Sequential Role of Plasmacytoid Dendritic Cells and Regulatory T Cells in Oral Tolerance

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BACKGROUND & AIMS: Orally induced tolerance to environmental allergens prevents deleterious, systemic, delayed-type hypersensitivity responses via immune suppression mechanisms believed to include either anergy/deletion of specific effector T cells or active suppression by CD4+CD25+ regulatory T cells (Tregs). The aim of this study was to investigate whether and how antigen (Ag) penetration through the gut orchestrates these 2 distinct mechanisms. METHODS: Using a model of allergic contact dermatitis (ACD) mediated by hapten-specific cytolytic CD8+ T cells and a T-cell transfer model of contact hypersensitivity in CD3ε-deficient mice, we studied the outcome of Ag gavage on CD8+ effectors and Tregs. RESULTS: Full protection from ACD by gavage with the relevant allergen required 2 coordinated events taking place first in gut-associated lymphoid tissues and then systemically. Allergen gavage induced deletion by plasmacytoid dendritic cells of a large fraction of Ag-specific CD8+ T cells in liver and mesenteric lymph nodes and also triggered the suppressive function of Treg of secondary lymphoid organs. Residual Ag-specific CD8+ T cells conditioned during this mucosal step are fully susceptible to suppression by activated Treg, which completely prevented their differentiation into ACD effectors, upon re-exposure to the allergen via the skin. CONCLUSIONS: Thus, oral tolerance initiated in gut-associated lymphoid tissues by the plasmacytoid dendritic cells-mediated deletion of Ag-specific T cells is completed systemically by CD4+CD25+ T cells. Biotherapies able to increase the susceptibility of effector T cells to the suppressive function of Treg may be valuable for the treatment of autoimmune and inflammatory diseases.

Oral tolerance is a physiologic mechanism that prevents the development of local and systemic T cell-mediated inflammatory responses to commensals, dietary proteins, and xenobiotics (such as drugs, dyes, conservatives, and flavor enhancers).1 Impaired oral tolerance is believed to contribute to mucosal inflammation in response to the gut flora in Crohn’s disease, gluten-driven enteropathy in celiac disease, and immediate and delayed-type food hypersensitivities.2 That oral administration of antigen (Ag) prior to systemic immunization prevents the development of organ-specific CD4+ and CD8+ T cell-mediated autoimmune pathologies and allergic diseases has been extensively documented in rodents.3

Oral tolerance is an active process that involves the transient activation of Ag-specific T cells in both gut-associated lymphoid tissues and peripheral organs4–6 and results in T-cell hyporesponsiveness to subsequent Ag re-exposure.7 Two types of mechanisms, which are thought to be largely dependent on the dose of oral Ag, contribute to mucosal tolerance.1,3 In situations favoring systemic spreading of Ag (eg, after feeding a single high dose or multiple low doses of Ag), clonal deletion of CD4+ T cells by apoptosis has been demonstrated in studies using T cell-receptor transgenic mice.8 Under similar circumstances, anergy, eg, a state of reversible functional paralysis of T cells characterized by inability to respond to Ag in vitro or in vivo, has also been documented and could precede T-cell deletion by apoptosis.6 Alternatively, feeding low doses of Ag has been shown to trigger the differentiation of transforming growth factor (TGF) β-producing T helper (Th) 3-type cells9 and CD4+CD25+ T cells,10,11 which can suppress pathologic inflammatory responses upon adoptive transfer.12 There is thus ample evidence that Ag penetration through the gut favors emergence of regulatory T cells (Tregs), but whether anergy/deletion and active suppression represent alternative or synergistic mechanisms involved in the induction and/or maintenance of oral tolerance remains unclear.

Most of our current knowledge on the mechanisms of oral tolerance relies on experimental models of CD4+ T cell-mediated diseases. However, there is now increasing evidence that CD8+ effector T cells are key players in several chronic inflammatory diseases affecting epithelial tissues such as the gastrointestinal tract (ie, celiac disease13 and inflammatory bowel disease14) or the skin (ie,

