Abstract

Introduction

Literature data have shown that the consumption of dietary proteins may cause modulatory effects on the host immune system, process denominated oral tolerance by bystander suppression. It has been shown that the bystander suppression induced by dietary proteins can improve inflammatory diseases such as experimental arthritis. Here, we evaluated the effects of oral tolerance induced by ingestion of ovalbumin (OVA) on TNBS-induced colitis in mice, an experimental model for human Crohn’s disease.

Methods and Results

Colitis was induced in BALB/c mice by instilling a single dose of TNBS (100 mg/kg) in ethanol into the colon. Tolerized mice received OVA (4 mg/mL) dissolved in the drinking water for seven consecutive days, prior to or concomitantly with the intrarectal instillation. Control groups received protein-free water and ethanol by intrarectal route. We observed that either the prior or concomitant induction of oral tolerance were able to reduce the severity of colitis as noted by recovery of body weight gain, improvement of clinical signs and reduction of histological abnormalities. The in vitro proliferation of spleen cells from tolerant colitic mice was lower than that of control mice, the same as the frequencies of CD4\(^+\) T cells secreting IL-17 and IFN-\(\gamma\). The frequencies of regulatory T cells and T cells secreting IL-10 have increased significantly in mice orally treated with OVA. The levels of inflammatory cytokines (IL-17A, TNF-\(\alpha\), IL-6 and IFN-\(\gamma\)) were lower in supernatants of cells from tolerant colitic mice, whereas IL-10 levels were higher.

Conclusion

Our data show that the modulation of immune response induced by oral tolerance reduces the severity of experimental colitis. Such modulation may be partially attributed to the
increase of Treg cells and reduction of pro-inflammatory cytokines in peripheral lymphoid organs of tolerant mice by bystander suppression.

Introduction

Recently, inflammatory bowel disease (IBD) has been receiving more attention from physicians and researchers due to the increase in its incidence in human populations [1–5]. IBD comprises a set of related diseases, collectively called Crohn’s Disease (CD) and Ulcerative Colitis (UC), which origin has been attributed to the breakdown of tolerance to self-antigens in the intestinal mucosa [6]. The mechanisms involved in these autoimmunities are diverse and cannot be considered mutually exclusive. They may include a combination of factors among which one can highlight the unbalanced production of cytokines and interleukins such as interleukin (IL)-9 IL-10, IL-35, transforming growth factor (TGF)-β. Several other molecules, transcription factors and receptors are associated with colitis, including Cytotoxic T-Lymphocyte Antigen (CTLA)-4, Leukocyte Activation Gene (LAG) -3, indoleamine (IDO), perforin/antagonists and Glucocorticoid-Induced Tumor Necrosis Factor Receptor (GITR) [7–11].

Recent literature, however, emphasizes the role of Th17 cells in inflammatory diseases such as colitis. T helper (Th) 17 cells and other IL-17-producing-cells play a crucial role in intestinal inflammatory diseases. IL-17 together with IL-22 appear to be related to induction of colitis, since these cytokines initiate and amplify the local inflammatory signs and promote the activation of regulatory mechanisms directly against the cells of the intestinal epithelium [12,13]. In turn, IFN-γ induces the production of inflammatory cytokines by cells of the innate immune system, contributing to an increased tissue inflammation seen in colitis [4,12,14,15]. Therefore, modulation of Th17 and INF-γ-secreting cells may affect the inflammation in colitis.

On the other hand, IL-10 produced by macrophages and regulatory T cells may skew the response into a regulatory one, leading to a reduction in inflammation [15]. In addition, several results suggest that tolerogenic dendritic cells (DC) lead to the generation of induced regulatory T (Treg) cells (CD4⁺CD25⁺Foxp3⁺) and an increase in the number of lymphocytes expressing the suppressor molecule CTLA-4 [16–19].

Accordingly, several studies have attempted to modulate the inflammatory response observed in experimental colitis by the induction of a tolerogenic role [16,20–23]. Specifically, oral tolerance by dietary antigens administration is a tool to the modulation of the immune system. The suppression of immune response on oral tolerance is due to the low responsiveness of local or systemic immune system, triggered by such protein antigens [24–28]. Despite the great interest in the subject, the effects of oral tolerance by bystander suppression in colitis are not yet fully understood [16,21,29,30].

The aim of this study was to evaluate the effects of oral tolerance on TNBS-induced colitis in mice of BALB/c. Tolerance was induced by oral administration of ovalbumin (OVA) in the previous and subsequent induction of colitis. Parameters as body weight, histology of target tissues, cell proliferation, cytokine production and expansion of regulatory CD25+Foxp3+ T cells and Th17 cells on spleen cell cultures were evaluated here. Our data suggested that oral tolerance to OVA modulates the immune responses against TNBS in BALB/c mice by bystander suppression. Suppression of the immune response is reflected in the increase in regulatory T cells and decreased production of pro-inflammatory cytokines that together markedly ameliorated the severity of the colitis.


Materials and Methods

Animals

SPF female BALB/c mice (four weeks old) were obtained from the Multidisciplinary Center for Biological Research (CEMIB) of the University of Campinas (UNICAMP) Campinas, SP, Brazil, and housed in plastic cages in groups of five. They were maintained in specific pathogen-free environment at 25˚C ±1 and photoperiod of 12/12 hours, and fed with autoclaved Nuvilab CR-diet and water ad libitum for at least 4 weeks before being used in experiments. The methods described in this manuscript were carried out in accordance with the ‘Guide for the Care and Use of Laboratory Animals’, as promoted by the Brazilian College of Animal Experimentation (COBEA), and was approved by the Ethics Committee for Animal Experimentation of University of Campinas. (CEUA/UNICAMP. Protocol #3077–1). All experimental procedures were performed under proper anesthesia and all efforts were made to minimize animal suffering. Mice general health was daily monitored for signs of inflammation such as rectal swelling, rectal bleeding, soft stool or weight loss. On day 5 after TNBS instillation, all mice were sacrificed by cervical dislocation after anesthesia with a mixture of ketamine (60 mg/kg, Ketalar; Pfizer) and xylazine (6 mg/kg, Rompun; Bayer) (i.p.).

