 1009–1017
142 Lian Z. X., Okada T., He X. S., Kita H., Liu Y. J., Ansari A. A. et al. (2003) Heterogeneity of dendritic cells in the mouse liver: identification and characterization of four distinct populations. J. Immunol. **170:** 2323–2330
143 Homann D., Jahreis A., Wolfe T., Hughes A., Coon B., van Stipdonk M. J. et al. (2002) CD40L blockade prevents autoimmune diabetes by induction of bitypic NK/DC regulatory cells. Immunity **16:** 403–415
144 Mayer L. and Shao L. (2004) Therapeutic potential of oral tolerance. Nat. Rev. Immunol. **4:** 407–419