ECCO Fellowships & Grants Reports

2012

Jessica Claire Wilson (ECCO Fellowship 2012)

Title of Fellowship: An Epidemiological Study on the Natural History of Patients with Inflammatory Bowel Disease (IBD)
Host institute: University Hospital Basel, Basel, Switzerland
Supervisor: Prof. Christoph Meier

FINAL REPORT:
Achievements of the Project
The project examined whether IBD related factors or treatment therapies were associated with an altered risk of developing extra-intestinal manifestations (EIMs).
Using data from the UK Clinical Practice Research Datalink, incidence rates for a first diagnosis of cancer, pulmonary event, cardiovascular event, autoimmune disease, and serious infection requiring hospitalisation were calculated in IBD and non-IBD patients. A matched case-control analysis comprising IBD patients was conducted. Risk estimates for the above outcomes were calculated in relation to IBD severity, duration, and type and duration of IBD therapy.
IBD patients had an increased incidence for all outcomes. Variation in EIM incidence by IBD type was observed. The risk of all outcomes was significantly increased as disease severity, and for cancer and cardiovascular event risk as IBD duration, increased. Aminosalicylate users had a 26% reduction in cancer risk. Current long term aminosalicylate use slightly reduced the risk of a cardiovascular event and autoimmune disease. Pulmonary event risk was increased with past aminosalicylate use. All Interstitial lung disease/ pulmonary fibrosis cases (n=16) were prescribed aminosalicylates.
The findings confirm previous reports of an increased incidence of EIMs in IBD patients. Results indicated IBD severity as a likely factor in EIM development, and that IBD treatment altered EIM risk. Aminosalicylate use reduced the risk of several EIMs, indicating the potential influence of systemic inflammation in their development. An increased risk of pulmonary events was observed with aminosalicylate use.

Discrepancy with the Initial Aims
Assessment of biologics on EIM risk and analysis by therapy of those with a serious infection requiring hospitalisation could not be achieved due to limited patient numbers in each category.

Publication Plan
One paper on the project is currently under review. Two further papers will be submitted shortly.

Timon Eric Adolph (ECCO Fellowship 2012)

FINAL REPORT:
Title of Fellowship: PhD project: Endoplasmatic reticulum stress and autophagy converge in the NFkB signaling pathway
Host institute: University of Cambridge, Cambridge, United Kingdom
Supervisor: Prof. Arthur Kaser

The discovery of a coding polymorphism in ATG16L1 (T300A) conferring risk for Crohn's disease (CD) 1, now known as one of the three most important genetic risk factors of the disease 2, uncovered that autophagy is somehow involved in the pathogenesis of CD. Several additional genetic risk factors of CD are associated with autophagy, including the risk gene with the strongest effect size, NOD2 3-5. Autophagy is a cellular catabolic process in which intracellular macromolecular structures, including proteins and their complexes, organelles or invading bacteria are processed for degradation 5,6. However, it remained largely elusive how autophagy defects lead to overt inflammation 1. The unfolded protein response (UPR) is another cellular homeostatic process that is initiated to compensate for protein misfolding in the endoplasmic reticulum (ER) and thereby allows cells to cope with ER stress that arises from such misfolding 7. ER stress frequently occurs in the intestinal epithelium of inflammatory bowel disease (IBD) patients although the genetic and environmental origin of this observation is incompletely understood 8,9. We aimed at determining the interaction between the UPR and autophagy in intestinal epithelial cells (IECs), and particularly Paneth cells, as we hypothesized that ATG16L1 and autophagy would protect against ER stress-induced intestinal inflammation.

In our study entitled "Paneth cells as a site of origin for intestinal inflammation" we identified a compensatory bi-directional crosstalk between ER stress and autophagy in Paneth cells 10. We discovered that ER stress converts otherwise innocuous autophagy defects, modelled by IEC-specific Atg16l1 deletion, into overt spontaneous ileitis that remarkably closely phenocopies human small intestinal Crohn's disease. ER stress-induced intestinal inflammation was driven by IRE1-mediated NF-!B activity, a process which was tightly controlled by autophagy. Finally, we established that spontaneous intestinal inflammation can solely arise from ER stress specifically in Paneth cells, providing direct evidence for the delicate role of this cell type in mammalian gut homeostasis 10. As carriers of the T300A variant exhibit ER stress specifically in Paneth cells in active and inactive CD patients, and, if present in homozygous form, also in healthy individuals 9, the mechanism reported in our paper may provide the first credible model how genetic risk in an autophagy gene may lead to disease.

A recent paper has revealed the specific mechanism whereby the ATG16L1 T300A variant results in hypomorphic autophagy. Specifically, the ATG16L1T300A protein is prone to caspase-3-mediated cleavage and hence degradation following death receptor signalling, intracellular infection, or metabolic stress 11. This means that only under these conditions, the highly prevalent (>50% of the normal population) ATG16L1T300A variant becomes manifest as blunted capacity to induce autophagy 11. ER stress serves as such caspase-3-inducing cellular stress mechanism 8, hence is predicted to prompt

the degradation of ATG16L1T300A. Altogether, our model 10 provides an intriguing, and to our knowledge the first, framework for understanding the pathogenic mechanisms arising from autophagy gene – environment interaction that lead to Crohn's disease.

We are grateful for the ECCO fellowship awarded to Timon Erik Adolph. The work on this project performed in the Kaser laboratory has been supported by a grant from the European Research Council (ERC) and additional grants.

Johan Burisch (ECCO Grant 2012)

Title of Grant: New Inception cohort in Europe: Is there an east-west-gradient in IBD?
Institute: Herlev University Hospital, Herlev, Denmark

FINAL REPORT:
Achievements of the project
The aim of this thesis was to create a prospective European population-based inception cohort of incident IBD patients in order to investigate whether an East-West gradient in the incidence of IBD exists in Europe. Furthermore, we investigated possible differences throughout Europe during the first year subsequent to diagnosis in terms of clinical presentation, disease outcome, treatment choices, frequency of environmental risk factors, as well as patient-reported health-related quality of life (HRQoL) and quality of care (QoC). Finally, we assessed resource utilization during the initial year of disease in both geographic regions.

A total number of 31 centres from 14 Western and 8 Eastern European countries covering a total background population of approximately 10.1 million participated in this study. During the inclusion period from 1 January to 31 December 2010 a total number of 1,515 patients aged 15 years or older were included in the cohort. Annual incidence rates were twice as high in Western Europe (CD: 6.3/100,000; UC: 9.8/100,000) compared to Eastern Europe (CD: 3.3/100,000; UC: 4.6/100,000), thus confirming a gradient in IBD incidence. The incidence gradient could not be explained by marked differences in environmental factors prior to IBD diagnosis. In fact, Eastern European patients had higher frequencies of dietary risk factors than Western European patients, while the remaining risk factors occurred just as frequently. Furthermore, the availability of diagnostic tools and the diagnostic strategy did not differ, and in fact was better in Eastern Europe in terms of the use of colonoscopies and diagnostic delay.

In terms of socio-economic characteristics as well as clinical presentation at diagnosis Eastern and Western European IBD patients did not differ significantly. However, regarding treatment choices during the initial year of disease the use of biological therapy was significantly higher in Western Europe for both CD and UC, while Eastern European centres used 5-ASA more often in CD and UC. In both regions patients were treated earlier and more frequently with immunomodulators compared to previous cohorts. But despite these differences in treatment, disease course – including hospitalisation and surgery rates during the first year of disease – were similar in both regions and the majority of

patients were in clinical remission at follow-up. Finally, generic and disease-specific HRQoL improved in all IBD patients and at twelve months follow-up the majority of patients had a good disease-specific HRQoL score.

Differences in how, and from whom, patients received disease-specific education and information were noted between the geographic regions; for instance IBD specialist nurses were not used in Eastern European IBD centres. Expenses for the cohort during the initial year of disease exceeded four million Euros with most money spent on diagnostics and surgery. Biological therapy accounted for one fourth costs in Western European CD patients.

Discrepancy with the initial aims
None

Future perspectives
Follow-up 2010-2015 is currently in progress and will form the basis of Johan Burisch MDSci thesis.

Publications:
The project has so far resulted in the following publications which formed the basis for Johan Burisch PhD thesis:

- Burisch J, Pedersen N, Cukovic-Cavka S, et al. Environmental factors in a population-based inception cohort of inflammatory bowel disease patients in Europe - An ECCO-EpiCom study. J Crohns Colitis 2013;:in press. doi:10.1016/j.crohns.2013.11.021
- Burisch J, Pedersen N, Cukovic-Cavka S, et al. East-West gradient in the incidence of inflammatory bowel disease in Europe: the ECCO-EpiCom inception cohort. Gut 2014;63:588–97. doi:10.1136/gutjnl-2013-304636
- Burisch J, Vegh Z, Pedersen N, et al. Health care and patients' education in a European inflammatory bowel disease inception cohort: An ECCO-EpiCom study. J Crohns Colitis 2014;:in press. doi:10.1016/j.crohns.2013.12.023
- Burisch J, Weimers P, Pedersen N, et al. Health-related quality of life improves during one year of medical and surgical treatment in a European population-based inception cohort of patients with Inflammatory Bowel Disease - An ECCO-EpiCom study. J Crohns Colitis 2014;:in press. doi:10.1016/j.crohns.2014.01.028
- Burisch J. Crohn's disease and ulcerative colitis. Occurrence, course and prognosis during the first year of disease in a European population-based inception cohort. Dan Med J 2014;61:B4778.
- Burisch J, Pedersen N, Cukovic-Cavka S, et al. Initial Disease Course and Treatment in an Inflammatory Bowel Disease Inception Cohort in Europe: The ECCO-EpiCom Cohort. Inflamm Bowel Dis 2014;20:36–46. doi:10.1097/01.MIB.0000436277.13917.c4
- Burisch J, Cukovic-Cavka S, Kaimakliotis I, et al. Construction and validation of a web-based epidemiological database for inflammatory bowel diseases in Europe An EpiCom study. J Crohns Colitis 2011;5:342–9. doi:10.1016/j.crohns.2011.02.016
- Burisch J, Vardi H, Pedersen N, et al. Costs and resource utilization during the initial year after diagnosis in a European inflammatory bowel disease inception cohort – An ECCO-EpiCom study. Submitted 2014

Tim Raine (ECCO Grant 2012)

Title of Grant: Immunophenotyping of atypical lymphocytes in human bowel in the context of genetic risk for IBD
Institute: Addenbrooke's Hospital, Division of Gastroenterology and Hepatology, Department of Medicine, University of Cambridge, United Kingdom

FINAL REPORT:
Genome-wide association studies (GWAS) have identified genetic variants within multiple risk loci as predisposing to common immune mediated diseases. The immune response underlying many such diseases may be shaped within the environment of the intestinal mucosa. Most risk variants act through effects on transcriptional regulation in a highly cell-type specific manner, but a critical challenge in translating genetic data into functional insight is to identify which genes and which cell types these variants affect. Through work supported through this ECCO grant, we isolated the four major T cell populations of the intestine from pinch biopsies obtained from healthy human subjects, and used these to generate the first high quality transcriptomes for each subset of these poorly understood immunocytes. Using strictly defined anatomic, physiological and pathological criteria, we were able successfully to minimise the inter-individual variability that often confounds human studies, while an optimized experimental workflow, precise polychromatic flow cytometric sorting, and robust computational analysis further increased data reliability. By subjecting these transcriptomes to in silico analysis, we generated insight into activity within these cell populations through analysis of genes showing differential expression between intestinal and peripheral blood T cells. Comparing these transcriptomes and integrating GWAS data we developed a novel approach to the prioritisation of GWAS positional candidate genes. We identified selected genes, some known to contribute to intestinal immune homeostasis and others unexpected, with intestinal T cell subset-specific enrichment of these transcripts within GWAS risk loci for intestinal and specific non-intestinal immune mediated diseases. These data implicate intestinal T lymphocytes, or related cells with shared transcriptional regulatory mechanisms, in susceptibility to these disorders.

Publication plan: A manuscript describing this work is currently under revision at major journal in the field.

Colin de Haar (ECCO Grant 2012)

Title of Grant: Is defective resolution of inflammation involved in IBD pathogenesis?
Institute: Erasmus MC, Dept. of Gastroenterology and Hepatology, Rotterdam, The Netherlands

SYNOPSIS:
The etiology of IBD is still not clear; this is probably because there are different subtypes of Crohn's disease or Ulcerative Colitis that, although they may have a resembling disease phenotype, can have a completely different etiology. A lot of research over the last few decades has focused on the immune response against

intestinal (invading) microbes. This response could be either to weak to keep the microbes at bay, or too strong causing a lot of collateral damage during the clearance process, both of which will lead to chronic intestinal inflammation.
In this project we will investigate yet another important part of the immune response: resolution. Resolution of inflammation is an important anti-inflammatory process, and impairment of this process is suggested to result in persistent inflammation that could lead to autoimmune disease. As such, we will investigate whether IBD patients have a defect in the resolution process called efferocytosis. This process involves of the uptake of apoptotic cells, by phagocytes, and the subsequent induction of a tolerogenic phenotype in these phagocytes. The uptake of the apoptotic cells prevents the potential spreading of pathogens that are able to survive in the apoptotic cells, whereas this tolerogenic phenotype of the phagocytes prevents further activation of the immune response. In this project we will look at the expression of factors on the apoptotic cells (neutrophils) as well as the phagocytes (monocyte-derived macrophages) that ensure the proper uptake of the apoptotic cells. In addition, we will functionally test the efferocytosis by means of the apoptotic cell-uptake and the subsequent induction of the tolerogenic phenotype in the phagocytes. This study will show if a subset of IBD patients suffers from defective efferocytosis, probably leading to poor resolution of inflammation. Details on where these defects are located will provide new interesting therapeutic targets for this subset of IBD patients.