Oral tolerance

The induction of oral tolerance to OVA was performed as described elsewhere [31] and depicted in Fig 1. Briefly, 4mg/mL OVA (Rhoster Commerce and Industry Ltda, Vargem Grande Paulista, SP, Brazil) was added to water supply of BALB/c mice for 7 consecutive days. The control mice received protein-free water.

Induction of experimental colitis

Experimental colitis was induced in BALB/c mice according to indications of Neurath and colleagues [29,32]. Mice were anesthetized and instilled with 100 μL of 1 mg/mL TNBS (2,4,6—
trinitrobenzenesulfonic acid; Sigma, USA) solution in 50% ethanol by intrarectal route. Control animals received 100 μL of 50% ethanol (Fig 1).

**Evaluation of clinical signs of colitis**

All groups were weighed daily until sacrifice. Clinical signs such as diarrhea, rectal prolapse, bleeding and cachexia were registered and assigned as scores, ranging from 0 to 2, with 0: no change, 1: slight change, and 2: severe change.

**Histological analysis**

On 5 days after TNBS instillation, mice were euthanized and the distal portion of the large intestines were removed and fixed with 4% paraformaldehyde. The pieces were cleared and embedded in paraffin. The sections were stained with hematoxylin and eosin. For the histological analysis, the sections were evaluated for the presence of folds, hemorrhage, infiltration of leukocytes on two distal portions of the intestines (1 to 2 cm and 2 to 3 cm from the rectum). The thickening of the wall of the colon was measured in micrometers in distal portions, with Infinity Analyze Nikon H600L program (100X). A score ranging from 0 to 20 was assigned [32,33].

**Spleen cell proliferation**

On day 5 after colitis induction, mice of all groups were sacrificed and spleens were aseptically removed. The spleens were macerated individually and erythrocytes in cell suspensions were lysed. Cells were pelleted at 200 g for 10 min and cell concentration was adjusted to 1 x 10⁶ cells/mL in RPMI medium (Sigma, USA) supplemented with 10% fetal bovine serum (CultiLAB). Cells were stained with 1.25μM Carboxyfluorescein diacetate succinimidyl ester (CFSE) according to manufacturer’s instructions (Invitrogen, USA). To determine the maximum uptake, aliquots of the cell suspensions stained with CFSE were fixed with 1% formaldehyde and analyzed by flow cytometer. Stained cells were seeded at 4x10⁵ cell/well in sextuplicate, and Concanavalin A (ConA) was added to each well at final concentration of 2.5μg/mL. Plates were incubated at 37°C in humidified incubator, with 5% CO₂ for 72 hours. After the incubation period, cells were fixed with 1% formaldehyde and proliferation was assessed in CD4⁺ CSFE⁺ cells by flow cytometer [34]. Acquisitions were performed with FACScalibur flow cytometer (Becton-Dickinson) and analyzes were done with the FCS Express 5 Plus Research Edition software (FCS Express Launcher). Results were expressed as proliferation index (fold change) calculated in relation to control group. Cells not stained with CFSE were also cultured in the presence of ConA and their supernatants were collected for dosage of cytokines.

**Phenotypic profile of T lymphocytes**

The frequencies of T CD4⁺CD25⁺Foxp3⁺ (Treg cells), T CD4⁺IL17⁺, T CD4⁺IFNγ⁺ and T CD4⁺IL-10⁺ cells in the cultures were assessed by flow cytometer. Briefly, cell suspensions were washed and initially stained with anti-CD4-PE (Clone GK1.5) and anti-CD25-FITC (Clone 7D4). Following, cells were permeabilized by the addition of fixation/permeabilization buffer (Cytofix / Cytoperm fixation/permeabilization kit, Becton-Dickinson, BD) and stained with anti-Foxp3-APC (clone FJK-16S), anti-IL-17-APC (clone eBIO17B7) or Alexa Fluor 647 (Clone TC11-18410), anti-IFNγ-APC (Clone XMG1.2), IL-10-APC (clone JESS-16E3) according to manufacturer’s instructions. Acquisitions were performed with FACScalibur flow cytometer and analyzes were done with the FCS Express 5 Plus, Research Edition software.
Th1/Th2/Th17 cytokines determination

IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-γ and TNF-α were quantified in culture supernatants of spleen cells by flow cytometry by using Multiplex CBA kit (BD Cytometric Bead Array Th1/Th2/Th17, San Diego, USA) according to manufacturer’s instructions. Cells were acquired in FACSCalibur cytometer and analyzed with FCAP Array TM Software Version 3.0 (BD).

Statistical analysis

The statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). The statistical significance of differences between control and experimental groups was determined by one-way ANOVA, followed by multiple comparisons Bonferroni’s test. Results were expressed as mean ± Standard Error Mean (SEM). Values were considered significant at P > 0.05. All data are representative of at least three independent experiments.

Results and Discussion

The gastrointestinal tract is in constant contact with dietary proteins, commensal microorganisms and potentially pathogenic microorganisms. To ensure the maintenance of homeostasis of the organism, the immune system of the intestinal mucosa must be able to tolerate antigens from diet and commensal microbiota and generate protective responses against harmful antigens. The ability of intestinal mucosa-associated lymphoid tissue (MALT) to suppress systemic immune response against ingested proteins is known as oral tolerance. Oral tolerance has been demonstrated in various experimental models and for different antigens [20,24,26,35,36]. Nowadays, other routes are under evaluation in order to develop oral tolerance strategies for immunotherapy. In this sense, eye-induced tolerance is a promising therapeutic tool in the treatment of complicated autoimmune diseases such as CIA [37,38] or EAE [39–41]. Several tolerance protocols are being proposed for controlling inflammatory manifestations, in particular autoimmune diseases such as colitis [42–48].

The TNBS-induced colitis is a widely used experimental model for the study of inflammatory bowel disease (IBD), since their clinical symptoms and immunological response are similar to those observed in human intestinal diseases [33,49–55]. BALB/c mice are often used for induction of colitis by TNBS administration because they develop a milder disease than SJL strain, but with significant clinical and immunological signals [49,54,56].