Abbreviations used in this paper: CHS, contact hypersensitivity; DNFB, 2,4-dinitrofluorobenzene; DTH, delayed-type hypersensitivity; MLN, mesenteric lymph node; pDC, plasmacytoid dendritic cells; Tregs, regulatory T cells.
allergic contact dermatitis. We previously documented that the hapten 2,4-dinitrofluorobenzene (DNFB), which covalently binds to self protein and generates modified self peptides presented by major histocompatibility complex (MHC) class I molecules, induces upon immunization and challenge via the skin or the colon, CD8+ T-cell-mediated delayed-type hypersensitivity (DTH) responses that reproduce the pathophysiology of allergic contact dermatitis and inflammatory bowel disease, respectively. In the model of allergic contact dermatitis, hapten gavage prior to skin sensitization induces tolerance and inhibits CD8+ T-cell priming and prevents the development of delayed-type contact hypersensitivity (CHS) by a mechanism involving both CD4+CD25+ Treg and plasmacytoid dendritic cells (pDC). In the present study, we demonstrate that pDC-mediated depletion of Ag-specific CD8+ T cells in gut-associated lymphoid tissues and Tregs inhibition of residual effector T-cell priming are 2 sequential and complementary events that are required to achieve complete suppression of systemic CD8+ T cell-mediated DTH responses to orally administered Ag.

Materials and Methods

Mice

Female C57BL/6 mice were purchased from Charles River Laboratories (L’Arbresle, France). C57BL/6-Ly5a, Ii−/−, I-AB−/−, interleukin (IL)-10−/−, and CD3ε−/− mice were bred as homozygotes in the “Plateau de Biologie Expérimentale de la Souris” (PBES; Ecole Normale Supérieure de Lyon) under specific pathogen-free conditions and were used between 6 and 12 weeks of age. All experiments were previously approved by the Animal Care and Use Committee according to institutional guidelines.

Contact Hypersensitivity to DNFB and Hapten Gavage

Five to 7 mice per group were sensitized epicutaneously on abdominal skin with 25 μL of 0.5% DNFB (Sigma, St. Quentin Fallavier, France) diluted in acetone-olive oil, i.e., vehicle (4:1, vol/vol) and ear challenged 5 days later with 2 × 4 μL of 0.15% DNFB applied on both sides of the right ear. CHS was determined by the mouse ear swelling test as previously described. For oral tolerance induction, 300 μL of 0.1% DNFB or 1% OXA in acetone-olive oil (1:10, vol/vol) was delivered by gavage.

Purification of CD8+ T Cells and CD4+CD25 Tregs

Liver leukocytes were isolated by enzymatic digestion as described elsewhere. CD8+ T cells were isolated by positive selection as previously described and were routinely >94% CD8+. For certain experiments, enriched CD8+ T cells were stained with anti-CD8α-FITC, anti-CD45.1-PE, biotinylated anti-CD45.2, and Streptavidin-PercPC5.5 and FACSorted (FACSvantage, BD Biosciences, France) into CD8+CD45.1+ and CD8+CD45.1− cells (>99% purity). For isolation of CD4+CD25+ T cells, CD25+ cells were first enriched by MACS using anti-CD25-PE monoclonal antibodies (mAb) and anti-PE microbeads (Miltenyi Biotech, Paris, France). Enriched CD25+ cells were then incubated with FITC-anti-CD4 mAb, and CD4+CD25+ cells were obtained by FACSorting (>99% pure).

In Vivo Depletion Experiments

pDC depletion was achieved by intraperitoneal (IP) injections of either anti-Gr1 mAb or anti-BST-2 mAb at days −1, 0, +2, and +5, with respect to day 0 of hapten gavage, as previously described. Depletion of CD25+ Tregs was performed by multiple IP injections of 100 μg anti-CD25 PC61 mAb as indicated in the figure legends. This treatment resulted in >80% depletion of CD4+CD25+ T cells in spleen and lymph nodes (data not shown).

Hapten-Specific CD8+ T-Cell Responses

Bone marrow-derived dendritic cells or syngeneic spleen cells from naive animals were irradiated (21 grays) and used as antigen-presenting cells for in vitro restimulation of hapten-specific CD8+ T cells, as previously described. For proliferation studies, 2 × 105 CD8+ cells were cultured in round bottom, 96-well plates in the presence of DNBS-pulsed or unpulsed bone marrow-derived dendritic cells for 3 days. The proliferative response was assessed by [3H]thymidine incorporation (1 μCi/well) during the last 8 hours.

Statistical Analysis

Statistics were performed using the Student t test. P values < .05 were considered to be statistically significant.