Marc Ferrante (ECCO Grant 2012)

Title of Grant: Influence of microbiota on intestinal stem cell behaviour and differentiation in patients with inflammatory bowel diseases
Institute: University Hospital Gasthuisberg, Department of Gastroenterology, Leuven, Belgium

SYNOPSIS:
The exact pathogenesis of Crohn's disease (CD) remains incompletely understood, but a loss of tolerance to normal gut microbiota seems crucial. Interactions between host and microbes take place at the intestinal epithelial surface which exists of enterocytes, goblet cells, entero-endocrine cells and Paneth cells. All these cells arise from crypt-based intestinal stem cells (ISC) and are constantly renewed. The impact of bacteria on ISC behaviour and differentiation has not been explored yet, due to the lack of good long-term intestinal culture models. The recently developed 3 dimensional human intestinal organoid system, may serve as an ideal model to study this impact (Sato, Stange, Ferrante, et al Gastroenterology 2011).

We hypothesize that ISC from CD patients and controls behave differently after stimulation with luminal microbiota leading to an altered differentiation into progeny and altered release of anti-microbial peptides by Paneth cells.
Intestinal stem cell behaviour will be compared between tissue samples obtained from CD patients (active or inactive disease) and controls, by evaluating specific markers of ISC and their progeny. Furthermore, these markers will be evaluated in a human intestinal long-term culture. Starting from biopsy samples, crypts will be isolated and plated in a laminin-rich matrigel. Under optimal culture

conditions, these crypts undergo multiple crypt fission events, while simultaneously generating villus-like epithelial domains in which all differentiated cell types are present.

Subsequently, human organoids will be challenged with bacterial-derived compounds (e.g. muramyl dipeptide and lipopolysaccharide) as well as complete microbiota such as the pro-inflammatory Escherichia coli and the anti-inflammatory Faecalibacterium prausnitzii. We will evaluate the inflammatory response after these challenges, as well the impact on stem cell behaviour and differentiation, and organoid barrier function. Findings will be confirmed in co-cultures with dendritic cells and T-cells sorted by FACS analysis.

Harry Sokol (ECCO Grant 2012)

Title of Grant: Role of Card9 in IBD pathogenesis and intestinal homeostasis
Institute: Gastroenterology department, Saint Antoine hospital & Faculté de Médecine Saint Antoine, Micro-organismes, molécules bioactives et physiopathologie intestinale, Paris, France

SYNOPSIS:
A deviation of the gut microbiota composition called dysbiosis has been pointed out in Inflammatory Bowel Diseases (IBD). Concomitantly, genome wide association studies have identified several susceptibility loci in genes involved in the interactions with microorganisms. Polymorphism in the gene encoding Card9 (Caspase Recruitment Domain 9) has been associated with Crohn's disease (CD) and ulcerative colitis (UC). Card9 is an adapter protein playing a central role for the integration of signals downstream of pattern recognition receptors. However, its role in the gastrointestinal tract, notably regarding the intestinal microorganisms, has not been investigated yet. Card9 is highly expressed in macrophages and dendritic cells. Card9 plays a major role in the sensing of fungi via several C-type lectins and is also involved in the innate immunity toward bacteria and virus. Card9 is thus a key adapter protein for innate immunity toward a wide range of microorganisms including many intestinal commensals and pathogens. We hypothesized that Card9 might play a role in IBD pathogenesis and more widely in shaping intestinal immunity.
The aim of the current proposal is to decipher the role of Card9 in IBD pathogenesis and in intestinal homeostasis. To address this question, we will use Card9 KO mice. In preliminary data, Card9 KO mice have an impaired intestinal IL17A and IFN□□response at baseline and in the context of DSS and Citrobacter rodentium induced colitis. These preliminary results, coupled with the association of Card9 polymorphism with CD and UC, confirm the importance of Card9 in intestinal immunity. We hypothesize that Card9-dependant microbiota sensing, and notably fungi sensing is involved in shaping the intestinal immunity.
While most of the studies performed on the intestinal microbiota so far focused on bacteria, we will extend the investigation toward fungi microbiota using in vitro experiments, in vivo mouse models and samples from IBD patients.

2011

Lael Werner (ECCO Fellowship 2011)

Title of Fellowship: Linking TNFα inhibitors and Notch-1: Novel implications in inflammatory bowel diseases
Host institute: Charité, Campus Virchow-Klinikum, Berlin, Germany
Supervisor: Prof. Andreas Sturm

FINAL REPORT:
What was already known about this subject?

- Inflammatory bowel diseases are chronic inflammations postulated to be caused by a deregulated mucosal T cell function.
- TNFα inhibitors are efficient biological therapies in IBD, although much is lacking in understanding their physiological mode of action.
- Reciprocal regulation of TNFα and Notch pathways has been reported in a number of scientific articles, however their involvement in IBD, and interaction is unknown.

What are our new findings?

- TNFα inhibitors do not only induce T cell apoptosis, but also potently inhibit activation and cell cycling of T cells.
- TNFα is responsible for inducing apoptosis by anti-TNFα, but not for cell-cycle restriction.
- By connecting Notch and TNFα signaling, we show that Notch-1 is activated by TNFα inhibitors, Notch-1 binds to TNFα, and Notch-1 inhibition averts anti-TNFα-induced T cell-cycle arrest but not apoptosis.
- Notch-1 mucosal expression differs in inflamed and non-inflamed mucosa and expression of Notch-1 increases in vivo after treatment with TNFα inhibitors.

How might our project impact on clinical practice in the foreseeable future?

- Our report points to Notch as a novel player in IBD, hopefully paving the path to a novel therapeutic approach in IBD.
- As our study substantially expands current knowledge on TNFα inhibitors, this could help better selection of IBD patients for TNFα inhibitors treatment.

Bénédicte Brounais-Le Royer (ECCO Fellowship 2011)

Title of Fellowship: Effects of interleukin-15 inhibition on skeletal alterations in Inflammatory Bowel Disease
Host institute: Geneva University Hospital, Geneva, Switzerland
Supervisor: Dr. Serge Ferrari

FINAL REPORT:
Inflammatory bowel diseases (IBD) are commonly associated with bone loss. We

hypothesized that interleukin (IL)-15, a pro-inflammatory cytokine highly expressed in colitis and an osteoclastogenic factor, could play a central role in the skeletal complications of IBD. The aims of this work were to investigate the effects of IL-15 inhibition on colitis-associated bone loss, the role of IL-15 activated T cells on skeletal alterations and the role of IL-15 on osteoblasts. Thus, we evaluated the effects of an IL-15 antagonist, CRB-15, in the model of DSS-induced colitis and in IL-10 deficient (IL-10KO) mice.

First, we demonstrated that CRB-15 improved survival, early weight loss and the colitis clinical score in DSS-treated mice. CRB-15 also delayed bone loss preventing the suppression of osteoblastic markers of bone formation and reducing osteoclast progenitors but only transiently. However, after two weeks of treatment, CRB-15 decreased TNF! and increased IL-10 expression in bone, paralleling a reduction of osteoclasts.

Similarly, IL-15 inhibition decreased the severity of colitis and improves colon inflammation in IL-10KO mice. Moreover, CRB-15 prevented skeletal alterations and restored bone turnover increasing both bone formation by osteoblasts and as a consequence bone resorption by osteoclasts. In comparison, RANK-Fc strongly increased bone mass but was not able to restore formation. Finally, we demonstrated that IL-15 modulated osteoblast differentiation through T cells.

Together, these observations delineate the role of IL-15 on the systemic and skeletal manifestations of chronic colitis and provide a proof-of-concept for future therapeutic developments. However, this work did not allow to completely elucidate the role of IL-15 activated T cells on colitis-associated bone loss and to identify mediators produced by IL-15-stimulated T cells and responsible for inhibition of osteoblastic differentiation.

The results obtained in the model of DSS-induced colitis were published in the American Journal of Pathology on June 2013. The second manuscript concerning the effects of CRB-15 in IL-10KO mice is in preparation and will be submitted for publication before September.

Stefania Vetrano (ECCO Grant 2011)

Title of Grant: The role of Chemerin /ChemR23 axis in the pathogenesis of IBD
Institute: Istituto Clinico Humanitas, Milan, Italy

FINAL REPORT:
Chemerin is a chemoattractant protein for ChemR23-expressing cells such as dendritic cells, macrophages and natural killer cells, involved in the initiation of inflammatory responses. Large amounts of chemerin were found in various inflammatory conditions including IBD suggesting a possible involvement of chemerin in IBD, but currently there are no studies describing the link between chemerin/ChemR23 axis and intestinal inflammation. The aim of this study was to investigate the involvement of chemerin/ChemR23 axis in the pathogenesis of IBD.

Methods: Chemerin and ChemR23 expression were studied by confocal microscopy in colonic sections from 15 controls, 14 Crohn's disease (CD), and 11 ulcerative (UC) subjects. Primary epithelial cells (EC) were isolated from 10 fresh human colon tissues of normal subjects and active IBD patients and the levels of chemerin and ChemR23 mRNA were quantified by RT-PCR analysis. WT and ChemR23 knockout mice were followed for susceptibility in developing DSS-induced colitis. Colitis was induced by administration of 3% DSS ad libitum in drinking water for 12 days. Mice were monitored daily for weight loss, fecal blood and diarrhea and a disease activity index (DAI) was calculated. The damage of murine colonic mucosa was evaluated by endoscopic and histological scores.

Results: Chemerin was expressed in the normal intestinal mucosa mainly by epithelial cells and its expression was up-regulated in active IBD ($p<0,05$). On the contrary ChemR23 expression levels were low on the normal epithelial layer and higher in the cells from the lamina propria as assessed by confocal analysis and flow cytometry. Under inflammation the number of ChemR23-expressing cells increased in the inflamed intestinal mucosa compared to non-inflamed areas ($p<0,05$) . ChemR23 knockout mice developed a more pronounced colitis than WT mice, as assessed by weight loss ($p<0.05$), DAI ($p<0.05$), colon length, histological and endoscopic scores (all $p<0.05$), while no differences between two groups were observed at baseline before DSS treatment. The experimental colitis was repeated several times in ChemR23 deficient mice obtaining always the same results. 7 animals were included in each group for one experiment and 4 independent sets of experiments were performed.

Conclusions : These data showed that intestinal epithelial cells producing chemerin participate in the recruitment of ChemR23-expressing cells in the mucosa exacerbating the inflammatory response. The inhibition of chemerin activity therefore could useful in reducing inflammatory process and its manipulation could be representing a novel therapeutic approach for the treatment of IBD. On the other hand the loss of ChemR23 function worsens the intestinal inflammation suggesting the important role of ChemR23-expressing cells in the resolution of inflammation. Taken together these results demonstrated that chemerin/ChemR23 axis is involved in both the initiation and resolution of inflammation and could be considered a new potential player in IBD pathogenesis.

Franco Scaldaferri (ECCO Grant 2011)

Title of Grant: Adipose tissue-derived mesenchymal stem cell ameliorates chronic DSS colitis in mice and does not affect colonic cancer formation
Institute: Institute of Internal Medicine, Catholic University of Rome, Rome, Italy

FINAL REPORT:
The employ of mesenchymal stem cells in treatment of IBD is becoming a reality. Solid data, are in fact sustaining the strong immune-modulatory activity of these cells, in vitro, in animal models of immune mediated disorders like IBD and also in vivo. Sources of employed MSC vary from bone marrow, to adipose tissue to other sources, being the one from adipose tissue, the more intriguing and

versatile, because of their easier availability and modality of isolation. Thanks to Ecco grant support, I had the opportunity to confirm preliminary data of my application and to go deeper in mechanisms, so that my work could be suitable for publication, following few national meetings where I had the opportunity to present them and acknowledge Ecco.
In this project I explored the effect of adipose tissue derived stem cells (AMSC) in controlling acute and chronic colitis in mice and in affecting colonic cancer on chronic colitis. AMSC were isolated following standardized protocols from GFP expressing C57BL6 mice (Charles River, USA), to ensure traceability. They were grown in mesenchymal stem cells media (Stem Cell technologies, USA) and characterized by flow cytometry for expression of SCA-1, CD44 and CD106 and for being negative for CD31, CD34, Cell Lineage Cocktail and Tion cKIT. AMSC were used until the XII passage, as these expressions remained stable.
As expected, in DSS acute model of colitis on C57BL6 mice, AMSC clearly showed a statistically significant capacity to contrast weight loss and increase in disease activity compared to controls, particularly at day VI, VII, VIII ($p < 0.5$). This effect was dose dependent, although no statistical differences were found using 1 million cells one time infusion at day 3 or 3 million cells.
Having demonstrated a clinical efficacy of AMSC in acute model of colitis, we then assessed efficacy and colonic cancer modulation using azoxymethane-DSS (AOM) chronic model of colonic cancer associated to chronic colitis in C57BL6 mice. Briefly mice received an intra-peritoneal injection of AOM (10 mg/Kg) 7 days before starting 3 cycles of DSS at 2% in drinking water per 7 days, intervalled by 2 weeks of rest, when mice were exposed to regular water. AMSC treated mice, received 1 million of AMSC ip in 200 ul of buffer saline solution, every 3rd day of DSS administration, while controls received same amount of saline. Weight and disease activity were monitored 3 days a week. At sacrifice, count of macroscopic lesions was then followed by colonic sample processing for histology. AMSC-treated animals showed a lower disease activity index as well as a lower reduction in body weight compared to controls.
Incidence of colonic tumors in our facility was comparable to what expected, running at 70 % of animals in control groups, with tumor interesting the left portion of the colon and within the rectum, with a mean number of colonic lesions per mouse of around 3-4 macroscopic lesions. AMSC treated mice showed fewer tumors than controls, although this difference was not statistically significant. Microscopic analysis of the animals treated with AMSC showed fewer tumor lesions compared to controls, with an average of 2.5 tumors per animal treated, compared to about 3.5 tumors per animal control. The differences were not statistically significant ($p > 0.5$).
Finally, the presence of AMSC GFP positive within the tumor lesions was not found. We then examined the role of AMSC in modulating tumor growth and progression in nude mice from a human (HCT116) and murine (CT26) intestinal epithelial tumor cell line. To do so, we injected 5 million HCT116 or CT26, in the 4 sites in the back of 6 weeks old nude mice (Charles Rivers, USA), with or without AMSC in a ratio of 10:1 and 100:1. AMSC alone and HCT116/CT26 alone at same concentration served as a control. AMSC did not significantly change tumor size over time, but actually slightly reduced tumor growth, particular when used at lower concentrations.
Our data strongly support the findings that AMSC positively modulate acute and chronic colitis in animal model and this effect does not seem to be dependent on AMSC recruitment in intestinal mucosa.