Data presented here show that the consumption of OVA, either prior or concomitant to colitis induction, led to the reduction of the weight (Fig 2A) and of typical clinical signs of TNBS-induced colitis, such as diarrhea, rectal prolapse, bleeding and cachexia (Fig 2B). The subsequent intraperitoneal challenge with OVA led to decreased levels of serum antibodies in animals fed protein, indicating a systemic effect of oral treatment (Fig 2C).

To evaluate the possible effects of OVA consumption on the intestinal mucosa, the distal segments of the large intestine of mice from all groups were collected on the fifth day after the induction of colitis and evaluated histologically. As summarized in Fig 3, the oral treatment with OVA prior to or concomitant with induction of colitis was able to partially preserve the integrity of the colonic tissue of mice with colitis. This can be observed by the significant reduction of histological changes (bends, hemorrhage and leukocyte infiltration) in the tissues of animals tolerized to OVA (Fig 3A and 3B) as well as the preservation of the wall thickness of the colon (Fig 3C) compared to the control animals. Together, these results indicate a possible bystander suppression of the immune response in mice tolerized with OVA, able to alter the course of experimental disease.

Previous studies conducted in our laboratory showed that it is possible to induce oral tolerance to OVA in animals that exhibit a normal repertoire of receptors to the target antigen
Several mechanisms seem to be involved in the induction of tolerance, including anergy, clonal deletion and induction of Treg cells [24,58–61]. Weiner and colleagues [22,48,62] suggested that the mechanisms that operate in the induction of oral tolerance are directly related to the antigen administration regimen. Thus, administration of high doses of antigens at once would lead to clonal deletion or anergy, while multiple doses of antigen in low concentrations would be capable of promoting the suppression mediated by suppressor/regulators T cells. However, Siewert et al [63] found a high rate of Foxp3+ T cells from oral tolerant mice that had been induced by high dose of antigen. This finding indicates that higher doses of antigen did not lead to clonal deletion, but the generation of suppressive responses associated with oral tolerance. Some cytokines, such as granulocyte macrophage colony stimulating factor...
(GM-CSF) can modulate DC towards a tolerogenic profile acting as an anti-inflammatory or regulatory modulator in autoimmune diseases, depending on its dose and the presence of other cytokines. Tolerogenic DCs can increase the frequency and function of regulatory T-cells [64–69].

Data from our laboratory have shown that continued ingestion of low doses of OVA can interfere with the phenotypic distribution of intraepithelial lymphocytes (IELs) in the small intestine of wild type BALB/c. After ingestion of OVA, BALB/c mice showed increased frequency of CD4⁺Foxp3⁺ regulatory T cells and increased expression of regulatory cytokines on IELs [70].
To investigate the systemic effects of OVA intake on cellular immune response of colitic mice induced by TNBS, spleen cells were collected on the fifth day after the induction of colitis and assessed for their proliferative capacity and for the expansion of CD4+ T lymphocytes with regulatory and effector profiles. The results summarized in Fig 4 and in S1 Fig show that the proliferation of spleen cells from mice fed with OVA was significantly lower than that observed in cultures of spleen cells from the control group (Fig 4A), whereas the frequency of Treg cells (TCD4+CD25+ Foxp3+) and expression of Foxp3 was higher in cultures of cells from tolerized mice (Fig 4B and 4C). It was also observed an increase in the frequency of CD4+IL-10+ T cells in spleen cell cultures of mouse fed with OVA (Fig 4D) as well as a reduced frequency of CD4+IFN-γ+ T cells and CD4+IL17+ T cells (Fig 4F and 4H) compared with non-tolerant group.

Assessment of cytokines in the supernatants of spleen cells revealed no statistically significant differences in IL-2 secretion in the experimental groups (Fig 5A), but showed an increase in IL-10 levels (Fig 5B) and reduction in levels of IFN-gamma (Fig 5C) and IL-17 (Fig 5D) in the spleen cell cultures from mice tolerized with OVA compared to the non-tolerant group. The results presented here are consistent with data recently obtained by our group in the experimental model of arthritis [34].

Corroborating our data, the literature shows that regulatory T cells may play an important role in autoimmune diseases [15,50,71–75] and the expansion of this population can aid in the control of clinical manifestations [16,76–78]. Moreover, Th17 cells have a potential role in the induction of inflammatory bowel disease and its reduction must also contribute to the improvement of the clinical picture in several autoimmune conditions [12,76,79,80].

It is already known that CD4+CD25+Foxp3+ cells on the intestinal mucosa seems to be able to induce local generation of Th3 cells secreting TGF-β (LAP'), type 1 regulatory T (Tr1) cells and CD8+ T reg cells [48,81]. These regulatory T cells generated in the intestine migrate to secondary lymphoid organs such as Peyer’s patches and mesenteric lymph nodes, which inhibit the generation of nonspecific effector cells, by a mechanism known as bystander suppression [22,82]. In addition, the imbalance between proinflammatory and anti-inflammatory cytokines released by the intestinal mucosa determines the intensity and duration of the inflammatory response seen in experimental colitis [14,15,33,83–86].

Data from our laboratory has shown that it is possible to protect mice against experimental arthritis (CIA) by oral tolerization with OVA as a preventive or therapeutic intervention, although the antigens involved in tolerization and triggering the disease had been administered independently [34].

An increased frequency of both CD4+FoxP3+ and CD4+ IL10+ lymphocytes was also observed after in vitro restimulation of spleen cells from tolerized arthritic animals. These results, however, as well as those of Vaz and colleagues [87–90] show indirect effects of oral tolerance that do not fit perfectly in bystander suppression model.

By definition, the bystander suppression occurs when the immune response to a particular epitope suppresses the response to another epitope administered concurrently or immediately after the first one. The effects of bystander suppression is related to the secretion of cytokines such as TGF-β, IL-4 and IL-10, and by the action of regulatory T cells [42,91,92].

