ORAL TOLERANCE
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W.31. CD8+ T Cell Mediated Regulation of Mucosal Tolerance

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The role of CD8+ regulatory T cells in tolerance to oral antigens remains unclear. We have developed a novel model system in which C57BL/6 mice were fed SIINFEKL (the MHC class I immunodominant epitope of OVA) for 5 consecutive days, generating antigen specific CD8+ Tregs capable of mediating tolerance to subsequent immunization with whole OVA. Mice were fed OVA or PBS as positive and negative controls, respectively. Draining LN cells harvested from mice fed SIINFEKL or OVA prior to immunization with OVA produced significantly less IFNγ and IL17 when restimulated *in vitro* with OVA compared to PBS-fed mice. These mice also had significantly diminished DTH responses *in vivo* as measured by reduced ear swelling 24 h after injection of OVA. In contrast, OVA but not SIINFEKL-feeding suppressed a Th2-driven OVA-induced model of allergic lung inflammation. PBS- and SIINFEKL-fed mice had comparable cell numbers in airways and inflammatory infiltrates in the lungs, which were reduced in OVA-fed mice. Thus, while SIINFEKL feeding resulted in the generation of CD8+ Tregs capable of suppressing Th1 and Th17 cytokines, their inability to suppress Th2 responses suggests that these cells act through a different mechanism than their CD4+ counterparts generated in response to OVA feeding.

W.32. Sublingual Immunotherapy (SLIT) Induce Systemic Tolerance in Naïve Mice

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Background: SLIT is a clinically effective treatment of patients suffering from both pollen and house dust mite induced rhinitis and asthma. However little is known of the preventive potential of SLIT treatment. In the present study, we have investigated whether prohylactic SLIT-treatment of naïve mice can down regulate a subsequent systemic challenge. Methods: Naïve BALB/c or C57Bl/6 mice were SLIT treated with either Phleum pratense (Phl p) extract or OVA for two weeks followed by intraperitoneal challenges with alum-adsorbed Phl p or OVA. Subsequently, the mice were sacrificed and serum antibodies and T cell reactivity were measured. Results: Prophylactic SLIT treatment of naïve mice led to the induction of systemic tolerance as measured by the ability to down regulate a subsequent intraperitoneal challenge. Both T cell proliferation as well as secretion of IFN-γ, IL-4, IL-5 and IL-10 were reduced by more than 50% in SLIT treated mice compared to buffer-treated mice. Furthermore the serum levels of Ag-specific IgE were reduced. This could be demonstrated in both BALB/c and C57Bl/6 mice and by using Phl p extract as well as OVA as antigen. Finally, it was demonstrated that the effect of the prohylactic SLIT treatment persisted for at least 4 weeks. Conclusions: The results of the present study demonstrate that sublingual treatment of naïve mice leads to the induction of systemic tolerance, indicating that prohylactic treatment of individuals predisposed for allergy may be possible.

W.33. Lymph Node Stromal Cells Strongly Influence Immune Response Suppression

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Many pathogens and food antigens are initially encountered in the gut, where the decision is made to mount an immune response or induce tolerance. To analyse the balance between tolerance and immunity, mesenteric or peripheral lymph node fragments (mLNtx or pLNtx) were transplanted into rats and mice. Recently we showed that only stromal cells survived transplantation, and that they are able to affect T cell function and influence the IgA response after induction of an immune response. After tolerance induction via ovalbumin, a reduced delayed-type hypersensitivity response was detected in pLNtx compared to mLNtx animals. Reduced IL-10 expression, reduced numbers of regulatory T cells, and increased numbers of B cells were identified within the pLNtx fragments. The increase of B cells results in an immunoglobulin production. These findings show that pLNtx induce a tolerogenic phenotype not by T cell suppression but by B cell accumulation and antibody production.

W.34. A Role of PARP-1 in Oral Tolerance by Regulation of CD4+Foxp3+Treg Cells

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Poly (ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme that is functionally linked to DNA repair. Its activation is triggered by DNA strand breaks or kinks. A large body of evidence demonstrates that PARP-1 is activated during the inflammatory response. Recent studies demonstrate that mice lacking PARP-1 (PARP-1-/-) develop normally, but show protection and inhibition of experimental inflammation and autoimmune disease such as diabetes, septic shock and mucosal inflammation. These findings suggest that PARP-1 plays a critical role in immunity and inflammation, but the underlying mechanism remains unclear. By using an oral tolerance model, we show here that CD4+Foxp3+Treg cells were higher in the mesenteric lymph nodes in PARP-1-/- mice than that in wild type mice after continuous feeding of OVA protein (2 mg per day) for 7 days. The cell membrane-bound LAP-TGFβ expression on CD4+ T cells was also increased in PARP-1-/- mice. The TNFα cytokine was however dramatically reduced in PARP-1-/- mice. When challenged with OVA protein in the presence of CFA after OVA protein feeding, PARP-1-/- mice showed significantly lower levels of antigen-specific IL-2 production and reduced T cell

proliferation. Our results have revealed a role of PARP-1 in influencing mucosal tolerance by regulation of CD4+Foxp3+ Treg cells in the gut-associated lymphoid tissues. This research was supported by the intramural research program of NIH, NIDCR.