This finding is particular important as it represent one of the first attempt to assess colonic-cancer related safety issues in a therapy which is becoming a reality in treatment of IBD. Of note is that IBD per se is a condition associated to colonic cancer development, so, providing this data for new therapeutic interventions should be mandatory. Major limitation of our paper is that the origin of AMSC tested is murine, so a direct translation to cells of human origin needs caution.

Arie Levine (ECCO Grant 2011)

Title of Grant: GROWTH Study: Factors predicting relapse and adverse outcomes early in the disease in newly diagnosed pediatric Crohn's disease - a prospective, multi-center prognostication study by The ESPGHAN Porto group
Institute: Wolfson Medical Center, Holon, Israel

PROGRESS REPORT:
We would like to report the progress of the GROWTH study. Our goal of the study was to prospectively enroll 300 patients and to analyze the role of silent inflammation on the outcomes and to be able to predict the outcomes of the disease from the first attack.
We are glad to report that we have reached our desired goal of enrolling 300 patients foe the primary endpoint. The study requires a follow up of two years (or a minimum of 18 months) after enrollment in order to define adverse outcomes. We have preformed an interim study in 222 patients to define the outcomes of induction of remission by exclusive enteral nutritional and medical therapy in new onset pediatric Crohn's disease (the abstract of article is attached to this e-mail). The article has been submitted to the American journal of Gastroenterology in February 2013. We have used funds provided by the ECCO grant to collect and provide the analysis of the data for the study. The next milestone of the study will be to evaluate factors that cause growth retardation. This data should be available in July 2013. The data for the final study to predict relapse will only be available once all the patients will complete the follow up (estimated date is December 2014).

Outcomes of Induction of Remission by Exclusive Enteral Nutritional and Medical Therapy in New Onset Pediatric Crohn's Disease: Evaluation of the Porto IBD Group "Growth Relapse and Outcomes With Therapy" (GROWTH CD) Cohort Study
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This study is supported by grants from the European Crohn's Colitis Organization (ECCO) and the A.L Thrasher Foundation.

Introduction: Robust real-life evaluation of induction therapies in pediatric Crohn's disease (CD) is sparse. We attempted to evaluate clinical, inflammatory and composite outcomes of induction of remission therapies in a large pediatric prospective multicenter study.
Methods: Patients enrolled at diagnosis into the GROWTH CD study, were evaluated for disease activity, CRP, and fecal calprotectin at 8 and 12 week after starting treatment. The primary end point was week 12 steroid free- remission defined by PCDAI (<10 points or <7.5 without growth item) and CRP<0.5mg/dL. The protocol required tapering off corticosteroids (CS) by week 11.
Results: We analyzed 222 patients at disease onset, (mean age 12.9±3.2 years; 65% - mild, to moderate and 32% severe disease). Main evaluated treatment options included: 5-ASA, exclusive enteral nutrition (EEN n=43) and CS (n=114). CS treated patients had more severe disease. Clinical remission at week 12 was achieved in 155 (73%) patients; however CS free remission with normal CRP was present in only 33%. Steroids and EEN were equivalent for remission, although CS-treated children had lower week 12 calprotectin levels. In a post-hoc analysis of a subgroup of patients with mild-moderate disease, corrected for disease severity and immunomodulators, EEN was superior to CS for inducing CS free remission (OR 5.8 (95%CI 1.8-18.3) and composite normal PCDAI and CRP remission (OR 3.4 (95% CI 1.3-9).
Conclusions: Clinical remission rates were high with all therapies; however normal CRP and low calprotectin corticosteroid free remission are obtained infrequently at week 12.
Study Highlights: Outcomes of efficacy to measure successful remission in IBD studies to date are clinical and not inflammatory
*Complications of the disease are due to inflammation and not symptoms, abnormal CRP at remission predicts relapse
*This study demonstrates that only about a third of children treated at IBD Centers achieve combined clinical remission and normal CRP at first remission
*Factors predicting normal CRP remission are older age and milder disease
* Using a propensity score we demonstrate that Exclusive enteral nutrition is superior to corticosteroids for steroid free remission and normal CRP remission at diagnosis in mild to moderate disease

FC, fecal calprotectin; EEN, exclusive enteral nutrition; PCDAI, Pediatric Crohn's Disease Activity Index; NCR, Normal CRP Remission; CS, corticosteroids

Catherine Reenaers (ECCO Grant 2011)

Title of Grant: Investigation of autophagy pathway defects in innate immune cells in Crohn's disease
Institute: CHU Liege, Liege, Belgium

Report pending

Daniela Petrova Stoyanova (ECCO Travel Award 2011)

Title of Travel Award: Complex approach to patients with complicated course of inflammatory bowel disease; Noninvasive methods for follow-up of postoperative Crohn's disease
Host institute: Western General Hospital, Edinburgh, United Kingdom
Supervisor: Dr. Charlie Lees

FINAL REPORT:
During the summer of 2011 I faced the great challenge to spend 3 months in the Western General Hospital, Edinburgh as a clinical observer under the supervision of Dr. Charlie Lees and the team of GI consultants headed by Prof.Jack Satsangi.
The essential part of my stay included daily work in the GI wards, visits to outpatient clinic, endoscopy department, virtual nurse led clinic and multidisciplinary meetings as well as plenty of hours spent in the medical archive. I was really impressed by the range and quality of medical care for so many IBD patients and I carefully followed each step in diagnosing, assessing and treating the disease.
The scientific part of my stay was highlighted by the anti TNF audit aiming to explore the safety and efficacy of these drugs in WGH's ten years of clinical experience. The results of this project hopefully would be submitted in the literature soon. The survey of patients' files showed in detail the variety of manouevres to handle the most complicated cases of IBD by the joint team of gastroenterologists, surgeons, dietitians, phychologists, radiologists and pathologists.
The most important takeaway message for me from this stay emerged in the discussions with my supervisors – be brave to undertake new therapeutic strategies, but always put patient's safety first!
In conclusion I highly recommend WGH as a great place to share your IBD experience with experts! Moreover – who knows – you may catch a glimpse of the next royal wedding in the lovely town of Edinburgh….

2010

Emanuela Sala (ECCO Fellowship 2010)

Title of Fellowship: Molecular determinations of homing of stem cells in IBD

Host institute: IDIBAPS - Institut D'Investigacions Biomediques, Barcelona, Spain
Supervisor: Dr. Miquel Sans

FINAL REPORT:
The first task of this year of investigation was to isolate a pure population of mesenchymal stem cells (MSC) from C57Bl/6 wild type (WT) mice. In order to do this we set up an isolation protocol based on multicolor flow cytometric cell sorting. Briefly, whole bone marrow cells were negatively sorted for CD31, and Lineage cell detection cocktail and positively sorted for Sca-1. These sorted cells have been tested *in vitro* for the expression of the most common mesenchymal stem cells surface markers and for their differentiation potential. In accordance to literature, our MSC culture expressed high levels of Sca-1, CD44 and CD106, while completely negative for the hematopoietic surface markers CD45, CD34, Lineage cell detection cocktail and c-Kit, for the endothelial surface marker CD31 and negative for CD90. Furthermore, our MSC culture generated both osteoblasts and adipocytes under standard *in vitro* differentiating conditions. After sorting MSC maintained *in vitro* an undifferentiated state until at passage 12, therefore the cells have been used at passage 7- 8 in subsequent experiments.
The second task was to investigate the therapeutic efficacy of MSC in the treatment of acute DSS- induced colitis, which was induced in C57Bl/6 WT mice by administration of 3% DSS in drinking water *ad libitum* for 10 days. MSC ($3x10^6$ cells) were injected within the peritoneal cavity of mice at day 5 of DSS treatment. MSC administration significantly ameliorated acute DSS-induced colitis, in terms of weight loss, DAI, colon shortening, endoscopic and histological scores.
The third task of this project was to explore of the mechanisms through which these cells exert their therapeutic efficacy in the treatment of acute DSS-induced colitis. Our working hypothesis was that MSC via ip specifically migrate towards inflamed tissues where they contribute to the regeneration of damaged mucosa and mitigate the inflammatory response thanks to their immunomodulatory properties. To test this hypothesis, we injected $3x10^6$ of GFP-MSC in the peritoneal cavity of healthy and colitic mice and we evaluated their presence in the colon and mesentheric lymph nodes at different time points. Interestingly, in colitic mice a small fraction (<1%) of GFP-MSC was detected, with a peak value after 48h of injection, selectively in inflamed tissues including colon and mesentheric lymph nodes, while no GFP-MSC were found in not inflamed tissues. As a result of such a low percentage, we postulated that MSC therapeutic efficacy in the treatment of acute DSS-induced colitis could be independent from their homing capability to the inflamed gut. To remove any doubt, we encapsulated MSC into alginate based microcapsules which prevent cells from escaping but are completely permeable to soluble factors. We implanted $3x10^6$ of encapsulated MSC into the peritoneal cavity of colitic mice and when we compared the therapeutic efficacy of free MSC and encapsulated MSCs, no significant difference was observed between the two groups, neither in term of clinical (body weight and DAI) nor histological parameters or survival. Taking into consideration the above results we asked ourselves where free MSC administered via ip localize after the injection. We were finally successful in demonstrating that free MSC injected via ip remain in the peritoneal cavity where they aggregate along with macrophages and lymphocytes generating organized "structures". Whether these

"structures" exert a functional role in providing MSC therapeutic efficacy needs further clarification.
Finally, we investigated the contribution of the route of administration in providing MSC therapeutic efficacy in the treatment of acute DSS-induced colitis. Our experiments demonstrate that the same amount of MSC administered both intraperitoneally and subcutaneously ameliorate DSS-induced colitis, while the intravenous administration showed no efficacy.
Further efforts are now necessary to unravel the mechanism(s) through which these cells are efficient in treating DSS-induced colitis in order to optimize the therapy.