Interventions leading to bystander suppression may be of great interest in autoimmunity regulation. Thus, the suppressive response initiated by mucosal administration of dietary proteins associated with self-antigens has been gaining ground as a preventive or therapeutic intervention proposed for autoimmune diseases [24,42,48]. In this respect, several authors have shown that the oral tolerance to dietary antigens may prevent or inhibit the progression of systemic or organ-specific autoimmune diseases [34,35,93]. In this regard, data from our and others laboratories have shown that it is possible to reduce the immune response to a different antigen from that used to induce oral tolerance, even if this antigen is administered
Fig 4. The oral tolerance to OVA alters the proliferative response and cytokine producing cells from mice with TNBS-induced colitis. The induction of tolerance and colitis was performed as described in Fig 2. Mice were sacrificed five days after TNBS instillation. Spleens were aseptically removed; cells were labeled with CFSE and cultured at a concentration of 2x10⁶ cells / ml in the presence of Concanavalin A (ConA; 2.5μg / ml) for 72 hours at 37˚C and 5% CO2. Panel A: Spleen cell proliferation. Cells were fixed in 1% formaldehyde and the readings performed in flow cytometer (FACSCalibur, BD). Proliferation was calculated using the software FCS Express and represents the inverse of the ratio of the fluorescence exhibited by the cells after
several days after ingestion of tolerogen and parenteral challenge with tolerated antigen [34,38]. Recent work from Vaz group have shown that there is an expansion of regulatory T CD4$^{+}$ Foxp3$^{+}$ and T CD4$^{+}$ LAP$^{+}$ non-specific cells in the early stage of parenteral exposure to the antigen used in the oral treatment, phenomenon that is not seen in non-tolerant animals subjected to the same treatment [94]. Similarly to what we have shown with our work, other authors have demonstrated that increased IL-10 production correlates with the ability to reduce inflammatory responses associated with human and experimental colitis [95,96].

Fig 5. The oral tolerance to OVA alters cytokine levels in supernatants of ConA-stimulated spleen cells from mice with TNBS-induced colitis. Cultures of spleen cells were carried out as described in Fig 4. Cytokine levels were evaluated in the culture supernatants by using the CBA Multiplex kit (Cytometric Bead Array Th1 / Th2 / Th17, BD) and readings were performed in a flow cytometer (FACSCalibur, BD). Cytokine concentrations were determined using the array FCAP TM Version 3.0 Software (BD). Results were expressed as means ± S.E.M. obtained from two independent experiments (n = 5). ANOVA followed by Bonferroni a posteriori test was used to determine statistical significance.

doi:10.1371/journal.pone.0170205.g004

doi:10.1371/journal.pone.0170205.g005
Conclusions
Our results indicate that the development of oral tolerance to OVA was able to reduce the signs and the immune response in TNBS-induced colitis. The immunomodulation observed can be attributed to the expansion of regulatory T cells and IL-10 producing cells, by a mechanism known as bystander suppression. Further studies are in progress to evaluate the role of dendritic cells in the protection afforded by oral tolerance in this colitis model.

Supporting Information
S1 Fig. Oral tolerance reduces the proliferation of T lymphocytes, increases the proportion of CD25+ Foxp3+ Tregs and reduces IL-17-and INF-γ- producing-T cells on colitic mice. Five days after the TNBS installation, leukocytes were harvested from spleens of mice that received OVA by oral route, as described in Fig 2. Leukocytes from spleens of naïve and untreated TNBS mice were used as controls. The spleen cells were stained with CFSE and cultured at a concentration of 2x10⁶ cells / mL in the presence of Concanavalin A (ConA; 2,5μg/mL) for 72 hours at 37˚C and 5% CO2. CFSE: Cells were fixed in 1% formaldehyle and the readings performed in flow cytometer (FACSCalibur, BD). Cell proliferation was determined using flow cytometry and assessed by fluorescence decay of the probe in the gate of CD4+ cells. The frequency of CD25+ Foxp3+ Tregs in the different groups was evaluated in the gate of CD4+ cells. IFN-γ-, IL-10-, and IL-17-producing cells were evaluated in the gate of CD4+ cells as well. Histograms and plots are derived from a representative animal from two independent experiments (n = 5/each assay).

Author Contributions
Conceptualization: WMSCT PUS.
Data curation: LNP FGDS JB ATY PUS.
Formal analysis: LNP PUS.
Funding acquisition: PUS.
Investigation: LNP FGDS JB PUS.
Methodology: LNP FGDS JB WMSCT PUS.
Project administration: PUS.
Resources: ATY WMSCT PUS.
Supervision: PUS.
Validation: PUS.
Visualization: LNP PUS.
Writing – original draft: LNP PUS.
Writing – review & editing: LNP FGDS JB MRB ATY WMSCT PUS.

References
1. Engel M.A., Neurath M.F., New pathophysiological insights and modern treatment of IBD, J. Gastroenterol. 45 (2010) 571–583. doi: 10.1007/s00535-010-0219-3 PMID: 20213337
2. Cosnes J., Gower-Rousseau C., Seksik P., Cortot A., Epidemiology and natural history of inflammatory bowel diseases, Gastroenterology. 140 (2011) 1785–1794. doi: 10.1053/j.gastro.2011.01.055 PMID: 21530745

3. Molodecky N. A, Soon I.S., Rabi D.M., Ghali W.A., Chernoff M., et al., Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review., Gastroenterology. 142 (2012) 46–54.e42; quiz e30. doi: 10.1053/j.gastro.2011.10.001 PMID: 22081864

4. Geremia A., Biancheri P., Allan P., Corazza G.R., Di Sabatino A., Innate and adaptive immunity in inflammatory bowel disease., Autoimmun. Rev. 13 (2014) 3–10. doi: 10.1016/j.autrev.2013.06.004 PMID: 23774107

5. Ramos De Mattos B.R., Pereira M., Garcia G., Nogueira J.B., Paiatto L.N., Albuquerque C.G., et al., Inflammatory Bowel Disease: An Overview of Immune Mechanisms and Biological Treatments, (n.d.).

