The aim of our study is to compare the concentrations of several angiogenic factors in mucosal cultures from patients with inflammatory bowel disease (IBD). The mean disease duration was 7.7 years. According to endoscopic activity, 36% of patient had quiescent, 32% mild, 28% moderate, and 4% severe activity. All angiogenic factors mean levels in MCS were higher in affected than in non-affected controls: VEGFA (19.1±18.7 versus 8.7±10.5 pg/mL/mg) and Ang2 (21.3±15.7 versus 11.8±8.9 pg/mL/mg) (p < 0.05). There were no differences in MCS depending on endoscopic activity. Levels of VEGFA, Ang1 and Ang2 from the affected, and VEGFA and Tie2 from the non-affected mucosa, were lower when cultured with ADA than without it (Table 1).

### Table 1. Levels of angiogenic factors in MCS from IBD patients after culture

<table>
<thead>
<tr>
<th></th>
<th>Affected mucosa</th>
<th>Non-affected mucosa</th>
<th>p-value</th>
<th>Affected mucosa + ADA</th>
<th>Non-affected mucosa + ADA</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGFA</td>
<td>19.1±18.7</td>
<td>8.6±10.5</td>
<td>0.003</td>
<td>5.8±6.1</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>Ang1</td>
<td>15.7±8.8</td>
<td>12.5±9.3</td>
<td>0.021</td>
<td>9.1±7.1</td>
<td>0.069</td>
<td></td>
</tr>
<tr>
<td>Ang2</td>
<td>21.3±15.7</td>
<td>11.8±8.9</td>
<td>0.021</td>
<td>10.4±6.7</td>
<td>0.406</td>
<td></td>
</tr>
<tr>
<td>Tie2</td>
<td>12.0±5.5</td>
<td>8.8±7.0</td>
<td>0.911</td>
<td>5.3±5.2</td>
<td>0.021</td>
<td></td>
</tr>
</tbody>
</table>

Concentrations are expressed in pg/mL per mg of tissue. In UC patients, VEGFA and Ang1, and all angiogenic factors MCS mean levels were lower in the affected-mucosa and non-affected mucosa, and in the non-affected mucosa when cultured with ADA, respectively (Table 2).

### Table 2. Levels of angiogenic factors in MCS from CD patients after culture

<table>
<thead>
<tr>
<th></th>
<th>Affected mucosa</th>
<th>Non-affected mucosa</th>
<th>p-value</th>
<th>Affected mucosa + ADA</th>
<th>Non-affected mucosa + ADA</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGFA</td>
<td>20.4±13.9</td>
<td>7.0±7.2</td>
<td>0.015</td>
<td>5.0±6.0</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>Ang1</td>
<td>18.2±9.6</td>
<td>13.0±9.6</td>
<td>0.049</td>
<td>8.7±5.8</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>Ang2</td>
<td>27.2±14.4</td>
<td>14.5±9.8</td>
<td>0.074</td>
<td>12.2±7.8</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Tie2</td>
<td>12.7±4.6</td>
<td>7.5±3.7</td>
<td>0.720</td>
<td>4.4±3.1</td>
<td>0.026</td>
<td></td>
</tr>
</tbody>
</table>

Concentrations are expressed in pg/mL per mg of tissue. In contrast, in CD patients there were no differences in MCS angiogenic factors levels and the addition of ADA to the culture. ADA might downregulate the production of angiogenic factors in MCS. ADA addition to mucosal cultures affects angiogenic factors levels in samples from patients with UC differently than in patients from CD.

### P014

The effect of the anti-TNFα adalimumab on the levels of angiogenic factors in mucosal cultures from patients with inflammatory bowel disease (IBD)

P.M. Linares1, M.E. Fernández-Conteras2, A. Algaba2, M. Chaparro1, F. Bermejo2, J.P. Gisbert1.1Hospital Universitario de La Princesa, IP and CIBERehd, Gastroenterology Unit, Madrid, Spain, 2Hospital Universitario de Fuenlabrada, Gastroenterology Unit, Fuenlabrada, Spain

**Background:** Effectiveness of adalimumab (ADA) treatment could be related to the modification of different angiogenic proteins, including VEGFA, Ang1, Ang2 and their receptor Tie2. The aim of our study is to compare the concentrations of several angiogenic factors in colonic mucosa culture supernatant (MCS) in patients with IBD and matched controls and to analyze their modifications with the in vitro ADA addition to the culture.