W.35. Induction of Treg from PBMC by Interacting with Immunosuppressive Molecule B7-H3 on Oral Mesenchymal Stem Cells from Human Gingival Fibroblasts

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Despite high bacterial colonization, acute infections are rare in the oral mucosa. A recent study showed that mesenchymal stem cells show tolerogenic property. It is also known that periodontal tissues contain many kinds of mesenchymal stem cells such as the dental pulp stem cells, dental follicle cells and periodontal ligament stem cells. These reports lead us to the assumption that oral mesenchymal stem cells can contribute to the oral tolerogenic property. In this study, we attempted to establish mesenchymal stem cells from the human periodontal tissues, and investigated immunosuppressive function of the cells. A fibroblast-like cell line named H1C1 from the periodontal tissue of an extracted baby tooth from a 6 year-old child was established by limiting dilution method and used for further research. The cell line had abilities to differentiate into adipocytes, osteocytes and chondrocytes, therefore we termed H1C1 as the human mesenchymal stem cells. RT-PCR showed that H1C1 constantly expressed mRNA of co-stimulatory molecules B7-H1, B7-H2 and B7-H3, which are known as immunosuppressive molecules. When human PBMC were co-cultured with H1C1, the population of Tregs was increased compared with monoculture of PBMC, and neutralization of B7-H3 suppressed this induction. These results suggest that the human oral mesenchymal stem cells such as H1C1 have the abilities to suppress immunoreactions by co-stimulatory molecules and increasing regulatory T cells. In conclusion, we succeeded to establish the human oral mesenchymal stem cells named H1C1, which may contribute to the oral tolerogenic environment by cell-to-cell interactions and promoting the induction of regulatory T cells by B7-H3 molecule.

W.36. TGF-β Signaling in Peyer's Patch CD4+ T Cells is Required for Induction of Oral Tolerance

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TGF-β signaling plays key roles in the induction of oral tolerance (OT). Here, we assess the ability of CD4-dominant negative TGF-βRII (TbRII) mice to develop OT to understand the role of TGF-β signaling in the early events of OT. Mice were fed 30 mg of OVA and were orally immunized three times weekly with OVA plus cholera toxin (CT) one week after tolerization. One week after last immunization samples were collected and mononuclear cells isolated from spleen, Peyer's Patches (PPs), and mesenteric lymph nodes for analysis. DTH responses were determined by ear swelling after oral challenge. $CD4^+$ T cells from C57BL/6 mice were examined for expression of TGF-βRII at 24-hour intervals for 3 days and on days 5 and 7 after feeding 30mg OVA. OVA-specific antibody titers in plasma and fecal extracts, as well as DTH response of OVA-fed TbRII mice were essentially the same as TbRII mice fed PBS. These responses were significantly higher than those of tolerized C57BL/6 mice. Expression of TGF-βRII by $CD4^+$ T cells of spleen and MLN of wildtype mice was low or variable, respectively. PP $CD4^+$ T cells had significantly increased expression over base-line at 24 hours and remained high for all time points. These results indicate critical roles for TGF-β signaling in PPs during OT induction. This work is supported by NIH grants DE 0176707, DE 012242 and AG 025873.

W.37. Effect of Protein-free Diet in the Induction of Food Allergy and Oral Tolerance to Ovalbumin in Mice

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Our group had previously shown that food protein is critical for the development of the immune system. Adult mice reared since weaning in a diet where all proteins were replaced by amino acids (Aa diet) presented an immature immune profile (Int.Immunol., 2003 5(3):447–455). Herein, we evaluated oral tolerance as well as food allergy induction in these mice. BALB/c mice were fed, since weaning, a balanced diet containing 15–20% of either casein (Cas) or a mixture of casein amino acids (Aa). After 8 weeks (12-weeks-old) mice were fed OVA for 2 days. Seven days later they were immunized with OVA plus Al(OH)3 and boosted with OVA 14 thereafter. After 7 days mice were fed a 20% OVA solution for 7 days. Food allergy, as measured by weight loss and OVA consumption, was induced in mice reared on both control Cas and Aa diet although IL-4 levels were higher in Cas-fed mice. Oral tolerance to OVA was also induced in both groups when specific Ig and IgE antibodies as well as IL-4 production were measured. However, specific IgG1 antibodies were suppressed only in Cas-fed mice. In both groups, IFN-gamma production was associated with oral tolerance induction. Financial support: CNPq (Brazil), FAPEMIG.