6. Faria A.M.C., Weiner H.L., Oral tolerance., Immunol. Rev. 206 (2005) 232–59 . doi: 10.1111/j.0105-2896.2005.00280.x PMID: 16048553

7. Miyara M., Sakaguchi S., Natural regulatory T cells: mechanisms of suppression., Trends Mol. Med. 13 (2007) 108–16. doi: 10.1016/j.molmed.2007.01.003 PMID: 17257897

8. Kobayashi T., Okamoto S., Hisamatsu T., Kamada N., Chinen H., Saito R., et al., IL23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn’s disease., Gut. 57 (2008) 1682–9. doi: 10.1136/gut.2007.135053 PMID: 18653729

9. Takamatsu M., Hirata A., Ohtaki H., Hoshi M., Hatano Y., Tomita H., et al., IDO1 plays an immunosuppressive role in 2,4,6-trinitrobenzene sulfat-induced colitis in mice., J. Immunol. 191 (2013) 3057–64. doi: 10.4049/jimmunol.1203306 PMID: 23956437

10. Shen W., Durum S.K., Synergy of IL-23 and Th17 cytokines: New light on inflammatory bowel disease, Neurochem. Res. 35 (2010) 940–946. doi: 10.1007/s11064-009-0091-9 PMID: 19915978

11. Banks C., Bateman A., Payne R., Johnson P., Sheron N., Chemokine expression in IBD. Mucosal chemokine expression is unselectively increased in both ulcerative colitis and Crohn’s disease., J. Pathol. 199 (2003) 28–35. doi: 10.1002/path.1245 PMID: 12474223

12. Harbour S.N., Maynard C.L., Zindl C.L., Schoeb T.R., Weaver C.T., Th17 cells give rise to Th1 cells that are required for the pathogenesis of colitis, Proc. Natl. Acad. Sci. 112 (2015) 201415675.

13. Jiang W., Su J., Zhang X., Cheng X., Zhou J., Shi R., et al., Elevated levels of Th17 cells and Th17-related cytokines are associated with disease activity in patients with inflammatory bowel disease., Inflamm. Res. 63 (2014) 943–50. doi: 10.1007/s00011-014-0768-7 PMID: 25129403

14. Oh S.Y., Cho K.-A., Kang J.L., Kim K.H., Woo S.-Y., Comparison of experimental mouse models of inflammatory bowel disease., Int. J. Mol. Med. 33 (2014) 333–40. doi: 10.3892/ijmm.2013.1569 PMID: 24285285

15. Guo B., IL-10 Modulates Th17 Pathogenicity during Autoimmune Diseases, J. Clin. Cell. Immunol. 7 (2016) 1367–1671.

16. Matteoli G., Mazzini E., Iliev I.D., Mileti E., Puccetti P., et al., Gut CD103+ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction., Gut. 59 (2010) 595–604. doi: 10.1136/gut.2009.185108 PMID: 20427394

17. Huang H., Dawicki W., Zhang X., Town J., Gordon J.R., Tolerogenic dendritic cells induce CD4 +CD25hiFoxp3+ regulatory T cell differentiation from CD4+CD25—Foxp3 - effector T cells., J. Immunol. 185 (2010) 5003–5010. doi: 10.4049/jimmunol.0903446 PMID: 20670943

18. Pletinckx K., Doehler A., Pavlovic V., Lutz M.B., Role of dendritic cell maturity/cosimulation for generation, homeostasis, and suppressive activity of regulatory T cells, Front. Immunol. 2 (2011) 39. doi: 10.3389/fimmu.2011.00039 PMID: 22566829

19. Ko H.-J., Cho M.-L., Lee S.-Y., Oh H.-J., Heo Y.-J., Moon Y.-M., et al., CTLA4-Ig modifies dendritic cells from mice with collagen-induced arthritis to increase the CD4+CD25+Foxp3+ regulatory T cell population., J. Autoimmun. 34 (2011) 111–20. doi: 10.1016/j.jaut.2009.07.006 PMID: 19665867

20. Hyun J.G., Barrett T.A, Oral tolerance therapy in inflammatory bowel disease., Am. J. Gastroenterol. 101 (2006) 569–71. doi: 10.1111/j.1572-0241.2006.00437.x PMID: 16542293

21. Shan-Shan Z., Yu-Lan L., Therapeutic effects of mucosal tolerance on experimental colitis in rats., Eur. J. Gastroenterol. Hepatol. 21 (2009) 1145–52. doi: 10.1097/MEG.0b013e32830ed2b9 PMID: 19478860

22. Faria A.M.C., Weiner H.L., Oral tolerance: therapeutic implications for autoimmune diseases., Clin. Dev. Immunol. 13 (2006) 143–57. doi: 10.1080/17402520600876804 PMID: 17162357

