be stable in time, so they might be used for prediction of phenotype in Romanian patients with CD.

**P283**

High prevalence of P-ANCA in ulcerative colitis and primary sclerosing cholangitis. Results of analyses with lactoferrin-repleted granulocytes

L. Komorowski1, B. Teegen1*, K. Aulinger-Stöcker2, S. Niemann1, D.P. Bogdano3, D. Ludwig4, N. Homann2, W. Stöcker1, 1Inst. of Exp. Immunology, to EUROIMMUN AG, Lübeck, Germany, 2Medical Clinic I, Medical University2016-04-08T12:38:57Z

**Background:** Recently, we described an indirect immunofluorescence test (IIFT) for the sensitive detection of one of the ulcerative colitis (UC)-associated autoantibodies using high-salt stripped, lactoferrin-reconstituted granulocytes. In this study, the prevalence of P-ANCA in UC and primary sclerosing cholangitis (PSC) was re-evaluated.

**Methods:** Slides with ethanol-fixed human granulocytes were incubated 30 min in 1 M MgSO₄ to strip off their P-ANCA targets, including lactoferrin. Subsequently, the cells were repleted by 30 min incubation selectively with lactoferrin purified from human colostrum. Success of lactoferrin immobilization was controlled by incubation with a polyclonal antibody raised against recombinant lactoferrin. These cells were used in IIFT, in parallel to normal ethanol-fixed granulocytes, to detect ANCA against recombinant lactoferrin. These cells were used in IIFT, to detect ANCA against recombinant lactoferrin.

**Results:** Using standard ethanol-fixed granulocytes, P-ANCA were detected with prevalences of 72% in UC (CD 11%, PSC 42%, HBD 0%). Reactions with the lactoferrin target were as follows: UC 72%, CD 3%, PSC 42%, HBD 0%. The reactions with the two different substrates were not concordant in many cases: Of the P-ANCA negative sera, 55% reacted positive with the lactoferrin target in UC, 1% in CD, and 20% in PSC. With a combination of both substrates, the sensitivity of IIFT increased to 87% in UC and to 54% in PSC.

**Conclusions:** By recruitment of the new lactoferrin-repleted granulocyte substrate additionally to ethanol-fixed granulocytes, the sensitivity of P-ANCA can be raised by 15% in UC and by 12% in PSC without a significant reduction of specificity.

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Pathology of regulatory T cells in Crohn’s disease: impaired migration of CCR4+ Treg to inflamed mucosa or insufficient priming of naive Treg in the mesenteric lymph node – or both?


**Aim:** Since CD4+CD25+Foxp3+ regulatory T cells (Treg) prevent and treat established colitis in animal models of inflammatory bowel disease (IBD), they are in the focus for both, cell-based therapy and IBD pathology. We have demonstrated that 30 min incubation inactivated increase in IBD mucosa (Maul et al, Gastroenterology, 128, 2005) that is probably mediated by a defective CCR4-dependent Treg migration (Maul et al, Z Gastroenterol, 46, 2008). Analysing Treg phenotype in peripheral blood (PB) and from mesenteric lymph node (MLN), we aimed to get additional information on Treg migration and priming.

**Material and Methods:** Mononuclear cells from 23 patients with active CD and 11 healthy controls (HC) were isolated from PB and MLN (for HC; e.g. from intussusception, benign colon stenosis, etc.). FACs analysis was performed after staining for CD4, CD25, CD45RA, CCR4 and CD137 (41-BB).

**Results:** In CD, Treg were significantly enriched in MLN compared to PB (median 9.28%, range 4.37–19.4%, vs. 1.89%, range 0.12–4.21%, p < 0.001). Only in CD but not in HC, the percentage of CCR4+ Treg was significantly increased when comparing PB vs. MLN (median 82.25%, range 28.7–96.2%, vs. 24.75%, range 17.9–92.8%; p = 0.007). In CD, there was a significant decrease in PB CCR4+ Treg expressing CD45RA compared to HC (median 1.43%, range 0.33–8.96% vs. 11.2%, range 10.2–11.2%; p = 0.046). Only in CD, the percentage of CCR4+ Treg expressing CD45RA was significantly increased in MLN (median 14%, range 4.24–15.7%; p = 0.007) compared to PB. Interestingly, approximately 1.5% of MLN Treg express CD137 in CD.

**Conclusion:** As it has been already shown for the entire CD4+ population in CD, also PB CCR4+Treg show an expansion of a CD45RO+ subpopulation – presumably memory/effector-like Treg. MLN represents a pool/inductive site for Treg. In CD, MLN harbours an increased fraction of naive CD45RA+CCR4+ Treg. Only CD45RO-expression is associated with competence to recirculate through the intestinal lamina propria (LP). In CD, LP shows a reduced percentage of CCR4+ Treg (Maul et al, Z Gastroenterol, 46, 2008). Therefore, accumulation of naive CCR4+ Treg in the MLN may reflect insufficient priming. This is supported by the relatively low level of CD137-expressing Treg corresponding to antigen-specific activated cells.

**P285**

Mucosal nonimmune cells produce pro-inflammatory mediators in response to Toll-like (TLRs) and Nod-like receptor (NLRs) ligands: mesenchymal and endothelial cells as novel players of intestinal innate immunity

F. Scaldaferri IV1*, M.H. Phillips2, F. Rieder2, A. Schirbel2, G. West2, C. McDonald2, C. Flocchi1, 1Catholic University of Rome, Rome, Italy, 2the Cleveland Clinic Foundation – Lerner Research Institute, Cleveland, OH, USA.

**Introduction:** IBD involves an abnormal immune response to gut bacteria, mediated by TLRs and NLRs displayed on monocytes, dendritic (DCs) and epithelial cells. Other cell types, like mesenchymal and endothelial cells, can display TLRs and NLRs but their role in mucosal immunity is not well defined.

**Aims and Methods:** We investigated whether human intestinal microvascular endothelial cells (HIMEC) and human intestinal fibroblasts (HIF) respond to NLR and TLR stimulation producing pro-inflammatory cytokines and how these cells can be compared to professional innate immune cells, such as monocytes and DCs.

HIF and HIMEC were exposed to specific ligands for TLR2/1, 2/6, 4, 5, 9, Nod1 and Nod2 or TNF-a, and supernatants were assessed for IL-6 and IL-8 content, using ELISA system. Cytokine levels were compared to those produced at same conditions by same number of freshly isolated blood monocytes and GM-CSF/IL4-induced dendritic cells (DCs).

**Results:** HIF and HIMEC expressed mRNA for NODs and TLRs. Stimulation of TLR2/1, 2/6, 4, 5, 9, Nod1 and Nod2 or TNF-a, and supernatants were assessed for IL-6 and IL-8 content, using ELISA system. Cytokine levels were compared to those produced at same conditions by same number of freshly isolated blood monocytes and GM-CSF/IL4-induced dendritic cells (DCs).

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