**Methods:** Prospective study in patients with IBD and non-IBD controls that underwent endoscopy. Duplicates of colonic mucosa samples from affected and non-affected mucosa from each IBD patient were obtained for comparison. Both were washed and then cultured at 37°C in 5% CO2 medium under shaking for 24hrs. In one duplicate, ADA was added up to a final concentration of 10 µg/mL prior to culture. MCS levels in AP were determined by ELISA. Endoscopic ulcerative colitis (UC) and Crohn’s disease (CD) activity was ascertained by Mayo sub-score and SES-CD indexes, respectively.

**Results:** 28 patients with IBD (16 UC, 12 CD) and 21 controls were included. Mean age was 41±16 years, and 61% were women. The mean disease duration was 7±7 years. According to the metastatic process. We hypothesized that lymphatics-targeted therapy may be effective in CAC formation and would...
abate metastasis dissemination by maintaining lymphatic vessel junctions’ integrity.

Methods: The azoxymethane (AOM)/dextran sulfate sodium (DSS) CAC model was used. In addition, colitic mice were orthotopically injected with CT26 colon cancer cells. Modulation of lymphangiogenesis was obtained by systemic inhibition of VEGFR3 or adenosine overexpression of VEGFC. Tumor density and size were measured at the end of the AOM/DSS protocol by visual, endoscopic and histological inspection. In the xenotransplantation model, liver, lung and draining lymphnodes were processed for metastasis quantification by FACS analysis. Whole mounts of colons were stained to analyse area density and dimension of lymphatic vessels (LVs) within peritumoral and tumoral regions. Moreover, VE-cadherin distribution and expression were studied in vivo on LVs and in vitro on Human intestinal lymphatic endothelial cells (HILEC) stimulated with VEGF or with a VEGFR3 inhibitor.

Results: In both the AOM/DSS and the xenotransplantation models, systemic inhibition of VEGFR3 inhibited lymphangiogenesis in peritumoral and tumoral regions, reducing both area density and LVs dimension. In addition, it reduced tumor density and size, together with an abatement of metastatic dissemination, when compared to untreated animals. In contrast, tumor density and growth, including metastasis formation was enhanced by VEGFC, which in turn increased lymphangiogenesis within the same regions. Whole mount staining showed that VEGFC altered VE-cadherin expression both in vivo and in vitro, whereas VEGFR3 inhibitor kept endothelial junction integrity, thus linking the VEGF/VEGFR3 pathway to VE-cadherin-dependent metastasis dissemination.

Conclusions: Our findings demonstrate that VEGF/VEGFR3-dependent lymphangiogenesis plays a key role both in CAC growth and metastasis dissemination. Our study reveals a novel mechanism of control of lymphatic junction integrity which may be a promising target for the treatment of CAC and the associated metastatic process.

P017
The effect of intestinal alkaline phosphatase on intestinal epithelial cells, macrophages and chronic colitis in interleukin-10 deficient mice
C. Lee1*, J. Chun2, S.W. Hwang1, S.J. Kang1, J.P. Im2, J.S. Kim2, 1Healthcare sytem, Gangnamcenter, Seoul national university hospital, Internal medicine, Seoul, Korea, Republic of, 2Seoul National University College of Medicine, Department of Internal Medicine and Liver Research Institute, Seoul, Korea, Republic of

Background: Intestinal alkaline phosphatase (IAP) is an intestinal brush border enzyme that has been shown to function as a gut mucosal defense factor, but its defensive mechanism remains unclear. The aims of this study were to evaluate the effect of IAP on intestinal epithelial cells and macrophages, and on chronic colitis in interleukin-10-deficient (IL-10−/−) mice.