23. Kraus T. A, Mayer L., Oral tolerance and inflammatory bowel disease., Curr Opin Gastroenterol. 21 (2005) 692–696. PMID: 16220047
24. Pabst O., Mowat A.M.M., Oral tolerance to food protein., Mucosal Immunol. 5 (2012) 232–239. doi: 10.1038/mi.2012.4 PMID: 22318493
25. Whitacre C.C., Gienapp I.E., Meyer A., Cox K.L., Javed N., Treatment of autoimmune disease by oral tolerance to autoantigens., Clin. Immunol. Immunopathol. 80 (1999) S31–S39. PMID: 8811061
26. Fujihashi K., McGhee J.R., Mucosal immunity and tolerance in the elderly., Mech. Ageing Dev. 125 (2004) 889–98. doi: 10.1016/j.mad.2004.05.009 PMID: 15563935
27. Barone K.S., Tolarova D.D., Ormsby I., Michael J.G., Doetschman T., Induction of Oral Tolerance in TGF-β 1 Null Mice, J. Immunol. 161 (2012) 154–160.
28. Matteoli G., Mazzini E., Iliev I.D., Mileti E., Fallarino F., Puccetti P., et al., Gut CD103 + dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction., Gut. 59 (2010) 595–604. doi: 10.1136/gut.2009.185108 PMID: 20427394
29. Neurath B.M.E., Fuss I., Kelsall B.L., Sti E., Strober W., Antibodies to Interleukin 12 Abrogate Established Experimental Colitis in Mice., 182 (1995).
30. Duan L., Chen J., Zhang H., Yang H., Zhu P., Xiong A., et al., Interleukin-33 ameliorates experimental colitis through promoting Th2/Foxp3+ regulatory T cell responses in mice., Mol. Med. 18 (2012) 753–61. doi: 10.2119/molmed.2011.00428 PMID: 22426954
31. Simioni P.U., Fernandes L.G.R., Gabriel D.L., Tamashiro W.M.D.S.C., Induction of systemic tolerance in normal but not in transgenic mice through continuous feeding of ovalbumin., Scand. J. Immunol. 60 (2004) 257–266. doi: 10.1111/j.0300-9475.2004.01454.x PMID: 15320882
32. Neurath B.M.F., Fuss I., Kelsall B.L., Presky D.H., Waegell W., Strober W., Experimental Granulomatous Colitis in Mice Is Abrogated by Induction of TGF-β-mediated Oral Tolerance By Neurath Markus F., *Fuss Ivan,* Kelsall Brian L., *Presky David H.,* Waegell Wendy, – and Strober Warren*, 183 (1996).
33. Neurath M.F., Fuss I., Kelsall B.L., Stüber E., Strober W., Antibodies to interleukin 12 abrogated experimental colitis in mice., J. Exp. Med. 182 (1995) 1281–90. PMID: 7595199
34. Thomé R., Fernandes L.G.R.L., Mineiro M.F.M., Simioni P.U., Joazeiro P.P., Tamashiro W.M.D.S.C., Oral tolerance and OVA-induced tolerogenic dendritic cells reduce the severity of collagen/ovalbumin-induced arthritis in mice., Cell. Immunol. 280 (2012) 113–123. doi: 10.1016/j.cellimm.2012.11.017 PMID: 23298866
35. Wang X., Sherman A., Liao G., Leong K.W., Daniell H., Terhorst C., et al., Mechanism of oral tolerance induction to therapeutic proteins., Adv. Drug Deliv. Rev. 65 (2013) 759–773. doi: 10.1016/j.addr.2012.10.013 PMID: 23123293
36. Mowat A.M., Parker L.A., Beacock-Sharp H., Millington O.R., Chirdo F., Oral tolerance: overview and historical perspectives., Ann. N. Y. Acad. Sci. 1029 (2004) 1–8. PMID: 15806729
37. Farooq S.M., Kumar A., Ashour H.M., Eye-mediated immune tolerance to Type II collagen in arthritis-prone strains of mice., J. Cell. Mol. Med. 18 (2014) 2512–2518. doi: 10.1111/jcmm.12376 PMID: 25211510
38. Farooq S.M., Ashour H.M., Type II Collagen Induces Peripheral Tolerance in BALB/c Mice via the Generation of CD8+ T Regulatory Cells, PLoS One. 7 (2012) e48635. doi: 10.1371/journal.pone.0048635 PMID: 23136648
39. Farooq S.M., Ashour H.M., Eye-mediated induction of specific immune tolerance to encephalitogenic antigens., CNS Neurosci. Ther. 19 (2013) 503–10. doi: 10.1111/cns.12087 PMID: 23522052
40. Farooq S.M., Ashour H.M., In vitro-induced cell-mediated immune deviation to encephalitogenic antigens, Brain. Behav. Immun. 35 (2014) 64–69. doi: 10.1016/j.bbi.2013.09.016 PMID: 24095895
41. Farooq S.M., Elkhattab W.F., Ashour H.M., Th in vivo and in vitro induction of anterior chamber associated immune deviation to myelin antigens in C57BL/6 mice, Brain. Behav. Immun. 42 (2014) 118–122.
42. Bayrak S., Mitchison N.A., Bystander suppression of murine collagen-induced arthritis by long-term nasal administration of a self type II collagen peptide., Clin. Immunol. 113 (1998) 92–5. doi: 10.1006/clim.1997.4563 PMID: 9697989
43. Min S.-Y., Park K.-S., Cho M.-L., Kang J.-W., Cho Y.-G., Hwang S.-Y., et al., Antigen-induced, tolerogenic CD11c+ ,CD11b+ dendritic cells are abundant in Peyer’s patches during the induction of oral tolerance to type II collagen and suppress experimental collagen-induced arthritis., Arthritis Rheum. 54 (2006) 887–98. doi: 10.1002/art.21647 PMID: 16598971
44. Weiner H.L., Oral Tolerance for the Treatment of Autoimmune Diseases, (1997).
45. Rodrigues C.M., Martins-Filho O. a, Vaz N.M., Carvalho C.R., Systemic effects of oral tolerance on inflammation: mobilization of lymphocytes and bone marrow eosinopoiesis., Immunology. 117 (2006) 517–25. doi: 10.1111/j.1365-2567.2006.02327.x PMID: 16556266
46. Weiner H.L., Friedman A., Miller A., Khoury S.J., Al-sabbag h A., Santos L., et al., ORAL TOLERANCE: Immunologic Mechanisms and Treatment of Animal, (1994).

47. Da Cunha A.P., Weiner H.L., Induction of immunological tolerance by oral anti-CD3, Clin. Dev. Immunol. 2012 (2012) 4–9.

48. Weiner H.L., da Cunha A.P., Quintana F., Wu H., Oral tolerance., Immunol. Rev. 241 (2011) 241–59. doi: 10.1111/j.1600-065X.2011.01017.x PMID: 21488901

49. Srinivasan M., Summerlin D.-J., Modulation of the colonic epithelial cell responses and amelioration of inflammation by CD80 blockade in TNBS colitis., Clin. Immunol. 133 (2009) 411–21. doi: 10.1016/j.clim.2009.09.001 PMID: 19811954

50. Okamoto T., Uemoto S., Tabata Y., Prevention of trinitrobenzene sulfonic acid-induced experimental colitis by oral administration of a poly(lactic-coglycolic acid) microsphere containing prostaglandin E2 receptor subtype 4 agonist., J. Pharmacol. Exp. Ther. 341 (2012) 340–9. doi: 10.1124/jpet.111.190447 PMID: 22300734

51. Wu X.-F., Xu R., Ouyang Z.-J., Qian C., Shen Y., Wu X.-D., et al., Beauvericin ameliorates experimental colitis by inhibiting activated T cells via downregulation of the PI3K/Akt signaling pathway., PLoS One. 8 (2013) e83013. doi: 10.1371/journal.pone.0083013 PMID: 24340073

52. Neurath M.F., Fuss I., Kelsall B.L., Presky D.H., Waegel l W., Strober W., Experimental granulomatous colitis in mice is abrogated by induction of TGF-beta-mediated oral tolerance., J. Exp. Med. 183 (1996) 2605–2616. PMID: 8676081

53. Majewska-szcze panik M., Góral ska M., Marciańska K., Zemelka-wieck M., Strzępa A., Doro I., Epicutaneous immunization with protein antigen TNP-Ig alleviates TNBS-induced colitis in mice, (2012) 1497–1504.

