CELIAC DISEASE
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M.36. Distribution of Dendritic Cell Markers and Toll-like Receptors 2 and 4 in the Ileum from a Mouse Model of Gluten Sensitivity

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We examined the distribution of dendritic cell (DC) markers as well as Toll-Like receptors (TLR) 2 and 4 in the ileum from a mouse model of gluten sensitivity. Gluten-sensitive HLA-DQ8+/HCD4 transgenic mice were orally treated with saline (controls), NSAID (indomethacin), gliadin or gliadin+NSAID (n=5–8/group). Ileal sections were processed and studied by immunohistochemistry and immunofluorescence. In controls, CD103+ cells were mainly located in the intraepithelial compartment of the lamina propria (LP) expressing some of them TLR-2. CD11c was observed in the connective tissue of the LP and scant TLR-4+ cells were detected. In the NSAID group, no changes in CD103+ cells but significant ($p<0.05$) reduction of CD11c were observed. Changes ($p<0.05$) in distribution of both DC markers were detected in the gliadin group as well as for CD11c in the gliadin+NSAID group. No differences for TLR-4 were observed in all treated groups, whereas significant reduction of TLR-2+ cells including absence of TLR-2+CD103+ cells was detected in the gliadin+NSAID group. In this model, a diverse distribution of ileal-DC markers occurred under different oral challenges. In addition, absence of TLR-2+CD103+DCs in gliadin+NSAID treated animals suggested a potential activation of this DC subpopulation via recognition of bacterial antigens by TLR-2.

M.37. CD103+ Dendritic Cells Reside in Human Small Intestinal Mucosa but do not Increase in the Active Celiac Lesion

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Background: CD103+ DCs promotes regulatory T-cell differentiation, differentiation of IgA-secreting B-cells and expression of gut-homing receptors (CCR9 and α4β7) in murine small intestinal mucosa. Whether similar DCs exist in the human intestinal mucosa has not been determined. Aim: To determine whether CD103+ DCs are present in human duodenal mucosa and whether their frequency is altered in celiac disease. Method: Immunofluorescence staining of tissue was performed on cryosections from normal mucosal tissue (n=10) and from treated (n=11) and untreated, active celiac disease (n=10). Result: CD103+ DCs constituted ~ 40% of mucosal DCs (identified by CD11c expression) in healthy controls and treated celiac disease. The number of CD11c+ DCs in the active celiac lesion was significantly increased ($p<0.01$) compared with healthy controls and treated celiac disease. However, the number of CD103+CD11c+ cells was unchanged in the active lesion. Thus, in active celiac disease the fraction of CD103+ DCs was significantly reduced (23%, $p<0.05$). Conclusion: The higher representation of the CD11c+CD103- cells in active celiac disease suggests a selective recruitment of this DC subset to the active lesion. This raises the possibility that an imbalance in the numbers of functionally different mucosal DC subsets is involved in the immunopathology of celiac disease.

M.39. *In vitro* and *in vivo* Innate Immune Activation of Monocytes, Macrophages and Dendritic Cells by Gliadin Occurs via Toll Like Receptor 4

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Celiac disease (cd) is a small intestinal enteropathy caused by Th1 mediated inflammatory responses to gluten peptides that specifically bind to HLA-DQ2/-DQ8. However, only 2–5% of individuals expressing DQ2/DQ8 develop cd, indicating that additional mechanisms such as innate immune responses to gluten might be involved in disease pathogenesis. Our aim was to further examine specificity and nature of these responses.Upon stimulation with a peptic-tryptic digest of gliadin but not zein from corn human monocytic cell lines significantly upregulated secretion of IL-8, MCP-1 and TNF-α. Proteinase K digestion of gliadin but not LPS abrogated the stimulatory effects. Peritoneal macrophages from MyD88-/- or C3H/HeJ mice that lack TLR-4 responses did not secrete these cytokines upon gliadin stimulation as compared to macrophages from wild type mice. Human monocyte derived dendritic cells (DCs) of cd patients stimulated with gliadin secreted IL-8 and TNF-α comparable to those from non-celiac controls. Cytokine secretion in DCs was significantly decreased by preincubation with TLR4 neutralizing antibody. Furthermore, intraperitoneal injection of gliadin led to increased serum levels of TNF-α and KC (IL-8) similarly in C57BL/6 and Rag1-/- but not in MyD88-/- mice. Gliadin peptides can elicit an innate immune response *in vitro* and *in vivo*, with TLR-4 serving as the prominent innate immune receptor.

M.40. Duodenal Transport and Processing of Various Proteins and Peptides in Celiac Disease

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Background: Intestinal permeation to gliadin peptides is altered in celiac disease (CD). Two distinct mechanisms could be involved: a paracellular leakage through tight junctions and a transcytosis of IgA/gliadin complexes through the IgA receptor CD71. Methods: To further analyze the occurrence of both pathways, we compared the mucosal to serosal transport and

processing of horseradish peroxidase (HRP, 40 kD), immunostimulant gliadin peptide (p31-49, 2kD) and non immunostimulant gliadin peptide (p202-220, 2kD) in duodenal biopsies from 8 patients with active CD, mounted in Ussing chambers. Results: Total fluxes of HRP, p31-49 and p202-220 did not differ (6.2±1.4, 6.2±2.0, 7.5±3.1 µg/3 h.cm^2) but the processing during transport was strikingly altered. Indeed, percentage of HRP, p31-49 and p202-220 in intact form in serosal compartment after transport was of 0.6%, 49% and 12% respectively, indicating a specific protective transport of p31-49. No significant correlation (Pearson coefficient=0.02605, p=0.7026) was observed between p31-49 transport in intact form and the ionic conductance, an index of paracellular permeability, suggesting a predominant transcellular transport pathway. Conclusion: Comparative study of the transport of protein or peptides of various size and nature in active celiac patients indicates no major paracellular leak of immuno-stimulant gliadin peptides but confirms their specific transcellular transport pathway.

M.41. Involvement of the CD40/CD40 Ligand Pathway in Celiac Disease

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Background and Aims: The CD40/CD40 ligand (CD40L) system is critically involved in many immune disorders. Therefore, to determine the role of this pathway in celiac disease (CD), we explored CD40/CD40L expression in CD mucosa and investigated the *ex vivo* effect of CD40L blockade on T helper cell type 1 (Th1) cytokine production and expression of the Th1 transcription factor, T-bet. Methods: Duodenal biopsies were collected from 11 CD patients before and after 12 months of gluten-free diet (GFD), and from 10 controls. Treated CD biopsies were cultured for 24 h with 1 mg/ml peptic-tryptic digest of gliadin (PT-gliadin) with or without 10 microg/ml of anti-CD40L neutralising antibody. Interferon (IFN)-gamma and interleukin (IL)-17 were measured in culture supernatants by ELISA. T-bet, CD40 and CD40L were determined by immunoblotting on mucosal homogenates. Results: CD40 and CD40L were higher in active CD mucosa than in controls, and normalised after GFD. PT-gliadin induced a significant ($p<0.001$) up-regulation of IFN-gamma and IL-17. CD40L blockade significantly ($p<0.005$) inhibited the PT-gliadin-induced up-regulation of both IFN-gamma and IL-17. T-bet expression was significantly ($p<0.001$) down-regulated in celiac biopsies treated with anti-CD40L antibody. Conclusions: Our findings provide evidence for a key pathogenic role of the CD40/CD40L pathway in active CD.