Caterina Strisciuglio (ECCO Fellowship 2010)

Title of Fellowship: Autophagy in immune cell-cell interactions in the gut
Host institute: Leiden University Medical Center, Leiden, The Netherlands
Supervisor: Prof. Daan Hommes

FINAL REPORT:
Over the last few years, a large number of studies have analyzed associations between Crohn disease (CD) and the occurrence of single nucleotide polymorphisms (SNP). Among the genes that have been implicated by these studies are ATG16L1 and IRGM, both of which are involved in a process termed autophagy. We wanted to understand the link between decreased autophagy and the pathogenesis of CD., We focused in particular on the role of autophagy in the regulation of
dendritic cells (DC), which are important players in the induction of tolerance and are abundantly present in the intestine. In our previous work we have shown that a lack of autophagy results in a pro-inflammatory phenotype of DC and that it alters their interaction with the surrounding cells. Indeed, we have demonstrated that dendritic cells in which the autophagy pathway was inhibited, either by siRNA or using the pharmacological inhibitor 3-methyladenine (3-MA), show an increased interaction time and strength with T cells. Since the strength of this interaction is related to the strength of the T cell activating signal , this likely contributes to the increased immunogenicity of these dendritic cells .
In project conducted last year we hypothesized that autophagy also plays a role in regulating the interactions between DC and epithelial cells and thereby in the induction of immune tolerance in the gut. DC-epithelial cell interactions are characterized by the presence of tight junction proteins (TJ), which are present on both type of cells. Lamina propria DC sample luminal antigens by opening the TJs between epithelial cells and protruding their dendrites between the cells, while forming new tight junctions and maintaining epithelial barrier function. This mechanisms allows DC to take up luminal antigens under non-inflammatory conditions, resulting in the induction of tolerance. Among the TJs proteins complex we have focused on E-cadherin, which has recently been shown to be related to inflammatory DC .We found that autophagy is involved in regulating E-cadherin levels, as the E-cadherin molecule (partly) localizes to autophagosomes and stimulation of autophagy by rapamycin decreases E-cadherin expression. This means, that a defect in autophagy, might alter tight junction formation, and thus DC-epitheleal cell interactions. To test this hypothesis further, we inhibited autophagy in either DC or epithelial cells, or both and co-cultered these cells in

an in vitro model system for luminal sampling. In line with our hypothesis, we found that decreased autophagy in either cell type resulted in the decreased formation of transepithalial protrusions and a decrease in uptake of luminal antigen. Furthermore, the DC recovered from these cultured showed a more pro-inflammatory phenotype, with higher expression of co-stimulatory markers and a pro-inflammatory cytokine profile. This data indeed indicates a decrease in tolerogenic antigen processing. We are currently writing the manuscript of these data

The data on DC and epithelial cell interactions we have obtained thus far are derived from experiments using peripheral blood monocyte-derived DC. Although this is a good model system, which allows for manipulation of the populations, it does not directly reflect intestinal immunity. Therefore we will confirm our findings using intestinal DC derived from patient biopsies.
Moreover, the SNPs described in patients do not result in complete loss of protein expression, but rather a decreased expression and decreased autophagy. Although our previous results were reproducible when only partial inhibition was achieved, we will confirm the relevance for human disease by repeating the experiments using DC obtained from CD patients carrying the SNP.
The aim of the current project is to determine whether autophagy related SNP in humans regulate the induction of tolerance in the gut, thus confirming in vivo the results thus far obtained in vitro.

Gwo-Tzer Ho (ECCO Grant 2010)

Title of Grant: Modulating primary intestinal epithelial defence and immune response in inflammatory bowel disease - development of an integrated inducible epithelial gene transfer and expression system
Institute: Institute of Genetics and Molecular Medicine, Edinburgh, United Kingdom

FINAL REPORT:
The main aim of this grant is to develop an *in vitro* integrated system, which allows IBD susceptibility genes involved in epithelial homeostasis or barrier function to be conditionally modulated. Therefore, the effects of these genes can be tested in response to paracrine inflammatory milieu (e.g. tumour necrosis factor) or luminal challenge (oxidative stress, whole or bacterial components such as lipopolysaccharide for examples). Our desired outcome is that this system can be used to screen for epithelial targets that represent viable therapeutic targets for further mechanistic studies. The proof concept will be based on the utilisation of the multidrug resistance gene-1 (MDR1) initially, which then can be expanded to a list of key epithelial genes identified from recent genome-wide association studies in IBD.

Progress and Outcomes: We have focused on 2 approaches namely i) an *in vitro* system based on HCT8 and T84 human intestinal epithelial cell lines; ii) an inducible system for mouse embryonic stem cells based on the similar principle. In the latter, the goal is to generate conditional modulation/overexpression of gene-of-interest (GOI) *in vivo*. Our original approach was to combine 2 separate technologies using the Flp Recombinase-Mediated Integration (Flp-InTM System)

and the ponA-inducible expression cassette that includes the E/GRE recognition sequence as the inducible element. However, to allow for a simplified approach to encompass functional mouse genetics *in vivo*, we have modified our choice to using the tetracycline based, Tet-On 3G system. This part of the study is performed in collaboration with MRC Human Genetics Unit, University of Edinburgh with the objective of producing an inducible Tet-On system MDR1-expressing mouse line, taking advantage of the in-house universal transgenesis vector (UTV) which allows multi-fragment Gateway cloning thereby MDR1 expression driven by Tet- (or villin-) promoter. We have generated stably knock-down long term MDR1 intestinal epithelial cell lines to provide the platform for the bi-directional modulation work where we have demonstrated that epithelial MDR1 expression can modulate specific NFKB response, promoting a proinflammatory response to bacterial components in particular, bacterial flagellin (*Abstract included below*). These findings have been presented at the Keystone Symposium for Mucosal Biology: A Fine Balance between Tolerance and Immunity (2nd March 2011, Vancouver, Canada); and the British Society of Gastroenterology: 'Host-bacterial interactions in inflammatory bowel disease – the role of MDR1 gene' (16th March 2011). All bearing reference to original funding from ECCO. A collaboration has been developed to generate inducible epithelial protein, vimentin as a novel receptor for adherent-invasive *E. coli* to exploit a further angle of host-bacteria interactions (Dr C Stevens, University of Edinburgh – Project Grant to Chief Scientist Office, Scotland April 2011). We are currently optimising our *in vitro* system in order to determine the appropriate functionality of MDR1 overexpression and its effects on direct epithelial barrier function. There have been issues with variability in the induction of gene expression and this is subject to on-going current work.

Epithelial multidrug resistance-1 (MDR1) gene modulates specific NFKB-response to bacterial flagellin and promotes a pro-inflammatory response in the intestinal epithelium

Rhona Aird[1], Bo Liu[2], Satsangi J[1], Balfour Sartor[2], Gwo-Tzer Ho[1,2]
University of Edinburgh, Scotland, UK
University of North Carolina, Chapel Hill, USA

The MDR1 gene encodes an ATP-dependent efflux transporter that is highly expressed on the apical aspect of the intestinal epithelium. Multiple lines of evidence suggest a role for MDR1 in the pathogenesis of inflammatory bowel diseases, in particular ulcerative colitis (UC). M*dr1a*-deficient mice develop spontaneous colitis, which is potentiated by the luminal flora. Germline variations of MDR1 gene are associated with, and colonic expression of MDR1 is significantly lower, in UC. The underlying mechanism by which the loss of MDR1 function results in increased susceptibility to colitis is not well-understood. Here we show that *in vitro*, *mdr1a*-deficient colonic epithelial cells have selectively higher NFKB transcriptional activity and activation of 2 NFKB-dependent genes, IL-8 and IKBα, specific to flagellin stimulation but not to bacterial CpG, TLR2, TLR4 and NOD2 ligands. *Ex vivo* data showed that bone-marrow derived macrophages have profoundly diminished response to flagellin (in comparison to bacterial lysate and LPS stimulation) with no differences between MDR1-deficient and wild-type mice backgrounds, implicating an epithelial-specific effect. Rather unexpectedly, in cultured epithelial monolayers, prolonged flagellin stimulation

resulted in earlier reduction in epithelial barrier function as measured by transepithelial electrical resistance; and increased commensal bacteria translocation (using adherent invasive *E. coli* LF82) following MDR1 downregulation. *In vivo*, *mdr1a*-deficiency in C57/BL6 strain has higher Cd11b/c myeloid cells in mesenteric lymph node (MLN) and basal innate immune activation (IL12/23p40) with increased susceptibility and decreased recovery from DSS colitis along with significantly elevated inflammatory cytokine profiles of IL12/23p40 and IL33. We postulate that colonic epithelial MDR1 modulates specific epithelial response to flagellin, altering the homeostatic balance from the usually protective effect of epithelial TLR5-NFKB engagement to one with an increased pro-inflammatory effect.

Michel Maillard (ECCO Grant 2010)

Title of Grant: Modulation of gut immune homeostasis via the Toll-interacting protein (Tollip)
Institute: Lausanne University Hospital (CHUV), Lausanne, Switzerland

FINAL REPORT:
Toll-Interacting Protein Modulates Gut Flora Composition and Susceptibility to Colitis

The bacterial flora is required for colitis development in both human inflammatory bowel disease (IBD) and murine models thereof (Nell et al., 2010). Activation of Toll-like receptors (TLR) and the nuclear factor-κB (NF-κB) are key events that allow a crosstalk and maintain equilibrium between the flora and the host at steady state and following physical, chemical or immune stresses. Our work focuses on the role of Toll-interacting protein (Tollip) on gut homeostasis. Tollip is an ubiquitously-expressed 274-amino-acid protein that regulates TLR-2, TLR-4 and IL-1R signals through binding of the IL-1 receptor-associated kinase (IRAK) and inhibition of its phosphorylation (Burns et al., 2000; Zhang and Ghosh, 2002). IECs and antigen-presenting cells (APCs) constitutively or inducibly express Tollip (Melmed et al., 2003; Otte et al., 2004). These data altogether implicate Tollip in the negative regulation of TLR and IL-1R signals – which are both known to play major roles in gut immune balance. However, the regulatory role of Tollip in intestinal homeostasis remains unknown *in vivo.*

Initial characterization of Tollip deficient mice showed normal development, no obvious evidence of spontaneous inflammatory disorders and no gross defect in immune cell activation or cytokine responses (Didierlaurent et al., 2006). During this funding period, we characterized the intestinal inflammatory status, permeability, epithelial cell turnover and composition of gut microflora of Tollip deficient versus WT mice at steady state. Unchallenged Tollip deficient mice had no spontaneous colitis development and epithelial turnover was comparable to WT controls. Likewise, tight junction integrity and epithelial permeability were unaffected. Using a 16s ribosomal RNA screen of the bacterial flora, we found that Tollip deficiency, although not associated with spontaneous colitis, had a dramatic impact on intestinal microbial content leading to overgrowth of the *segmented filamentous bacteria*, which were previously reported to influence Th17 cell induction (Ivanov et al., 2009). Increased SFB colonization in the ileum was further confirmed by electron microscopy. We next explored whether SFB overgrowth was associated with increased susceptibility to gut inflammation. We

thus challenged WT or Tollip deficient mice dextran-sulfate sodium (1.5%) to induce colitis. Starting at day 5 of DSS exposure, Tollip-/-mice had increased body weight loss compared to WT mice - a difference that became significant after day 7. This correlated with increased rectal bleeding scores, increased histological scores of colitis and elevated pro-inflammatory cytokines transcription (IL-1β and IL-6). Using bone-marrow chimeras, we found that susceptibility to acute DSS-induced colitis was mainly attributable to lack of Tollip expression in non-hematopoietic cells. Analysis of expression in various cell types showed a predominant Tollip expression in colonic epithelial cells. As a result, Tollip deficiency was associated with several epithelial cell abnormalities. In vitro, knock-down of Tollip expression in epithelial cells (Caco-2) using an siRNA strategy led to increased lipopolysaccharide- and peptidoglycan-induced NFκB activity. In vivo, after acute colitis induction, tight junction dissolution appeared earlier in Tollip deficient mice and this was associated with increased epithelial permeability and increased apoptosis.
Th17-associated cytokines play a major role in the development of chronic colitis in IL-10 deficient mice. We thus asked whether overgrowth of the Th17-associated SFB in Tollip deficient mice impacts colitis susceptibility and Th17-cytokine expression in IL-10 deficient mice. Histological analysis of colons from Tollip-/-IL-10-/-mice showed high histological scores of colitis that were significantly greater than IL-10 deficient controls. This was associated with higher IL-17 and IFN-γ expressions Tollip-/-IL-10-/-mice compared to IL-10 -/-controls.
In conclusion, our studies show that Tollip deficiency leads to marked changes in the bacterial flora and early colonization with *segmented filamentous bacteria*. These changes were associated with several epithelial defects that lead to increased susceptibility to acute and chronic colitis.

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Yoav Mazor (ECCO Grant 2010)

Title of Grant: Matrix metalloproteinases (MMPs) and ADAMs in IBD
Institute: Rambam Medical Center, Haifa, Israel

PROGRESS REPORT:
MMPs and ADAMs in inflammatory bowel disease: 1 year update report
Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases, capable of degrading different extracellular matrix proteins. The matrix metalloproteinases are inhibited by specific endogenous tissue inhibitors of metalloproteinases (TIMPs). ADAM (a disintegrin and metalloproteinase) protein family are transmembrane glycoproteins known to be involved in cell adhesion and

proteolytic ectodomain processing of cytokines and adhesion molecule. MMPs, TIMPs and ADAMs have been shown to play a role in the inflammatory and repair cascade in the inflamed intestine. Evidence exists for expression and activity of these enzymes in inflammatory bowel disease (IBD), but no study has systematically delineated the deferential expression of the different MMPs and ADAMs in different disease manifestations.

The first goal of our project was to map the differential expression of MMPs, TIMPs and ADAMs in the gut of IBD patients. After appropriate informed consent, biopsies from the terminal ileum and colon of IBD patients and controls were collected during colonoscopy. The mucosal macroscopic appearance, disease classification and demographic details were noted. Real Time PCR using selected primers and probes for indentifying a range of MMPs, ADAMs and TIMPs were used. A total of 75 biopsies from 31 patients with crohn's disease (CD), ulcerative colitis (UC) and healthy controls were collected up to now. All biopsies were analyzed for MMP 1,2,3,7,8 and 9, ADAM 8,10,12, 17,19 and 28, and TIMPs 1-4. Results were as following ($p<0.05$ for all comparison): Expression of ADAM12 was greater in the normal appearing colon of CD patients compared to healthy controls. Expression of MMP12 was greater in the normal appearing colon of UC patients compared to healthy controls. Expression of ADAM12 was greater in inflamed colon compared to non-inflamed colon of CD patients, but was greater in non-inflamed colon compared to inflamed colon of UC patients. Expression of ADAM10 was greater in non-inflamed terminal ileum compared to inflamed terminal ileum of CD patients. Expression of MMP9 was greater in inflamed colon of UC patients compared to healthy controls.