Results

Oral Encounter With Ag in Mesenteric Lymph Nodes and Liver Induces Transient Activation of CD8+ T Cells

Complete suppression of the CHS response in B6 mice was achieved by a single gavage with 0.1% of DNFB administered between 5 and 14 days prior to skin sensitization (Supplementary Figure 1A and B). Because hapten gavage prevents priming of hapten-specific CD8+ CHS effectors in response to skin sensitization, we examined the impact of oral Ag delivery on CD8+ T cells.
CD8+ T cells from mesenteric lymph nodes (MLN), and to a lesser extent from liver (but not spleen), from day 3 (but not day 6) DNFB-fed mice slightly proliferated in vitro in response to in vitro restimulation with the hapten (Figure 1A), although they were unable to secrete IFN-γ (data not shown). Thus, the oral tolerogen caused a transient activation of specific CD8+ T cells in MLN and liver but did not induce their differentiation into effector T cells.

Gavage Renders Oral Ag-Specific CD8+ T Cells Hyporesponsive to Systemic Immunization

Oral tolerance is initiated in MLN and liver17,19 by a step of CD8+ T-cell hyporesponsiveness mediated by oral Ag presentation by pDC.17 Using our CD8+ T-cell transfer model of CHS in T-cell-deficient CD3e−/− mice (Figure 1B), we examined in more detail the Ag specificity and mechanism of this orally induced CD8+ T-cell unresponsiveness. CD3e−/− mice developed a CHS response of similar intensity and kinetics when transferred with MLN CD8+ T cells from either vehicle-fed, day 3 DNFB-fed, or day 7 OXA-fed donors. Alternatively, CD8+ T cells obtained 7 days after DNFB gavage were impaired in their ability to give rise to skin inflammation (Figure 1C and D). Skin draining lymph nodes of day 5 DNFB-sensitized CD3e−/− recipients reconstituted with CD8+ T cells from DNFB-fed donors contained highly reduced numbers of
CD25<sup>+</sup>CD44<sup>high</sup>CD62L<sup>low</sup> activated CD8<sup>+</sup> T cells (Figure 2A) and IFN-γ-producing CHS effectors (Figure 2B), as compared with the CD8<sup>+</sup> T progeny derived from vehicle- or OXA-fed B6 donors. This reduced propensity to differentiate into CHS effectors after DNFB gavage was more prominent for CD8<sup>+</sup> T cells originating from MLN and liver as compared with spleen (Figure 2C). Moreover, in contrast to intragastric DNFB delivery, exposure to DNFB via the skin before systemic sensitization was unable to generate tolerant CD8<sup>+</sup> T cells (Figure 2D).

Thus, Ag encounter in MLN and liver, but not spleen, induced within 7 days Ag-specific CD8<sup>+</sup> T-cell hyporesponsiveness and suppression of the CHS.

**Ag-Specific CD8<sup>+</sup> T Cells From DNFB-Fed Animals Are Neither Anergic nor Suppressive**

Anergy, deletion, or conversion into Tregs might be the mechanism underlying orally induced CD8<sup>+</sup> T-cell hyporesponsiveness. Because anergy is reversible and requires continuous exposure to Ag, we first tested whether parking of tolerant CD8<sup>+</sup> T cells in Ag-free CD3ε<sup>-/-</sup> recipients for an extended period of time before skin immunization could reverse CD8 tolerance. MLN CD8<sup>+</sup> T cells from DNFB-fed mice remained tolerant for at least 8 weeks after transfer into CD3ε<sup>-/-</sup> mice (Figure 3A), even after 2 consecutive DNFB immunizations (Figure 3B), ruling out anergy as the mechanism of CD8 tolerance. CD8<sup>+</sup> T cells from tolerant mice did not affect the capacity of CHS effector cells to proliferate (Supplementary Figure 2) and to pro-
produce IFN-γ in vitro (Figure 4D). Thus, orally induced CD8⁺ T-cell hyporesponsiveness is not associated with conversion into suppressor cells nor anergy and thus likely involves deletion.