Methods: Human intestinal epithelial cells COLO 205 and peritoneal macrophages from IL-10−/− mice were pretreated with IAP and then stimulated with lipopolysaccharide (LPS). IL-8 secretion from COLO 205 cells and TNF-α, IL-6, IL-12 from peritoneal macrophages were measured by ELISA. Electrophoretic mobility shift assay to assess the DNA binding activity of NF-κB and immunoblotting assay was used to assess IkBα phosphorylation/degradation in COLO 205. For the in vivo study, colitis was induced in IL-10−/− mice with piroxicam, the mice were treated with 100 or 300 units of IAP by oral gavage for 2 weeks. Colitis was quantified by histologic scoring, and the phosphorylation of IkBα in the colonic mucosa was assessed using immunohistochemistry.

Results: IAP significantly inhibited LPS-induced inflammatory cytokine production in both IECs and peritoneal macrophages. IAP also attenuated LPS-induced NF-κB binding activity and IkBα phosphorylation/degradation in IECs. Oral administration of IAP significantly reduced the severity of colitis and down-regulated colitis-induced IkBα phosphorylation in IL-10−/− mice.

Conclusions: IAP may inhibit activation of intestinal epithelial cells and peritoneal macrophages, and attenuates chronic murine colitis. This finding suggests that IAP supplementation is a potential therapeutic option for inflammatory bowel disease.

P018
The anti-inflammatory cytokine IL-10 is an important mediator of the action of the TLR-9 agonist DIMS0150
C. Admyre, P. von Stein, T. Knittel, O. von Stein, A. Zargari*. InDex Pharmaceuticals AB, R&D Department, Stockholm, Sweden

Background: DIMS0150 is an oligonucleotide that acts as a Toll like receptor 9 (TLR-9) agonist and has shown positive effects on response and remission rates in treatment refractory patients with ulcerative colitis. DIMS0150 induces a variety of anti-inflammatory cytokines including IL-10. We have previously shown that DIMS0150 restores the sensitivity of cells to the action of steroids in vitro by monitoring the expression level of steroid response genes such as the CD163 scavenger receptor. To gain further insights into the mechanism of action of DIMS0150 we analyzed whether these sensitizing effects are mediated by the anti-inflammatory cytokine IL-10.

Methods: PBMCs were isolated from buffy coats of patients with ulcerative colitis and moderate to severe disease activity despite steroid treatment. PBMCs were cultured in the presence or absence of dexamethasone, DIMS0150, IL-10 or antibodies directed against the IL-10 receptor. CD163 expression levels were analyzed by cytometric bead array RT-PCR. in cell culture supernatants.

Results: In PBMCs derived from DIMS0150 treatment naive UC patients, CD163 induction by steroids was suppressed compared to healthy controls in vitro. The combined steroid-DIMS0150 treatment in vitro elevated the CD163 expression to the level of healthy controls in a dose dependent manner. The same effect was observed when cells were cultured in the presence of IL-10 but in the absence of DIMS0150 illustrating the known IL-10 steroid sensitizing effect. To elucidate whether the DIMS0150 effect was mediated by IL-10, cells were cultured in the presence of dexamethasone, DIMS0150 and an IL-10 antagonist. In this setting the DIMS0150 sensitizing effect was heavily diminished demonstrating that DIMS0150 mediates its effect through IL-10, which is known to be induced upon DIMS0150 treatment. In accordance with these finding, CD163 levels were restored to the level of healthy controls in PBMCs derived from a UC patient following treatment with a single rectal administration of 30 mg DIMS0150 in vivo.

Conclusions: The effects of TLR-9 activating oligonucleotide DIMS0150 in vitro are mediated by the inflammatory cytokine IL-10. DIMS0150 has shown promising effects in the treatment of patients with ulcerative colitis, which might be mediated through the interleukin-10 pathway in vivo.