Based on these observations and on the putative pathophysiologic role of the selected molecule our next goal was to design siRNA molecules aimed at reducing tissue inflammation and enhancing repair. As a preliminary model of siRNA therapeutic use we have selected to inhibit the expression of MMP-9, previously implicated in IBD tissue inflammation. siRNA probes were designed and purchased from Thermo scientific (Dharmcon). We used a pool of 4 sequences ("ON-TARGET plus smart pool"). For testing the effect of MMP-9 siRNA, CaCo2 cells growing on DMEM/F-12(HAM)1:1 medium supplemented with 20% FCS were grown O.N. without antibiotics. 40,000 CaCo2 cells per 24 well were transfected with 3 pmol MMP9 siRNA or control none-target siRNA (Dharmcon) using INTERFERin transfection reagent according to manufacture instructions. TNFα 100ng/ml was added 1 h after transfection. At 24h, 48h and 72h RNA was extracted from these cells using QIAGENE RNeasy kit, cDNA was synthesized from equal amount of RNA, and MMP9 mRNA levels were determined using real time RT-PCR normalized to GAPDH expression. Maximum decrease in MMP9 mRNA level in cells transfected with MMP9 siRNA was 75% relative to control level and was achieved after 24 h.

We further assessed the effect of siRNA application on protein using MMP-9 zymography. . The cells were grown 24 h before examination of MMP9 level in the supernatant without serum. The supernatant medium was concentrated 10 times using Amicon ultra-0.5 centrifugal filter device (Millipore) and then samples were assayed using zymography in which samples are separated by electrophoresis under non-denaturating conditions on acrylamide gels containing gelatin. Commasie blue staining of these gels leave white holes where gelatinase like MMP9 are present and active. Protein levels of MMP9 72h after transfection are decreased with MMP9 siRNA.

In further experiments we will complete the analysis of MMP, TIMP and ADAMs expression in IBD tissue. We will explore more efficient means of siRNA delivery in order to improve their effect on cellular function. Furthermore, we will also select an appropriate animal model to test their effect in vivo.

Michael Scharl (ECCO Grant 2010)

Title of Grant: The role of Protein tyrosine phosphatase N2 in the regulation of cytokine-induced apoptosis in the intestinal epithelium
Institute: Clinic of Gastroenterology and Hepatology at the University Hopital Zurich, Switzerland

FINAL REPORT:
We have previously shown that the Crohn's disease (CD) susceptibility gene, protein tyrosine phosphatase N2 (PTPN2), regulates IFNγ-induced signalling and epithelial barrier function in T_{84} intestinal epithelial cells (IEC). The aim of this study was to investigate whether PTPN2 is also regulated by IFNgamma and/or TNFα and if PTPN2 controls IFNgamma/TNFα-induced signalling and apoptosis in IEC. To address our aims, T_{84} IEC were used for all cell studies. Protein levels were assessed by Western blotting, mRNA levels by RT-PCR and cytokine levels by ELISA. PTPN2 knock-down was induced by siRNA. Imaging was performed by immunohistochemistry or immunofluorescence.
TNFα treatment elevated PTPN2 mRNA as well as nuclear and cytoplasmic protein levels and □caused cytoplasmic accumulation of PTPN2, similar th what we observed before for IFNgamma treated cells. Biopsy specimens from patients with active CD showed strong immunohistochemical PTPN2 staining in the epithelium, which is also in good line with our previous mRNA data. Loss of PTPN2 resulted in increased TNF□-induced phosphorylation of p38 and ERK, but not of ERK and NFκB. In TNFα and IFNγ co-treated cells, loss of PTPN2 enhanced the extent of apoptosis as measured by increased levels of cleaved (activated) caspases-3 and -7 as well as of increased cleavage (inactivation) of the cell survival protein, PARP. By nuclear staining with DAPI, we could confirm that loss of PTPN2 results in increased cytokine-induced fragmentation of cell nuclei. Additionally in PTPN2-deficient cells, we found a large number of apoptotic bodies, indicative for the onset of apoptotic events in these cells.
TNF□ induces PTPN2 expression in IEC. Loss of PTPN2 promotes TNFα-induced MAPK signalling and favours the onset of apoptosis in IEC. These data indicate that PTPN2 activity could play a crucial role in the establishment of chronic inflammatory conditions in the intestine, such as CD.

Zoran Milenkovic (ECCO Travel Award 2010)

Host institute: Centre Hospitalier Regional Universitaire de Lille - Hospital Claude Huriez, Lille, France
Supervisor: Prof. Jean-Frédéric Colombel

FINAL REPORT:
From 1st of april untill 29th of june 2010.,supported by ECCO Travel Award,as clinical observer I visited one of the leading IBD centers in Europe,Hospital

Claude Huriez in Lille. That was great opportunity for me,as young gastroenterologist interested in area of IBD,to meet different strategies in treatment of IBD patients (hospitalised and outpatients),especially in administrations of different anti-TNF agents,also therapy regimens for most severe and complicated clinical cases.
Mostly,I worked with Professor J.F.Colombel,observed examinations during IBD consultations,where I had been introduced with implementation of guidelines in treatment regimens of IBD patients.Also,we colaborated in literature searching,papers analysis,disscusions of all kind of subjects related to IBD,which was for me quite new experience and chalenge in following the path of IBD science.From that,many ideas for possible projects and papers raised.
Since we realised that it wouldn´t be possible to start a project or paper in three months, we analysed and discussed several topics for possible projects in future, for example, Possible Role of PET scan in IBD Management, or, Possible Relation of Presence of Inflamatory Masses and Trough Level of Infliximab , etc.
During everyday visits at departments of Gastroenterology Clinic in Hospital Claude Huriez (Nutrition and Endoscopy),I faced great hospitality of all stuff members and very good communication with other doctors in sharing knowledge and practice in IBD area.I had the chance to observe implementation of advanced diagnostic procedures with which I hadn"t been familiar previously and which are not presented in routine practice in my country and institution (such as endomicroscopy and capsule endoscopy,for example).
I emphasise excelent organisation and technical support in everyday work in hospital.
I would rate my stay in Lille as excelent,inspirative,productive,chalenging,by all means.
I can say, for that reason-ECCO mission acomplished!
After one year of my staying in Lille, I can say that outcomes are more than productive – I participated in two IBD studies,raised number of treated IBD patients, improved work and collaboration with national organisation of IBD patients (UKUS,wich became a member of EFCCA this year). These days,I participate as coordinator in forming a National register of IBD patients in Serbia,also work on project for immunization of IBD patients.
Upon all, during last congress of ECCO,in Dublin, on February 23rd, I had very successful presentation about my experience in Lille, at YECCO ´s Workshop, in role to motivate young members and doctors to apply for Travel Award.

Ana Maria Catuneanu (ECCO Travel Award 2010)

Host institute: John Radcliffe Hospital, Oxford, United Kingdom

Report pending

Itay Maza (ECCO Travel Award 2010)

Title of Travel Award: Purpose and time of visit: one week visit, to observe and study IBD biobanking

Report pending

2009

Francesca Fava (ECCO Fellowship 2009)

Title of Fellowship: Measuring the impact of anti-tumour necrosis factor-alpha (TNF-α) treatment on the faecal microbiota in inflammatory bowel disease (IBD)
Host institute: Istituto Clinico Humanitas-IRCCS in Gastroenterology (Milan, Italy
Supervisor: Dr. Silvio Danese

FINAL REPORT:
Projects I was directly involved with during my fellowship:
1) The impact of anti-tumour necrosis factor-alpha (TNF-α) treatment on faecal microbiota in inflammatory bowel disease (IBD).
Recent studies showed that the gut microbiota of T-bet-deficient mice were a live transmission agent capable of transferring a TNF-α-driven colitis, which was effectively cured by anti-TNF-α treatment. TNF-α blockade is successfully employed for the treatment of Crohn's disease and ulcerative colitis, although its impact on the gut microbiota in IBD has not been tested.
Aim of this study was to test whether changes in mucosal inflammation upon anti-TNF-α treatment modulate the gut microbiota in IBD patients compared to chronic inflammatory pathologies lacking gastrointestinal symptoms (i.e. psoriasis -PS- and rheumatoid arthritis –RA-) and non-treated healthy controls.
Patient and volunteer recruitment and sample collection:
13 CD (of which n=10 with moderate to severe active disease, CDAI>150, and n=3 with mild disease, CDAI<150), 6 UC (of which n=5 moderate to severe active disease, Mayo score>6, and n=1 with mild disease, Mayo score <6), 4 PS (Psoriasis Area and Severity Index, PASI ≥ 12) and 7 healthy control volunteers were recruited so far. Disease activity indexes (DAI) (i.e. CDAI, MAYO score, DAS-28, PASI, respectively for CD, UC and PS) were analysed at each timepoint in IBD and PS. Faecal and blood samples were collected at weeks 0, 2, 6, 14, 22, 30 of anti-TNF-α treatment (Infliximab, 5mg/kg or Adalimumab, 160-40 mg). One faecal and one blood sample was collected from 7 healthy controls. Intestinal biopsies were collected from 5 IBD patients (of which n=4 CD and n=5 UC) before and after anti-TNF-α treatment.
Sample analysis:
10 out of 13 CD, 5 out of 6 UC and all PS patients responded to treatment (response=decrease of CDAI>70, Mayo score>3, 75% baseline PASI score). 7 CD, 3 UC and all PS patients responded to treatment at week 2; 3 CD and 3 UC responded at week 6.
Microbiota profiling of faecal samples of IBD, PS and healthy individuals was carried out through FISH using 16S rRNA-targeted oligonucleotidic probes specific to enumerate the dominant members of the human gut microbiota.
Lower baseline levels of Eubacterium rectale/Clostridium coccoides gp, Faecalibacterium prausnitzii, Bacteroides spp, slightly lower Bifidobacterium spp, and higher Enterobacteriaceae spp were observed in IBD compared to PS and healthy individuals.
Anti-TNF-α treatment increased the levels of Bifidobacterium spp in CD responders during induction of treatment, but the values tended to baseline

during maintenance (Week0: 7.6±1.1, Week2: 8,0±0.9; Week6: 7.9±0.7; Week14: 7,4±0,6; Week22: 7,7±0,7 Log10[bacteria/g feces]). In UC responders there were no major changes in Bifidobacterium spp numbers (Week0: 8.0±1.0, Week2:8.2±0.5, Week6:7.9±0.9, Week14:8.3±0.2, Week22:7.7±0.7 Log10[bacteria/g feces]). F.prausnitzii increased after induction treatment in UC, but tended to revert to baseline values during maintenance (Week0: 8.1±0.4, Week2: 8.7±0.3, Week6: 9.0±0.1, Week14: 8.4±0.7, Week22: 7.9±1.6 Log10[bacteria/g feces]), while in CD the levels decreased during induction and increased during maintenance (Week0: 7.5±1.2, Week 2: 7.4±0.9, Week6: 7.2±0.8, Week14: 6.7±1.2, Week22: 7.5±1.4 Log10[bacteria/g feces]). Bacteroides spp numbers increased as early as induction phase in UC patients (Week0: 8.6±0.2, Week 2: 9.1±0.3, Week6: 9.2±0.1, Week14: 8.9±0.1, Week22: 9.1±0.1 Log10[bacteria/g feces]), and during maintenance therapy in CD patients (Week0: 7.9±1.0, Week 2: 7.8±1.2, Week6: 8.4±0.8, Week14: 7.8±1.1 , Week22: 8.7±0.4 Log10[bacteria/g feces]). Numbers of Eu. rectale-C. coccoides group also showed a modest increase in both CD and UC patients (Week0: 8.8±0.6, Week 2: 8.8±0.6, Week6: 8.8±0.7, Week14: 8.9±1.2, Week22: 9.3±0.3 Log10[bacteria/g feces] in CD, and Week0: 8.9±0.7, Week 2: 9.1±0.1, Week6: 9.4±0.1, Week14: 9.3±0.1, Week22: 9.2±0.1 Log10[bacteria/g feces] in UC). Enterobacteriaceae numbers showed a decrease in CD and especially in UC patients when reaching maintenance therapy (Week0: 7.5±0.5, Week 2: 7.8±0.5, Week6: 7.2±0.5, Week14: 7.4±0.5, Week22: 7.1±0.9 Log10[bacteria/g feces] in CD, and Week0: 7.1±0.5, Week 2: 7.4±0.5, Week6: 7.2±0.5, Week14: 6.7±0.1, Week22: 6.3±0.1 Log10[bacteria/g feces] in UC). Atopobium numbers showed variable counts at different timepoints and also high inter-individual variability in CD (Week0: 7.4±1.0, Week 2: 8.0±0.6, Week6: 7.4±1.1, Week14: 6.8±1.2, Week22: 8.4±0.1 Log10[bacteria/g feces]), while they did not show major changes in UC (Week0: 8.2±0.1, Week 2: 8.5±0.2, Week6: 8.7±0.1, Week14: 8.3±0.1, Week22: 8.5±0.2 Log10[bacteria/g feces]). Numbers of bacteria belonging to C. hystolyticum remained almost unaltered (Week0: 7.3±0.3, Week 2: 7.2±0.5, Week6: 7.3±0.5, Week14: 7.0±0.4, Week22: 7.4±0.3 Log10[bacteria/g feces] in CD, and Week0: 7.4±0.4, Week 2: 7.1±0.3, Week6: 7.0±0.1, Week14: 7.0±0.3, Week22: 7.4±0.4 Log10[bacteria/g feces] in UC). Also Lactobacillus-Enterococcus species were unchanged (Week0: 8.1±0.7, Week 2: 7.8±0.8, Week6: 7.8±0.8, Week14: 8.0±0.4, Week22: 8.1±0.4 Log10[bacteria/g feces] in CD, Week0: 8.3±0.4, Week 2: 8.1±0.4, Week6: 8.1±0.5, Week14: 6.7±2.1, Week22: 8.3±0.1 Log10[bacteria/g feces]).