54. te Velde A.A, Versteg e M.I., Hommes D.W., Critical appraisal of the current practice in murine TNBS-induced colitis., Inflamm. Bowel Dis. 12 (2006) 995–999. doi: 10.1097/MIB.0b013e3181005bca PMID: 17012970

55. Eri R., Kodumudi K.N., Summerlin D.J., Srinivasan M., Suppression of colon inflammation by CD80 blockade: evaluation in two murine models of inflammatory bowel disease., Inflamm. Bowel Dis. 14 (2008) 458–70. doi: 10.1002/ibd.20344 PMID: 18186109

56. Lindsay J., Van Montfrans C., Brennan F., Van Deventer S., Drillenburg P., Hodgsen H., et al., IL-10 gene therapy prevents TNBS-induced colitis., Gene Ther. 9 (2002) 1715–21. doi: 10.1038/sj.gt.3301841 PMID: 12457286

57. Simioni P.P.U.P., Fernandes L.L.G.R.L., Gabriel D.L., Tamashiro W.M.S.C., Effect of aging and oral tolerance on dendritic cell function, Brazilian J. ... 1497–1504. http://www.scielo.br/scielo.php?pid=S0100-879 X2010000100010&script=sci_arttext (accessed March 27, 2015).

58. Garside P., Mowat A M., Oral tolerance., Semin. Immunol. 13 (2001) 177–85. doi: 10.1006/smim.2001.0310 PMID: 11394960

59. Gomes-Santos A.C., Moreira T.G., Castro-Junior A.B., Lemos L., Cruz D.N., et al., New insights into the immunological changes in IL-10-deficient mice during the course of spontaneous inflammation in the gut mucosa., Clin. Dev. Immunol. 2012 (2012) 560817. doi: 10.1155/2012/560817 PMID: 22400037

60. Goubier A., Dubois B., Gheit H., Joubert G., Villard-Truc F., Asselin-Patur el C., et al., Plasmacytoid Dendritic Cells Mediate Oral Tolerance, Immunity. 29 (2008) 464–475. doi: 10.1016/j.immuni.2008.06.017 PMID: 18789731

61. Bhatthacharya P., Budnick I., Singh M., Thiruppathi M., Prabhalakar B.S., GM-CSF-induced, bone-marrow-derived dendritic cells can expand natural Tregs and induce adaptive Tregs by different mechanisms., J. Leukoc. Biol. 89 (2011) 235–249. doi: 10.1189/jlb.0310154 PMID: 21048215
66. Rowin J., Thiruppathi M., Arhebamen E., Sheng J., Prabhakar B.S., Meriggiol M.N., Granulocyte macrophage colony-stimulating factor treatment of a patient in myasthenic crisis: effects on regulatory T cells., Muscle Nerve. 46 (2012) 449–53. doi: 10.1002/mus.23486 PMID: 22907239

67. Haddad C.S., Bhattacharya P., Alharshawi K., Marinelaarena A., Kumar P., El-Sayed O., et al., Age-dependent divergent effects of OX40L treatment on the development of diabetes in NOD mice, Autoimmunity. 49 (2016) 298–311. doi: 10.1080/08916934.2016.1183657 PMID: 27245356

68. Gathungu G., Kim M.-O., Ferguson J.P., Sharma Y., Zhang W., Ng S.M.E., et al., Granulocyte—Macrophage Colony-Stimulating Factor Autoantibodies, Inflamm. Bowel Dis. 19 (2013) 1671–1680. doi: 10.1097/MIB.0b013e318281f506 PMID: 23749272

69. Bhattacharya P., Thiruppathi M., Elishabrawy H.A., Alharshawi K., Kumar P., Prabhakar B.S., GM-CSF: An immune modulatory cytokine that can suppress autoimmunity, Cytokine. 75 (2015) 261–271. doi: 10.1016/j.cyto.2015.05.030 PMID: 26113402

70. Ruberti M., Fernandes L.G.R., Simioni P.U., Gabriel D.L., Yamada A.T., Tamashiro W.M.D.S.C., Phenotypical and functional analysis of intraepithelial lymphocytes from small intestine of mice in oral tolerance., Clin. Dev. Immunol. 2012 (2012) 208054. doi: 10.1155/2012/208054 PMID: 22400033

71. Thomas S., Baumgart D.C., Targeting leukocyte migration and adhesion in Crohn's disease and ulcerative colitis., Immunopharmacology. 20 (2012) 1–18. doi: 10.1007/s10787-011-0104-6 PMID: 22205271

72. Lee S.K., Choi B.K., Kim Y.H., Kang W.J., Kim K.H., Sakaguchi S., et al., Glucocorticoid-induced tumour necrosis factor receptor family-related receptor signalling exacerbates hapten-induced colitis by CD4+ T cells., Immunology. 119 (2006) 479–87. doi: 10.1111/j.1365-2567.2006.02459.x PMID: 17177830

73. Ramsdell F., Foxp3 and natural regulatory T cells: Key to a cell lineage?, Immunity. 19 (2003) 165–168. PMID: 12932350

74. Kiesler P., Fuss I.J., Strober W., Experimental Models of Inflammatory Bowel Diseases., Cell. Mol. Gastroenterol. Hepatol. 1 (2015) 154–170. doi: 10.1016/j.jcmgh.2015.01.006 PMID: 26000334