**Deletion of Oral Ag-Specific CD8⁺ T Cells by pDC Does Not Require Treg nor IL-10**

Oral tolerance relies both on pDC¹⁷ and on CD4⁺CD25⁺ Tregs.¹² DNFB feeding generated in MLN Ag-specific CD4⁺ Tregs that were able to transfer suppression (Supplementary Figure 3), and we previously demonstrated that CD25⁺ cells represented the major CD4⁺ T cells involved in oral tolerance.¹² Although IL-10 produced by Treg is not necessary for oral tolerance,¹² we found that IL-10 deficiency impairs oral tolerance (Supplementary Figure 4A and B) and that environmental IL-10 is required for CD4⁺CD25⁺ Treg restoration of oral tolerance in invariant chain (li⁻/−) mice (Supplementary Figure 4C). We thus tested the respective contribution of Tregs, pDC, and IL-10 to gavage-induced CD8⁺ T-cell deletion. Data using the CD3e⁻/⁻ transfer model showed that pDC depletion of DNFB-fed CD8⁺ T-cell donors, induced by anti-Gr1- or anti-BST2-specific mAb treatments, prevented CD8⁺ T-cell deletion (Figure 5A), whereas CD8 tolerance was maintained when DNFB-fed donors had a temporary (anti-CD25 mAb injection) or permanent (Ab⁻/− mice) Treg defect or were treated with a neutralizing anti-IL-10 mAb (Figure 5B), indicating that neither Treg nor IL10 contributes to oral Ag-specific CD8⁺ T-cell deletion.

**CD4⁺CD25⁺ Tregs Contribute to Oral Tolerance by Controlling Residual CD8⁺ T-Cell Priming in Response to Systemic Immunization**

Because orally induced CD8⁺ hyporesponsiveness, highly efficient both in MLN and liver, only modestly affected Ag-reactive CD8⁺ T cells in spleen, we hypothesized that Treg might be operating on these residual T-cell pools at the time of systemic immunization to achieve oral tolerance. We previously reported that anti-CD25 mAb injection before Ag gavage prevented establishment of oral tolerance.¹² As shown in Figure 5C, depletion of CD25⁺ T cells at the time of sensitization was sufficient to abrogate oral tolerance and restored a normal CHS response, supporting that Treg contributed to oral tolerance by controlling the systemic phase induced by Ag re-encounter via the skin.

**Hapten Gavage Renders CD8⁺ T Cells Susceptible to Tregs and Increases the Suppressive Function of CD4⁺CD25⁺ T Cells**

To determine whether orally induced CD8⁺ T-cell deletion is a prerequisite for the ability of Tregs to completely prevent the CHS response during oral tolerance, we compared the susceptibility of splenic CD8⁺ T cells isolated from vehicle- or DNFB-fed mice to CD4⁺CD25⁺ T cell-mediated suppression (Figure 6A). CD8⁺ T cells from vehicle-fed mice differentiated into IFN-γ-producing effectors and initiated a CHS response when transferred into CD3e⁻/⁻ mice. These responses were only modestly diminished by cotransfer of Ag-activated CD4⁺CD25⁺ T cells (Figure 6B and 6C). Although, as expected, CD3e⁻/⁻ recipients of CD8⁺ T cells from DNFB-fed donors generated reduced numbers of specific CD8 effectors and mounted a lower CHS response, these responses were even more dramatically reduced by cotransfer of Tregs isolated from DNFB-fed mice (Figure 6B and 6C). This clearly demonstrates that hapten gavage renders circulating Ag-specific CD8⁺ T cells more sensitive to suppression by CD4⁺CD25⁺ Tregs. In addition, CD4⁺CD25⁺ Tregs from DNFB-fed donors have an increased suppressive function as compared with vehicle-fed Treg because they almost completely pre-
vented in vivo activation (Figure 6D) and differentiation into functional CHS effectors (Figure 6E) of residual CD8<sup>+</sup> T cells from DNFB-fed donors. Thus, Ag gavage enhances susceptibility of specific CD8<sup>+</sup> T cells to Tregs control but also activates CD4<sup>+</sup>CD25<sup>+</sup> Tregs and increases their suppressive function.

Transfer of CD4<sup>+</sup>CD25<sup>+</sup> Tregs at the Time of Systemic Immunization Is Sufficient to Restore Orally Induced Tolerance in I<sup>–/–</sup> Mice