β-defensins in faecal samples were analysed before (T0) and after (T14) anti-TNF-α treatment by ELISA. High inter-individual variation was observed in β-defensin levels at baseline and after therapy. The therapy did not seem to influence faecal β-defensin levels in both CD and UC patients (Week0: 65.0±57.5 and Week14: 43,6±34.0 ng/ml for CD, Week0: 70.9±53.8 and Week14: 92.5±129.0 ng/ml, for UC).

Gene expression of a set of 48 genes encoding for inflammatory molecules, tight junctions proteins, defensins (i.e. TLR2, TLR3, TLR4, TLR5, TLR7, TLR9, DEFA3, DEFB4, DEFB1, DEFA6, DEFA5, F11R (JAM-A), CLDN1, TJP1 (ZO-1), OCLN, CTNNB1, CCL1, CCL2, CCL3 (MIP1A), CCL4 (MIP1B), CCL5 (RANTES), CCL7, CCL11, CCR2, CCR3, CCR4, CCR6, CXCL1, CXCL2, CXCL10, CXCL13, IL10, IL12A, IL12B, IL1A, IL1B, IL6,IL8, TNF, TGFB1, IFNG, IL2, IL4, IL13, IL17, IL22, IL25, and 18S and GAPDH as housekeeping genes) was measured in intestinal biopsies

of IBD patients using Running TaqMan Low Density Arrays on 7900HT Real Time PCR Systems. Analysis of the results are being performed.

2) NEUTROPHILS EXTRACELLULAR TRAP FORMATION IN IBD.
The role of innate immune system in IBD was also another of my current research themes. Particularly I have focused on testing the hypothesis that Crohn disease (CD) and ulcerative colitis (UC) might be associated with a defect of innate immunity, represented by an impaired response of granulocyte neutrophils to stimulation with bacterial components. The formation of neutrophil extracellular traps (NETs) from peripheral blood after stimulation with bacterial antigens (i.e. LPS, MDP, faecal water from IBD or healthy) has been compared between IBD patients and healthy subjects.

3) PILOT STUDY ON THE EFFECTS OF PROBIOTIC FERMENTED DAIRY DRINKS ON THE INTESTINAL IMMUNE RESPONSE IN HEALTHY ADULTS (industrial sponsor).

4) NOD-2 GENOTYPING OF IBD PATIENTS.

Other training:
Animal models of colitis (DSS)
Cytofluorimetry
Organ culture of mouse and human intestinal biopsies
Confocal microscopy
RT-PCR for tissue gene expression

Sofia Maria Buonocore (ECCO Grant 2009)

Title of Fellowship: Identification of IL-23 dependent effector pathways in colitis
Institute: University of Oxford, Sir William Dunn School of Pathology , Oxford, United Kingdom

FINAL REPORT:
1. Introduction
The key role of IL-23 in the pathogenesis of autoimmune and chronic inflammatory disorders is supported by the identification of IL-23R susceptibility alleles associated with IBD [1], psoriasis and ankylosing spondylitis. IL-23 driven inflammation has primarily been linked to the actions of Th17 cells. Somewhat overlooked, IL-23 also has striking inflammatory effects on innate immune cells and can drive T cell- independent colitis [2, 3]. However the downstream cellular and molecular pathways involved in this novel innate intestinal inflammatory response are poorly characterized. The aim of this project is to identify the cellular source of key IL-23-dependent cytokines including IL-17 and IL-22 and assess their functional role in innate immune mediated colitis. The results of this work may identify more specific targets for the treatment of inflammatory bowel disease.

2. Results

We have shown that innate immune colitis in Rag-/- mice following infection with Helicobacter hepaticus is IL-23 dependent [2]. To identify the cellular and molecular pathways involved, we first analysed the expression of inflammatory cytokines in this model. Consistent with selective upregulation of IL-23 in the intestine, we observed significant increases in the expression of Th17 and Th1 signature cytokines including IL-17, IL-22 and IFN-□ by colonic lamina propria cells (cLP) from H. hepaticus infected Rag-/- mice but not from spleen cells (Fig. 1a). To determine whether IL-23 acts directly on innate cells to induce Th1 and Th17 cytokines, cLP cells were isolated from healthy colons of Rag-/-mice and stimulated with IL-12 or IL-23. Addition of IL-23 induced secretion of IL-17, IL-22 and IFN-□□ (Fig. 1b), whereas IL-12 induced IFN-□ only. To determine whether IL-17 and IFN-□ played a functional role in innate colitis, H. hepaticus infected Rag-/- mice were treated with neutralising □-IL-17 or □-IFN-□ mAbs. Blockade of either IL-17 or IFN-□ was sufficient to significantly reduce colitis (Fig. 1c and d), without affecting colonisation with H. hepaticus (data not shown). Similarly, systemic immune activation, assessed by splenomegaly, was also abrogated by IL-17 or IFN-□ blockade (Fig. 1e). Collectively, these results indicate that H. hepaticus induced IL-23 regulates the innate expression of effector cytokines such as IL-17 and IFN-□ that play functional roles in the intestinal innate inflammatory response.

To identify IL-23 responsive innate cells present in the inflamed intestine, we used a cell sorting approach. Using leukocyte lineage (Lin) markers CD11b, GR1 and B220, we found that cytokine expressing cells were CD45+Lin- and distinct from common innate cell populations (Fig. 2a). To identify these cells, we performed intracellular cytokine staining in combination with cell surface marker expression on IL-23 stimulated cLP cells from colitic mice. We found that IL-23 not only enhanced the frequency of IL-17+IFN-□- cells but also increased the frequency of IL-17+IFN-□+ cLP cells whereas the frequency of IL-17-IFN-□+ cells did not increase (Fig. 2b). Analysis of surface markers showed that the vast majority of the cLP IL-17 secreting cells expressed high levels of Thy1 (Fig 2c). Lin-Thy1hi cells in Rag-/- mice include a population of cells required for secondary lymphoid organ (SLOs) organogenesis, termed lymphoid tissue inducer cells (LTi)/LTi-like cells [4]. Similar to classical LTi-like cells, IL-17 expressing cells were found to be IL-7R+CD44+NKp46-CCR6+CD25+ROR□+ (Fig. 2d). They also expressed LTi related genes such as LT□ and □, TRANCE and CXCR5, recently found to be important for the recruitment of LTi-like cells during inflammation [5] (data not shown). However, IL-17- expressing innate lymphoid cells were also phenotypically distinct from LTi-like cells as they were CD4-c-kit- and also expressed SCA-1 (Fig. 2d) suggesting heterogeneity amongst Thy1+ innate lymphoid cells in the intestine. Further work will be needed to characterize Thy1hiSCA-1+ innate lymphoid cells and their functional role in various innate models of colitis.

3. References

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Figure legends:
Figure 1: IL-23 induced IL-17 and IFN-☐ are required for H.hepaticus-mediated innate IBD
a, Cytokine secretion following overnight culture of splenocytes or cLP cells from control or *H. hepaticus*–infected 129SvEvRag−/− mice (n=6 per group). b, Cytokine secretion by cLP cells from control 129SvEvRag−/− mice following overnight culture with IL-12 or IL-23 (n=6). Data represents mean ± sem. Colitis scores (c), splenomegaly (e), and representative colon photomicrographs (×50) (d) from H. hepaticus infected 129SvEvRag−/− mice treated with blocking ☐-IL-17 and/or ☐-IFNγ or isotype (Iso) control mAbs throughout the course of infection. Data represents two pooled experiments (n=5-12 per group). *P<0.05; **P<0.01; ***P<0.001.

Figure 2: IL-23-responsive innate lymphoid cells in inflamed colon are Thy1hiSCA-1+ ROR☐t+.
a, IL-23 drives cytokine production by CD45.2+ Lin- cLP cells. Cytokine secretion by Lin+ and Lin- cLP cells isolated from H. hepaticus infected 129SvEvRag-/- mice following overnight culture in the presence or absence of IL-12 or IL-23. b,c IL-17, IFN-γ and Thy1.2 expression in Lin- cLP cells from *H. hepaticus*–infected 129SvEvRag−/− mice following overnight culture with or without IL-23. d, Phenotypic analysis of Lin- IL-17+ cLP cells from H. hepaticus-infected 129SvEvRag−/− mice, using specific antibodies (black line) and isotype controls (grey line). Results are representative of ≥ 2 independent experiments.

Margarita Elkjaer (ECCO Grant 2009)

Title of Fellowship: Virtual Hospital System in IBD: Patient centred monitoring and web-guided therapy with 5-ASA in ulcerative colitis "Constant-care": Impact on quality of life and cost benefit
Institute: Herlev University Hospital, Gastrointestinal Unit, Herlev, Denmark

PROGRESS REPORT:
Project update: *Virtual Hospital System in IBD: Patient centred monitoring and web-guided therapy with 5-ASA in ulcerative colitis "Constant-care": Impact on quality of life and cost benefit.*

Since February 2009 the following have been completed:

1. All 316 UC patients have been included in the project.
2. All Danish patients have been completed 12 months follow-up.
3. Approximately 45 Irish patients are still in the study and will complete the 12 months follow-up in April 2010.
4. The second (historical) control group has been included in Copenhagen and Dublin.
5. The following lectures about Web-based self-management in patients with Ulcerative Colitis and 5-ASA treatment (Ph.D. project) have been given:
   i. 25.03.09, Stockholm, Swedish -invited speaker
   ii. 28.09.09, Annual meeting of Danish paediatric gastroenterologists, Copenhagen, Denmark
   iii.29.10.09, Padua, Italy- invited speaker
   iiii. 03.12.09, Cornell University, and 04.12.09 Columbia University, New-York, USA

6. **Publications:**
   i. Development of a Web-based concept for patients with ulcerative colitis and 5-aminosalicylic acid treatment.
   Elkjaer M, Burisch J, Avnstrøm S, Lynge E, Munkholm P
   Eur J of Gastroenterol and Hepatol 2009 Jun 18 (Epub ahead of print)
   ii. A new rapid home test for faecal calprotectin in ulcerative colitis.
   Elkjaer M, Burisch J, Voxen Hansen V, Deibjerg Kristensen B, Slott Jensen JK, Munkholm P.
   APT 2009 Oct 10 (Epub ahead of print)

7. **Posters:**
   i. Faecal calprotectin Home Test in Ulcerative Colitis-Is it possible?
   M. Elkjaer, J. Burisch, A. Røseth, VV. Hansen, BD. Kristensen, D. Bremnes, JK. Jensen, P. Munkholm
   ECCO, Hamburg, 2009, Vol 3 Issue 1, S27
   DDW, Chicago, May 2009, S1051
   ii. Monitoring of inflammation burden in UC be faecal calprotectin home test-Fact or Fiction?
   M. Elkjaer, J. Burisch, VV. Hansen, BD. Kristensen, JK. Jensen, P. Munkholm UEGW, 22.-25.November 2009, GUT supl vol 58, P1065, London, UK
   Imedex IBD meeting, 3.-6. December, Florida, US

8. **At present time we are evaluating the data on Danish patients and the first result seems to be promising.**

9. **Project in progress:**
   i. A Single Center, Investigator Initiated, Open Label, Pilot Study of Anti-TNF□ Chimeric Monoclonal Antibody (infliximab, Remicade®) Web-Based Treatment for Optimizing Disease Control in Patients with Crohn's Disease-all 28 patients have been included.

ii. Is there an East-West gradient incidence caused by environmental factors and genetic differences?" started 01.01.2010 –co-mentor
iii. Web-based IBS treatment solution. Master OSVAL II, KU, start 01.02.10- co-mentor

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Maria Papp (ECCO Grant 2009)

Title of Fellowship: The possible role of von Willebrand factor (VWF) and its cleaving protease (ADAMTS-13) in the vascular pathogenesis of inflammatory bowel disease
Institute: University of Debrecen, Medical & Health Science Center, Institute of Internal Medicine, Div. Of Gastroenterology, Debrecen, Hungary

FINAL REPORT:
During the 1st year, we completed the clinical part of the study: patient examination, selections, blood and data collections were done. Seven hundred plasma samples of 340 patients with Crohn's disease and ulcerative colitis were assayed for different VWF (antigen level, activity and multimerization) and ADAMTS-13 (antigen level, and activity) parameters. Statistical analysis of the total array of data shows moderate 1.3 and 1.4 fold increase compared to control group in case of activity and antigen level (mean VWF:CB=133%, SD=70%; VWF:Ag=144%, SD=62%,) and a decreased in high molecular weight multimers (VWF multimerisation level (MW25:5.8+/-1.3, reference: MW25:6.1+/-0.72); and no difference in ADAMTS-13 activity and antigen level. Their possible interaction with the clinical presentation of the disease is under evaluation. Serum level of C-reactive protein (CRP), lipopolysaccharide-binding protein (LBP), antiphospholipide antibodies and anti-microbial antibodies were also tested in the whole cohort. Of the 340 patients, 200 were recruited in the quiescent phase of the disease up to a 1-year follow-up study to evaluate the possible association of VWF and ADAMTS-13 with the disease activity. We registered the clinical flare-ups in the whole cohort. Efficacy of VWF and ADAMTS-13 parameters as predictors individually and also in comparison with CRP and LBP for clinical relapses in a Kaplan-Meier and a proportional Cox-regression analysis is pending. In addition to circulating VWF and ADAMTS-13, we aimed to study the local conditions in gut tissue samples. For this purpose, we set up the method for mRNA level of VWF and ADAMTS-13 detection. Briefly, freshly frozen samples on dry ice in Trizol reagent and stored at -80 Celsius degrees were homogenized after thawing and total RNA was extracted following the manufacturer's protocol. NanoDrop ND-1000 was used to determine the concentration of the RNA samples. cDNA was generated using High Capacity cDNA Archive Kit (Applied Biosystems) and mRNA levels were measured using individual TaqMan RT-QPCR assays on ABI HT7900 instrument (Applied Biosystems) CyclophilinA was used as internal control gene and relative mRNA expression values were determined by $\Delta\Delta$ Ct method. Our original plan to use colon and ileal biopsy samples taken during endoscopic intervention seems not to be suitable because they do not contain enough vessels. Accordingly, we have to use surgical samples either from recent operation or from the pathological archive. This latter requires different mRNA isolation but the same detection

methods. Our plan for the 2nd year is to prepare the manuscript of the clinical study and to complete the evaluation of the local conditions.