75. Cardoso C.R., Teixeira G., Provinciatto P.R., Godoi D.F., Ferreira B.R., Milanezi C.M., et al., Modulatory effects of 1,25-dihydroxyvitamin D3 on TH1/TH2 cytokines in inflammatory bowel disease: an in vitro study., Int. J. Immunopathol. Pharmacol. 22 63–71. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC6383127/

76. Boden E.K., Snapper S.B., Regulatory T cells in inflammatory bowel disease., Curr. Opin. Gastroenterol. 24 (2008) 733–41. http://www.ncbi.nlm.nih.gov/pubmed/19125486 (accessed December 15, 2014). PMID: 19125486

77. Walker L.S.K., Treg and CTLA-4: two intertwining pathways to immune tolerance., J. Autoimmun. 45 (2013) 49–57. doi: 10.1016/j.jaut.2013.06.006 PMID: 23849743

78. Liu Z.-J., Yadav P.-K., Su J.-L., Wang J.-S., Fei K., Potential role of Th17 cells in the pathogenesis of inflammatory bowel disease., World J. Gastroenterol. 15 (2009) 5784–8. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2791270&tool=pmcentrez&rendertype=abstract (accessed December 12, 2014).

79. Feng T., Qin H., Wang L., Benveniste E.N., Elson C.O., Cong Y., Th17 cells induce colitis and promote Th1 cell responses through IL-17 induction of innate IL-12 and IL-23 production., J. Immunol. 186 (2011) 6313–6318. doi: 10.4049/jimmunol.1001454 PMID: 21531892

80. Sun C.-M., Hall J.A., Blank R.B., Bouladoux N., Okka M., Mora J.R., et al., Small intestine lamina propria dendritic cells promote de novo generation of Foxp3+ T reg cells via retinoic acid., J. Exp. Med. 204 (2007) 1775–85. doi: 10.1084/jem.20070602 PMID: 17620362

81. von Herrath M.G., Harrison L.C., Antigen-induced regulatory T cells in autoimmunity., Nat. Rev. Immunol. 3 (2003) 223–32. doi: 10.1038/ni1029 PMID: 12658270

82. Talero E., Sánchez-Fidalgo S., de la Lastra C.A., Illanes M., Calvo J.R., Motilva V., Acute and chronic responses associated with adrenomedullin administration in experimental colitis., Peptides. 29 (2008) 2001–12. doi: 10.1016/j.peptides.2008.07.013 PMID: 18708104

83. Ardizzone S., Cassinotti A., Trabattoni D., Manzigna G., Rainone V., Bevilacqua M., et al., Immuno-modulatory effects of 1,25-dihydroxyvitamin D3 on TH1/TH2 cytokines in inflammatory bowel disease: an in vitro study., Int. J. Immunopathol. Pharmacol. 22 63–71. http://www.ncbi.nlm.nih.gov/pubmed/19309553 (accessed December 12, 2014). PMID: 19309553

84. Mügez G., Molnàr B., Tulassay Z., Sipos F., Changes of the cytokine profile in inflammatory bowel diseases., World J. Gastroenterol. 18 (2012) 5848–61. doi: 10.3748/wjg.v18.i41.5848 PMID: 23139600
87. Carvalho C.R., Vaz N.M., Specific responses to two unrelated antigens in mice made orally tolerant to one of them., Braz. J. Med. Biol. Res. 23 (1990) 861–4. http://www.ncbi.nlm.nih.gov/pubmed/2101328 (accessed February 18, 2015). PMID: 2101328
88. Carvalho C.R., Verdolin B.A., V de Souza A., Vaz N.M., Indirect effects of oral tolerance in mice., Scand. J. Immunol. 39 (1994) 533–8. http://www.ncbi.nlm.nih.gov/pubmed/8009172 (accessed February 18, 2015). PMID: 8009172
89. Carvalho C.R., Vaz N.M., Indirect effects are independent of the way of tolerance induction., Scand. J. Immunol. 43 (1996) 613–8. http://www.ncbi.nlm.nih.gov/pubmed/8658049 (accessed February 18, 2015). PMID: 8658049
90. Carvalho C.R., Verdolin B.A., Vaz N.M., Indirect effects of oral tolerance cannot be ascribed to bystander suppression., Scand. J. Immunol. 45 (1997) 276–81. http://www.ncbi.nlm.nih.gov/pubmed/9122617 (accessed February 18, 2015). PMID: 9122617
91. Gotsman I., Shlomai A., Alper R., Rabbani E., Engelhardt D., Ilan Y., et al., Amelioration of Immune-Mediated Experimental Colitis: Tolerance Induction in the Presence of Preexisting Immunity and Surrogate Antigen Bystander Effect, 297 (2001) 926–932.
92. Sinha S., Subramanian S., Miller L., Proctor T.M., Roberts C., Burrows G.G., et al., Cytokine switch and bystander suppression of autoimmune responses to multiple antigens in experimental autoimmune encephalomyelitis by a single recombinant T-cell receptor ligand., J. Neurosci. 29 (2009) 3816–23. doi: 10.1523/JNEUROSCI.5812-08.2009 PMID: 19321778
93. Oefner C.M., Winkler A., Hess C., Lorenz A.K., Holecska V., Huxdorf M., et al., Tolerance induction with T cell-dependent protein antigens induces regulatory sialylated IgGs, J. Allergy Clin. Immunol. 129 (2012) 1647–1655. doi: 10.1016/j.jaci.2012.02.037 PMID: 22502800
94. Castro-Junior A.B., Horta B.C., Gomes-Santos A.C., Cunha A.P., Silva Steinberg R., Nascimento D.S., et al., Oral tolerance correlates with high levels of lymphocyte activity., Cell. Immunol. 280 (2012) 171–81. doi: 10.1016/j.cellimm.2012.12.004 PMID: 23399844
95. Glocker E.O., Kotlarz D., Klein C., Shah N., Grimlacher B., IL-10 and IL-10 receptor defects in humans, Ann. N. Y. Acad. Sci. 1246 (2011) 102–107. doi: 10.1111/j.1749-6632.2011.06339.x PMID: 22236434
96. Guo B., Li Z., Endoplasmic reticulum stress in hepatic steatosis and inflammatory bowel diseases, Front. Genet. 5 (2014) 1–11.