Invariant chain-deficient (I<sup>–/–</sup>) mice, which have a partial deficiency in MHC class II restricted CD4<sup>+</sup> T cells, cannot be tolerized by DNFB gavage<sup>16</sup> except if CD4<sup>+</sup>CD25<sup>+</sup> T cells from naïve B6 mice were adoptively transferred prior to hapten gavage.<sup>12</sup> To confirm that Tregs essentially act in peripheral lymph to control the remaining Ag-specific CD8<sup>+</sup> T cells that have escaped deletion, I<sup>–/–</sup> mice were first fed with the hapten and then injected at the time of skin sensitization with CD4<sup>+</sup>CD25<sup>+</sup> T cells from DNFB-fed mice (Figure 7A). DNFB feeding of I<sup>–/–</sup> mice (without Treg transfer) was unable to prevent the priming of IFN-γ<sup>+</sup>CD8<sup>+</sup> effector cells (Figure 7B) or induction of CHS.<sup>12</sup> Treg transfer into vehicle-fed I<sup>–/–</sup> recipients did not induce tolerance (Figure 7B), demonstrating that Tregs alone are not sufficient to control the CD8<sup>+</sup> T-cell response. In contrast, oral tolerance could be restored if DNFB-fed I<sup>–/–</sup> mice were transferred with CD4<sup>+</sup>CD25<sup>+</sup> T cells at the time of skin sensitization. Thus, efficient CD8<sup>+</sup> T-cell tolerance induced via the oral route in I<sup>–/–</sup> mice requires first induction of T-cell hyporesponsiveness by Ag feeding and subsequently active suppression by CD4<sup>+</sup>CD25<sup>+</sup> T cells.

Discussion

This study demonstrates, in a pathophysiologic model of allergic skin contact dermatitis in mice, that the mechanism by which oral Ag gavage achieves complete suppression of T cell-mediated skin inflammation requires both effector T-cell deletion and control by Tregs. These 2 distinct mechanisms triggered by oral exposure to the allergen proceed in 2 sequential steps, which are compulsory to prevent Ag-specific DTH responses. During the first step, ie, “mucosal phase of oral tolerance,” which starts shortly after gavage, pDC cause a dramatic
loss of Ag-specific CD8+ T cells in the liver and MLN but not spleen. To achieve complete inhibition of DTH responses, a subsequent step of systemic tolerance that starts upon skin immunization allows Tregs activated by oral Ag to fully control the priming and differentiation of residual Ag-specific circulating peripheral T cells into CHS effectors.

Our functional studies on the mechanism of orally induced CD8+ T-cell tolerance provide the first in vivo evidence that partial deletion of specific CD8+ T cells by oral Ag presentation conditions the efficacy of Ag-specific suppression of CHS by Tregs. By following the fate and phenotype of CD8+ T cells that mediate the CHS response,15 and their ability to give rise to functional CHS effectors, we found that hapten feeding induces within 3 days the transient activation of hapten-specific CD8+ T cells in MLN and liver but not spleen. This observation is consistent with our recent data on DNFB distribution after gavage17 and with previous studies showing that orally induced systemic tolerance is preceded by a transient phase of Ag-specific T-cell activation, which occurs preferentially in gut-associated lymphoid tissues5,6,19 and liver.21 Using adoptive transfer of CD8+ T cells into T cell-deficient mice, we demonstrated that DNFB feeding generated hyporesponsive CD8+ T cells that were impaired in their ability to differentiate into CHS effectors in response to skin immunization of the recipients, confirming our previous results.17 CD8+ T-cell hyporesponsiveness was Ag-specific and long lasting, occurred primarily in MLN and liver (but not spleen), and was selectively induced after exposure to the Ag by the oral route but not via the skin. Importantly, CD8+ T cells from hapten-fed mice did not convert into suppressive cells able to suppress the priming of naïve CD8+ T cells. Because T-cell anergy is an IL-2 reversible process that requires continuous exposure to the Ag,20 our data strongly support that deletion rather than anergy is responsible for CD8 hyporesponsiveness after mucosal encounter with the Ag. Indeed, Ag-specific CD8+ T cells from hapten-fed mice remained Ag hyporesponsive up to 2 months after reconstitution of the recipients (ie, in the absence of chronic Ag exposure). Moreover, in CD8 co-