Stefania Vetrano (ECCO Grant 2009)

Title of Fellowship: The protein C pathway in inflammatory bowel disease: a novel mediator of cross-talk between dendritic and epithelial cells
Institute: Istituto Clinico Humanitas, Milan, Italy

FINAL REPORT:
INTRODUCTION: The protein C (PC) pathway is a well characterized anti-coagulant system. Endothelial protein C receptor (EPCR) and thrombomodulin (TM) are expressed at high levels selectively in the microvasculature, and convert protein C (PC) to its activated form (aPC), which, together with EPCR and protease activated receptor-1 (PAR-1) displays potent anticoagulative and barrier protective properties, the latter characterized by antiapoptotic and anti-inflammatory activities. We have previously shown that the PC system controls microvasculature inflammation in the gut.
AIMS & METHODS: The aim of this study was to explore if the protein C pathway is also expressed by other cell-types in the gut and its functional role therein.The level of PC, TM, and EPCR expression was evaluated by confocal microscopy in colonic sections from controls, Crohn's disease (CD), and ulcerative (UC) subjects, and by flow cytometry and real-time pcr in human intestinal mucosa and monocyte-derived dendritic cells (DC), at baseline and after treatment with TNF-alfa;, LPS, and IL-10. The capacity to generate activated PC by DC was measured by colorimetric assay at baseline and after treatment with LPS, TNF-α and IL-10.

RESULTS: In healthy subjects, TM and EPCR were expressed beside by intestinal DC as shown by colocalization with CD11c. In both CD and UC patients expression in DC of TM and EPCR was significantly reduced. In both mucosal and monocyte-derived DC, inflammatory molecules such as TNF-α and LPS down regulated mRNA of EPCR and TM in DC, as well as their surface expression, while IL-10 induced up-regulation of their expression levels. Importantly, the PC pathway was proven to be functionally active in converting PC into its activated form by DC. TNF-α and LPS significantly ($p<0.05$) impaired the capacity to activate PC, while IL-10 increased DC conversion of activated PC.

CONCLUSION: These results show for the first time that the PC coagulation pathway is expressed and functionally active in the intestinal mucosa of healthy subjects. In addition, previously unrecognized cell types such as DC express the PC pathway components that mediate a new system for cell-cell cross-talk in the gut. The PC pathway is strongly down-regulated in IBD-DC, and inflammatory chemokines or bacterial products induce its down-regulation. Restoring the anti-inflammatory activity of activated PC could be therapeutically relevant for IBD, as this approach is successfully used in sepsis.

Jan Wehkamp (ECCO Grant 2009)

Title of Fellowship: WNT transcription factor Tcf-1 and ist role in protective innate immunity in inflammatory bowel diseases
Institute: Dr. Margarete Fischer Bosch Institute of Clinical Pharmacology, Stuttgart, Germany

SYNOPSIS:
Intestinal epithelial barrier function is increasingly recognised as an important factor in the pathogenesis of IBD. It functions as a mechanical shield and provides potent biological protection via the production of antimicrobial molecules (defensins). Ileal Crohńs disease (CD), is characterised by diminished antibacterial activity of the epithelium and a specific decrease of small intestinal Paneth cell b-defensins, HD-5 and HD-6. We have reported a causal link between the decrease of antimicrobial peptides and diminished expression of the Wnt pathway transcription factor TCF4. Wnt signalling contributes to intestinal epithelial renewal, by regulating stem cell maintenance and their transition to Paneth cells. Disturbed Wnt signalling alters innate immunity, suggesting a novel mechanism for the pathogenesis of ileal CD. We have explored the intensity of the disturbance in Wnt signalling in small intestinal CD and have identified impairment of TCF1, another transcription factor in the Wnt signalling cascade, which is also decreased in ileal CD. Other studies indicate regulation of TCF1 by TCF4, so the impairment of both factors is of considerable interest. They share a common consensus binding motif, so TCF1 could be a causative factor. Initial results suggest that TCF1 regulates Paneth cell b-defensin expression. With the support of the ECCO grant we aim further to investigate the influence of decreased TCF1 in small intestinal CD. We plan to analyze the role of TCF1 in regulation of HD-5 and HD-6 gene transcription. Functional studies will investigate the significance and interaction of TCF1 and TCF4 in the regulation of Paneth cell b-defensins. This will be correlated with genetic analysis. An understanding of the mechanisms that initiate inflammation is indispensible for preventative therapeutics, as opposed to current therapy that modifies inflammation once initiated.

Davide Checchin (ECCO Travel Award 2009)

Host Institute: John Radcliffe Hospital, Oxford, United Kingdom
Supervisor: Dr. Simon Travis

FINAL REPORT:
For three months I have had the honour of joining the Gastroenterological Unit of the University of Oxford. During that period I joined the team headed by Simon Travis spending my time following clinical activities and teachings and I performed a short study as well.
The research project was around dysplasia in UC patients and it implied a data collection through the oncologists, pathologists and endoscopy databases with a review of specimens. Reading through the notes I came up the hand made signature of Prof. Truelove, and, I have to admit, I was quite excited for that (...even taken a pictures of that!). The work has been quite long but we

achieved some results and now the abstract is running its way as a poster for the DDW. On the other hand I joined the clinical and University activities. I met expert and brilliant consultants - Keshav, Collier, Chapman - and shrewd registrars and fellows - Chandra, Cummings, O'Baley, - very human nurses and secretaries that guided me through the three months experience.
Surely I came home with my mind enriched with knowledge on IBD, but this is not the point. Spending my time abroad I've understood the importance of opening my mind to different scenarios, of learning a different way to approach the problems to improve my insight to managing myself. An extraordinary mentor in doing this has been Simon Travis, I have appreciated his devotion to work and his ability in managing the expectations of patients and in combining a really updated knowledge with a pragmatic medicine method. I'd like to thank him for his way to be a paragon and because he gave me the opportunity of joining his team: ECCO travel grant, a very recommended experience!

Michael Dam Jensen (ECCO Travel Award 2009)

Title of Travel Award: The impact of wirless capsule endoscopy on clinical decision-making in suspected and known Crohn's disease
Host Institute: John Radcliffe Hospital, Oxford, United Kingdom
Supervisor: Dr. Simon Travis

FINAL REPORT:
Supported by an ECCO Travel Award, I visited Department of Gastroenterology in Oxford, England. The purpose of the stay was to improve my knowledge about managing patients with complicated Crohn’s disease and ulcerative colitis.

During my stay, I participated in ward rounds, the IBD Clinic, and Endoscopy Unit giving me the opportunity to see the full spectrum of patients with inflammatory bowel diseases including highly complicated patients requiring multidisciplinary treatment. Discussing strategies for diagnosis and treatment was very educational. Furthermore, I experienced an excellent academic environment in which education and research was paramount. It was very inspiring.

During my stay, I did a retrospective study on the clinical impact and benefit of capsule endoscopy in patients with suspected and known Crohn’s disease. Results were presented and discussed the last day of my stay. Further data collection has been initiated in Odense, Denmark.

I would like to thank ECCO for granting me the Travel Award, and express my gratitude to Dr. Simon Travis for providing an educational stay in Oxford.

Joana Maria Tinoco da Silva Torres (ECCO Travel Award 2009)

Title of Travel Award: Observational Internship in Inflammatory Bowel Diseases
Host Institute: Centre Hospitalier Regional Universitaire de Lille - Hospital Claude Huriez, Lille, France

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Supervisor: Prof. Jean-Frédéric Colombel

FINAL REPORT:
From October the 1st until the end of December 2009, I had the wonderfull opportunity to make an internship as a clinical observer in *"Service des Maladies de l´Appareil Digestif et de la Nutrition" in Centre Hospitalier Regional Universitaire de Lille*, France. Professor Jean-Frédéric Colombel was my Ecco Host. Hepatogastroenterology Service in Lille is a reference international centre, distinguished in Gastroenterology and particularly in the area of IBD, that contacts with a large volume of cases, and that has an intense scientific work in this field. I had the chance to work with brilliant doctors and skilled scientists, in a Service where team work was very stimulated.
During my stage, and in order to profit the most as possible, I divided my time between IBD consultation, the Infirmary of Nutrition (where most of IBD cases needing hospitalisation are admitted) and Endoscopy.
In **IBD consultation** I worked mostly with Professor Colombel and Professor Antoine Cortot. Both have an enormous experience in the field of IBD. I watched the implementation of guidelines on the daily basis and had the opportunity to develop my knowledge and skills in the diagnostic and therapeutic area in IBD. The enormous volume of patients seen in consultation provided me a better understanding of the indications, contraindications and pharmacology of the therapies used in relevant clinical situations, such as the importance of achieving mucosal healing as a way of changing the natural history of what can be an extremely disabling disease. I also observed the utilization of new biologic agents by the inclusion of patients in phase III clinical protocols (golimumab, vedolizumab, Anti-IL-12/IL-23, etc).
In **Nutrition Infirmary,** I contacted mostly with patients with IBD (fulminant ulcerative colits, complicated Crohn) and short bowel syndrome. The work in this sector of the Service was very interesting, allowing me a better Knowledge of the indications for enteral and parenteral alimentation in order to be able to implement nutritional therapies.
In **Endoscopy sector** I had the chance to observe the routine utilization of endoscopic scores in IBD, as well the utilization of new endoscopic techniques in the surveillance of dysplasia in IBD (narrow band imaging endoscopy, cromoendoscopy, confocal endomicroscopy).
During these three months, I also had the opportunity to assist to several **scientific reunions** in the field of IBD, such as the 1st meeting of discussion of the project ORIGIN, the meeting "Cutting edge of cell therapy in IBD", etc.
I worked with Professor Colombel , Professor Cortot and Professor Frank Broly in a new TPMT mutation found in a patient who developed immunosuppression while on standard doses of azathioprine - accepted for publication as Letter to the Editor *in Inflammatory Bowel Diseases.* I also worked with Professor Colombel in a case study of a patient with paroxysmal nocturnal hemoglobinuria suffering from recurrent ischemic episodes, complicating her disease – waiting decision from *Nature Reviews Gastroenterology and Hepatology.*

Lille Gastroenterology team was always warm full and made feel at home.
I am very gratefull to Professor Colombel and to Professor Cortot. They are brilliant doctors and scientists and wonderful persons that kept me always motivated and transmitted me their "passion" for IBD.

I would also like to gratefully thank to the ECCO SciCom for the Travel award granted in the value of 1500 euros. It was helpfull in lodgment and travel expenses.