W.38. Role of Immunization and of the Regimen of Feeding in Oral Tolerance Maintenance

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Aging is reported to impair the susceptibility to oral tolerance induction, however, oral tolerance induced in young animals is maintained throughout their lives (Faria *et al*., Mech. of aging and development 1998, 102:67–80). We investigated oral tolerance maintenance using two different adjuvants (CFA and Al(OH)3) for immunization, in distinct times after feeding. Seven-week-old BALB/c mice were treated with either saline or OVA by gavage or continuous feeding. After 7, 30, 90, 180 or 360 days after oral treatment, all animals were i.p. immunized with OVA plus Al(OH)3 and boosted with OVA 14 days thereafter. Alternativelly, mice were s.c. immunized with OVA plus CFA 7, 30, 90 days after feeding. Oral tolerance was measured as inhibition of serum anti-OVA Ig, IgG1 and IgG2a production. Oral tolerance was not seen in animals immunized with CFA 90 days after OVA feeding. However, mice that were continuously fed, but not mice orally treated by gavage, and immunized with Al(OH)3 remained tolerant up to 360 days after oral treatment. Our results suggest that oral tolerance maintenance depends on the time between oral treatment and immunization, feeding regimen and type of adjuvant used. Financial support: Fogarty International Center/NIH, CNPq and FAPEMIG (Brazil).

W.39. A Novel Food Component Boosts Stress Protein HSP70 to Activate T Cell Regulation of Inflammation in Experimental Arthritis

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Locally up-regulated stress proteins are protective inhibitors of stress-mediated cell death. In the present study, however, we manipulated stress protein expression *in vivo* with a food component, and obtained evidence for immune recognition of stress proteins expressed in the tissue. We report that our food compound had an unprecedented capacity to co-induce cellular heat shock protein 70 (HSP70) expression *in vitro* and, upon intragastric administration, in Peyer's patches of mice *in vivo*. As a consequence, the compound specifically promoted T cell recognition of endogenous HSP70 as shown *in vitro* by the activation of a HSP70-specific T cell hybridoma and amplified T cell responses to HSP70 *in vivo*. Oral administration also increased the number of CD4+CD25+Foxp3+ T cells, systemically in the spleen and locally in the joint, and almost completely suppressed proteoglycan-induced experimental arthritis. Furthermore, protection against arthritis could be transferred with T cells isolated from fed mice. These findings illustrate that a food component can boost protective T cell responses to a self-stress protein and down-regulate inflammatory disease; the immune system responds to one's diet.

W.40. Oral Administration of the VR1 Ligand Capsaicin Confers Protection from Autoimmune Diabetes by Suppressing Autoreactive T Cells in an APC Dependent Manner

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Sensory nerve fibers innervating the pancreas play a critical role in the etiology of autoimmune diabetes. These sensory neurons express a nonselective cation channel vanilloid receptor 1 (VR1) which has been identified as a receptor for acid, temperatures > 43°C and Capsaicin (CP), the principal pungent ingredient in chili peppers. We have reported that dendritic cells (DCs), a key cell type in immune responses, have the receptor for CP, and engagement of this receptor has powerful immune consequences. However very little is known about the function of VR1 expressing pancreas resident antigen presenting cells (APCs) including DCs in Type 1 diabetes. Here we have explored the immunomodulatory activity of CP in the non-obese diabetic (NOD) mouse and found that oral administration of CP delays the onset of diabetes and reduces the incidence of diabetes in NOD mice in a dose dependent manner. Moreover, splenocytes from CP treated mice are able to transfer protection in recipient NOD mice. CP administration attenuates the proliferation of pancreatic antigen-specific autoreactive T cells in pancreatic lymph nodes (PLN) both *in vivo* and *in vitro* in two distinct mouse models of diabetes, NOD and RIP-OVA. We have further found that this CP dependent modulation of autoimmunity requires both VR1 expression and a population of myeloid CD11b+ cells present in the PLN. These results reveal that CP is an immunologically potent ligand that when administered orally suppresses autoimmune diabetes by inhibiting the proliferation of autoreactive T cells in an APC dependent manner.