Figure 5. Depletion of pDC but not CD4+CD25+ Tregs prevents orally induced T-cell hyporesponsiveness. (A and B) At day −7, C57BL/6, I-Aβ−/−, IL-10−/−, anti-CD25− (PC61 injection at days −8, −7, and −4), anti-Gr1−, or anti-BST2− (RB8.6-CS or 120G8 injections at days −8, −7, −5, and −2) mAb-injected mice were fed either with DNFB or vehicle. At day −1, CD8+ T cells were isolated from MLN, intravenously transferred into CD3–/–/– KO mice, and tested for their capacity to differentiate into IFN-γ SFC after DNFB skin sensitization of recipient mice. Results are expressed as in Figure 2C, with 100% being ascribed to CD8+ T cells from vehicle-fed mice. (A) One hundred percent corresponded to 4104 ± 79, 1480 ± 71, and 3962 ± 288 SFC per 10^6 CD8+ T cells for C57BL/6, anti-Gr1-treated mice, and anti-BST2-treated mice, respectively. (B) One hundred percent corresponded to 1355 ± 413, 1400 ± 285, 2980 ± 85, and 1272 ± 34 SFC per 10^6 cells for C57BL/6, anti-CD25-treated, I-Aβ−/−, and IL-10−/− mice, respectively. A and B are representative of 2 independent experiments. (C) C57BL/6 mice were fed with DNFB at day −7 and were injected or not with anti-CD25 mAb (PC61) at days −1, 0, and +3. Both groups of mice were sensitized by DNFB painting on abdominal skin at day 0 and ear challenged at day +5 with DNFB. The CHS response was determined by ear swelling (µm) 48 hours after hapten challenge. Horizontal bars represent the mean ear swelling response for each group of mice.
transfer experiments, DNFB-fed CD8+ T cells remained tolerant despite the IL-2 supply from the CHS effectors that differentiated from the progeny of cotransferred vehicle-fed CD8+ T cells. Finally, loss of Ag-reactive T cells occurred between 3 and 7 days after hapten feeding, consistent with the time frame of deletional tolerance by T-cell apoptosis after Ag feeding.8,21

Interestingly, oral Ag-induced T-cell deletion was mediated by pDC but required neither regulatory CD4+CD25+ T cells nor the immunosuppressive cytokine IL-10, which both contribute to oral tolerance in this CHS model12 (and this study). Thus, at variance to respiratory23 or transplantation24 tolerance, associated with the propensity of pDC to induce/activate CD4+ Tregs, our study identifies CD8+ T-cell deletion as the key functional property of pDC that is instrumental for oral tolerance.

Our study further provides an explanation for the intriguing contribution of Tregs to the induction and maintenance of oral tolerance3,12 and revealed 2 important findings. First, Ag gavage enhances both the suppressive efficacy of CD4+CD25+ Tregs and the suscepti-
The frequency of IFN-\(\beta\) by epicutaneous application of DNFB onto the shaved abdominal skin of mice. Immediately after T-cell transfer, recipient mice were sensitized and/or induction of Tregs.\(^{10,11}\) In this respect, intestinal inflammation is at present and might involve the expansion/activation of CD4\(^+\)CD25\(^+\) T cells by pDC accounts for the increased susceptibility of the remaining circulating CD8\(^+\) T cells to Treg suppression. However, it remains possible that the residual CD8\(^+\) T cells that have escaped deletion are “conditioned” during oral Ag presentation by pDC or other types of DC, by induction of molecules transducing inhibitory signal to the effector T cells or favoring interaction with and/or activation by Tregs. In this respect, the negative regulator of T-cell activation PD1 appears as a candidate, inasmuch as the PD1/PD1-L pathway is involved in Tregs mediated suppression\(^{27}\) and appears critical for oral tolerance.\(^{28}\) However, we could not detect any expression of PD1 or CTLA4 on hyporesponsive CD8\(^+\) T cells in our model (data not shown). IL-10, produced by non-Tregs cells in the gut microenvironment, appears essential for oral tolerance and might be responsible for the enhanced sensitivity of effector cells to Tregs, for example by increasing/maintaining expression of the TGF-\(\beta\) receptor and/or inhibiting the stimulatory functions of antigen-presenting cells.\(^{29}\)

In conclusion, this study demonstrates that full protection from pathogenic T-cell responses by orally induced tolerance requires the synergistic action of pDC and CD4\(^+\)CD25\(^+\) Tregs, which sequentially act to induce effector T-cell deletion and systemic suppression, respectively. This suggests that oral biotherapies aiming at strengthening Treg function in autoimmune, allergic, or inflammatory diseases should benefit from prior targeting of pDC to render effector T cells more sensitive to Treg suppression.

**Supplementary Data**

Note: To access the supplementary material accompanying this article visit the online version of *Gastroenterology* at [www.gastrojournal.org](http://www.gastrojournal.org) and at doi: 10.1053/j.gastro.2009.03.055.

**References**


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