W.42. Long-chain Dietary Fat Promotes Oral Tolerance via Chylomicron-dependent Antigen Transport through Mesenteric Lymph Nodes (MLN)

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Background and Aim: Immunological tolerance to dietary proteins (oral tolerance) requires antigen absorption and transport to mesenteric lymph nodes (MLN). We hypothesized that enterocytes secrete dietary antigens together with chylomicrons for subsequent transport to MLN. Methods: Chylomicron-dependent absorption of the model antigen ovalbumin (OVA) was studied in CaCo-2 cells and mice. Chylomicron formation in CaCo-2 cells was induced with Oleic Acid (OA) and in mice by gavaging

long-chain triglycerides (LCT). Chylomicron formation was prevented or blocked *in vitro* by using Butyric Acid (BA) instead of OA or by adding an inhibitor of chylomicron formation, Pluronic L-81. In mice, chylomicron formation was curtailed by replacing LCT with medium-chain triglycerides (MCT) or by adding Pluronic L-81. Results: CaCo-2 cells acquired OVA but degraded little OVA in lysosomes. Chylomicron formation stimulated basolateral release of cell-associated OVA *in vitro* and OVA absorption into the MLN *in vivo*. Moreover, chylomicrons isolated from mice gavaged with LCT plus OVA but not LCT plus bovine albumin stimulated DO11.10 T-cell proliferation, suggesting enrichment of chylomicrons with OVA. Interestingly, preventing or blocking chylomicron formation during OVA feeding significantly impaired oral tolerance as reflected by increased anti-OVA IgG production after subsequent peripheral sensitization with OVA. Conclusion: Enteroctytes secrete dietary antigens into the MLN during postprandial chylomicron formation, and this promotes oral tolerance. These findings reveal a novel immunomodulatory effect of dietary fat and could be exploited to suppress food allergies or auto-immune diseases.

W.43. Similar Patterns of Lymphocyte Activation in Orally Tolerant and Parenterally Immunized Mice

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In oral tolerance, T-dependent specific immune responsiveness is suppressed by previous ingestion of antigen. Activated regulatory T cells are believed to play a decisive role in oral tolerance. However, we found similar activation profiles of spleen T and B cells after oral tolerance induction or parenteral immunization of C57BL/6 mice to ovalbumin (OVA). Mice fed OVA diluted in drinking water for 3 days were immunized with 10 μg OVA plus Al(OH)3 7 days later and boostered (OVA only) 14 days thereafter. Immune mice received immunization but not oral OVA and control group were sham immunized with Al(OH)3. Serum specific immunoglobulins were measured by ELISA and spleen lymphocytes were stained for CD4, CD19, CD62L, CD25, CD45RB and MHCII 7 days after booster. Although specific antibody levels in tolerant mice were significantly lower than in immune mice, both groups presented same increase in CD4+CD25+CD45RBlow and CD19+CD62L- cells. B cells presented lower than normal levels of MHCII in tolerant and immune mice. This suggests that differences between tolerant and immunized animals should not rely on down activation and/or induction of regulatory cells during oral tolerance. Since both mice present same number of activated/regulatory lymphocytes, oral tolerance phenomena may depend on distinct activated lymphocyte repertoire.

W.44. Induction of Oral Tolerance is Compromised in Chimeric Mice Lacking MHC-II Expression on Non-hematopoietic Antigen-presenting Cells

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The intestinal barrier has the potential to regulate immune responses to luminal antigen using many mechanisms. To address the question of the role of epithelial MHCII molecules in induction of mucosal tolerance, a model of MHCII chimeric mice was used. B6 WT and MHC class II knock-out (KO) mice were γ-irradiated and adoptively transferred with bone marrow from WT B6 mice (WT->WT and WT->KO). Six weeks later, total splenocytes from B6 OT-II mice were iv injected (Day 0) into WT->WT and WT->KO mice. Mice were fed (Day 1) with 50 mg ovalbumin (OVA), or PBS in controls. On Day 9, mice were subcutaneously injected with OVA (200 μg) in IFA. On Day 16, tissues were collected for analysis. Oral tolerance induction was measured by 3H-thymidine incorporation into total inguinal lymph node cells cultured with OVA protein or OVA323-339 peptide. OVA-specific responses were detected in both WT->WT and WT->KO controls. Significant systemic suppression was detected in WT->WT OVA-fed mice. In WT->KO mice, the level of suppression was less profound and not statistically significant, suggesting that non-BM-derived MHCII plays a role in oral tolerance induction. The role of epithelial and other non-BM-derived components in this phenomenon is under further investigation.