Annalisa Crudeli (ECCO Travel Award 2009)

Host Institute: Centre Hospitalier Regional Universitaire de Lille - Hospital Claude Huriez, Lille, France
Supervisor: Prof. Jean-Frédéric Colombel

Report pending

2008

Alessia Grillo (ECCO Fellowship 2008)

Title of Fellowship: Role of ß-catenin signalling in primary subepithelial myofibroblasts of Crohn's disease
Host institute: University of Padova, School of Medicine, Padova, Italy

FINAL REPORT:
Thirty percent of Crohn's disease (CD) patients have a fibrotic phenotype causing recurrent ileal stenosis. Although intestinal sub-epithelial myofibroblasts (ISEFMs) in CD show an enhanced ability to produce and reorganize extracellular matrix proteins, the molecular mechanisms inducing this fibrogenic phenotype are not known. TAK1 (transformig growth factor activated kinase1) is a MAP3K activated by various factors able to ignite different signal transduction pathways. Since previous studies have linked TAK1 activity to hearth and kidney fibrosis, we investigated the expression of TAK1 in CD patients and assessed whether TAK1 is involved in ISEMFs fibrogenic phenotype. We also evaluated Tak1 expression and correlation with fibrotic markers in CD patients without clinical signs of fibrosis.
TAK1 and its activate posphorylated form (pTAK1) expression in ileum surgical specimens from CD and control patients and in primary ISEMFs were assessed by Western Blot and double labelling confocal immunohistochemistry (CIF). Level of TAK1 mRNA was determined in ileal biopsies from CD (n=17, without clinical and endoscopic signs of stenosis), control (n=14) and colitis (n=9) and correlated to fibrosis markers: pro-collagen1a, Timp1 and αSMA. The functional role of TAK1 in ISEMFs collagen synthesis, cellular migration (by quantitative RT-PCR and 3H-proline incorporation) and cell migration (in a wound healing assay) was determined using a specific Tak1 inhibitor and sh-RNA silencing.
TAK1 and p-TAK1 levels, as assessed by WB and CIF, were increased in CD patients as compared to controls. TAK1 and p-TAK mainly localized in lamina propria cells, identified as ISEMFs (αSMA positive). TAK1 mRNA increased 4,8-fold (p=0,01) as compared with controls and directly correlated with fibrotic marker expression (Tak1/pro-col1a R=0.370 p=0.022, Tak1/αSMA R=0.420 p=0.010). Primary ISEMFs from CD patients (n=4) showed higher TAK1 and pTAK1 expression, associated to increased pro-collagen1a mRNA levels

(p=0,01) versus control cells (n=5). TAK1 activation by TGF-β occurred within 30 min. ISEMFs treatment with a TAK1 inhibitor or TAK-1 silencing with sh-RNA significantly inhibited basal and TGF-β1 induced collagen production (by RT-PCR and 3H-proline incorporation) and migration.
We report here that: 1)TAK1 and pTAK1 are up-regulated in ISEMFs of CD patients, 2) TAK1 expression correlates with marker of fibrosis in ileal biopsies of patients without signs of stenosis, 3) TAK1 activation is required to induce a fibrogenic phenotype in ISEMFs. We speculate that TAK1 activation is a key event in the development of ileal fibrosis and may represent a marker for the development of ileal stenosis.

Christian Jakobsen (ECCO Grant 2008)

Title of Fellowship: Genotype-Phenotype interactions in Danish Paediatric IBD Patients
Institute: Hvidovre University Hospital, Hvidovre, Denmark

FINAL REPORT:
Aim of the project:

1. To analyse associations between single nucleotide polymorphisms (SNP) in IBD related genes and the risk of developing IBD in children under the age of 18 years in Denmark.
2. To compare the genetic impact on the phenotypic ? presentation in IBD patients under the age of 18 in Denmark.

Patients:
The goal was to include 500 IBD patients diagnosed below the age of 18 years. We have included 525 patients and 235 of these are < 15 years and from a population based cohort. The median follow up time in the large cohort was 5 years. We have registered full phenotypes on all the patients who were in included: age at diagnosis, time from debut to diagnosis, IBD in first degree relatives, disease localisation, disease behaviour, all medical treatment, surgical treatment, disease course, height and weight.

Genetics:
The DNA extraction from all the 525 patients will be done in the beginning of February 2009. The SNP analysis will be done immediately hereafter. The results from the analysis will be ready around the beginning of June for preparation of a scientific article.

Holm Uhlig (ECCO Grant 2008)

Title of Fellowship: Immunosuppressive drugs and Foxp3+ regulatory T cell activity in inflammatory bowel disease
Institute: University Children's Hospital, Leipzig, Germany

FINAL REPORT:
According to the ECCO grant proposal one year ago we performed the following experiments to investigate the influence of immunosuppressive therapy, in

particular the effect of ciclosporin A on cytokine secretion (IL-2) and regulatory T cells. The following experiments have been performed using animal models:

- The number, density, and frequencies of Foxp3+ cells were investigated in colon tissue sections using manual and semiautomatic image acquisition and automatic image analysis using the open source software Cell profiler. For this software we developed image analysis routines and statistical methods for investigating cell-cell interactions in tissue sections. The automatic image analysis has been validated by manual analysis of the tissue sections. We were able to show that computer based tissue analysis is sufficient to quantify complex histological interactions between cell types. Results of these studies are in press (Haendel et al. Cytometry).
- In collaboration with Dr A. Izcue and Professor F. Powrie, University of Oxford we investigated IL-2 knockout mice that were injected with IL-2 sufficient congenic CD45.1 CD4+ T cells. Our hypothesis was that Foxp3+ cells of the recipient could accumulate in contact to donor CD4+ cells (because of their IL-2 secretion). We did not observe such an accumulation. It is possible that the lack of cell cell accumulation is not due to wrong hypothesis but to lack of antigenic stimulation of the donor T cells in the host since we have not used an antigen specific system.
- Wild type mice were injected with PBS or cyclosporine A. We investigated the influence of ciclosporin treatment in the TNBS model of colitis. Using i.p. injection of ciclosporin in olive oil, ciclosporin has not been protective in this T cell mediated colitis model.
- In the TNBS model of colitis we investigated the influence of IL-2. For this experiment, Balb/c mice received recombinant IgG2b-IL-2. We used an IgG2b-IL2 fusion protein because of the short half life of native IL-2. Due to the limited availability of the fusion protein, we have not applied the 4-8 week T cell transfer model of colitis as proposed but the 1 week TNBS colitis model. So far, we have not been able to reproduce results that show a protective effect of IL-2 on the development of TNBS colitis (Stallmach et al. Gastroenterology 117: 866-76). The effect of this treatment on frequency and number and tissue distribution of Foxp3+ regulatory T cells is currently under investigation.
- We analysed the mRNA gene expression of Foxp3, IL-2, IL-10, TNF-a in tissue sections as well as in tissue compartments and single cells. For this analysis we used laser capture microdissection. We have shown, that IL-2 is preferentially expressed in the lymphoid follicles in that colon and that Ki67 positive cells are a direct source of IL-2 in situ.

In addition to the mouse experiments we investigated human tissue sections:

- The tissue distribution of Ki67, nuclear NFATc1 and nuclear NFkB was determined. For these experiments frozen tissues of appendix and tonsil have been used. We observed a dominant cytoplasmatic signal as compared to the active nuclear staining. So far there was no correlation between NFATc1/NFkB and Ki67 signals.

- Colon tissue sections of patients with steroid dependent ulcerative colitis as well as patients receiving steroids and cyclosporine A or other immunosuppressive drugs were analysed for the frequency and density of Foxp3+ regulatory T cells. We analysed the density and frequency of Foxp3+cell-Ki67+ cell-interactions

using fluorescence microscopy. We will furthermore perform a gene expression analysis using these tissues. The experiments were possible due to the collaboration with Dr Myles Fleming and Dr Baljit Singh, University of Oxford.

Publication: N. Händel, A. Fanger, M. Heindl, E. Klein, H.H. Uhlig. Cell-cell-neighbourhood relations in tissue sections – a quantitative model for tissue cytometry. Cytometry (in press)
We present initial results of our work as poster presentation at the 4th Congress of ECCO - Inflammatory Bowel Diseases 2009. (February 5 - February 7, 2009 in Hamburg): Haendel et al. Local Fuelling Of IL-2 Determines The Tissue Localisation Of CD4+Foxp3+ Regulatory T Cells - Proliferating Cells Signal Inflammation And Provide IL-2.

Silvio Danese (ECCO Grant 2008)

Title of Fellowship: The role of lymphangiogenesis in IBD pathogenesis
Institute: Istituto Clinico Humanitas-IRCCS in Gastroenterology, Milan, Italy

PROGRESS REPORT:
The lymphatic vasculature complements the blood vascular network and its well known task is to transport extravasated fluid unidirectionally from tissues back to the blood circulation. Beside this function, the lymphatic vasculature plays a fundamental role in immunity. Indeed, lymphocytes and dendritic cells (DC) enter the lymphatic capillaries in the periphery and migrate through the lymph nodes to elicit acquired immune responses. We investigated lymphangiogenesis in Crohn's disease (CD) and ulcerative colitis (UC) by quantifying mucosal lymphatic vascularization state, and we isolated and characterized in vitro the functional properties of primary human intestinal lymphatic endothelial cells (HILEC).
Control (n=15), CD (n=15) and UC (n=15) colonic mucosa were immunostained for the lymphatic antigen D2-40, and vessels quantified. HILEC cultures were isolated from intestinal specimens and characterized in vitro by fluorescence with anti-Prox-1, LYVE-1 and Pal-E antibodies. HILEC were stimulated with TNF-α and investigated for VCAM-1 and ICAM-1 expression by flow cytometry and fluorescence microscopy.DC adhesion and migration were measured by adhesion and migration assays.
Microvessel lymphatic density was significantly ($p<0.05$) higher in CD (23±3) and UC (23±2) compared to control mucosa (6±1). Purity of HILEC was determined by 99% expression of Prox-1 and LYVE-1 and absence of Pal-E. Resting HILEC expressed undetectable levels of VCAM-1 (0.1%) and physiological levels (42%) of ICAM-1. After TNF- α stimulation both VCAM-1 (58%) and ICAM-1 (92%) were potently up-regulated, and significantly ($p<0.01$) bound more DC (6 fold increase) than resting HILEC. Adhesion was VCAM-1 ($p<0.05$) and ICAM-1 dependent, as demonstrated by the use of blocking antibodies against each CAM. Similar results were obtained with migration.
Based on morphological and functional evidence, it is concluded that IBD lymphatic microvasculature undergoes an intense process of lymphangiogenesis. This expanded lymphatic network is characterized by functional HILEC that are able to express CAM after pro-inflammatory cytokine stimulation, and to adhere and migrate DC towards lymp nodes. Our results provide the first report of

isolation and culture of a primary intestinal lymphatic endothelial cell line, and offer an essential tool for studying lymphatics in IBD. Furthermore, this study paves the way for considering anti-lymphatic strategies for IBD therapy, as currently being tested in other autoimmune disorders.

Richard Day (ECCO Grant 2008)

Title of Fellowship: Assessment of Bioactive Microspheres as a Prospective Novel Treatment for Fistulae
Institute: Windeyer Institute, University College London, London, United Kingdom

PROGRESS REPORT:
Chronic perianal fistulae are a challenging manifestation of Crohn's disease and significantly impair the quality of life in patients. The ECCO project is currently developing a microspherebased device specifically tailored for the delivery of anti-TNF antibody (infliximab) into Crohn's disease fistulae. The ECCO grant has funded the purchase of a Nisco Encapsulator Var D (Figure 1) and consumables required for the production of microspheres.

Figure 1. The Nisco Encapsulator used to fabricate themicrospheres being evaluated in the project. The encapsulator consists of a vibration unit and control cabinet. Polymer solution is fed into the vibration unit via a syringe pump before being dropped into a container holding liquid nitrogen. A stroboscopic unit facilitates visualization of the droplets.

The encapsulator unit has enabled the production of much smaller microspheres than could achieved by the manual production previously used, providing microspheres at a more feasible size for delivery into fistula tracts (Figure 2).

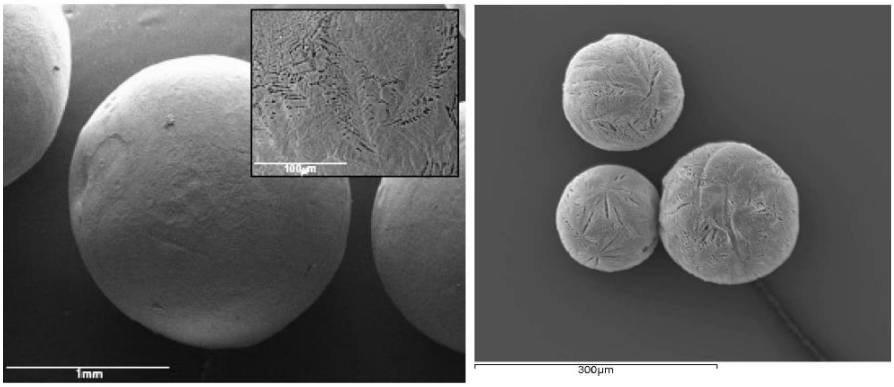

Figure 2. Microspheres produced manually (shown on the left) are too large for delivery into a fistula tract. The encapsulator unit enables the production of much smaller microspheres (shown on the right).

To date the project has investigated how different processing parameters affect the release profile and biological activity of the captured antibody. Three different approaches have been explored from which two are being further evaluated. The results from this part of the part of the project are being presented as an Oral Poster presentation at 4th Congress of ECCO 2009 (control number 500 - P82 'Fabrication and Assessment of Microspheres Loaded with Anti-TNF-_ as a Prospective Novel Treatment for Crohn's Disease Fistulae'). The next stage of the project will involve physical characterization of the antibody-loaded microspheres, further evaluation of the loading efficiency and release of antibody, and the effect on cell migration and cytotoxicity.

Fraser Cummings (ECCO Grant 2008)

Title of Fellowship: Biological markers to predit the outcome of acute severe ulcerative colitis
Institute: John Radcliffe Hospital, Oxford, United Kingdom

PROGRESS REPORT:
This is a prospective study to try and identify biological markers which will predict outcomes in acute severe ulcerative colitis (ASUC). The study protocol dictates that patients must meet the strict Truelove-Witts criteria to be considered for the study and that the FACS analysis is done on fresh blood samples rather than stored cells.

Recruitment
The study originally aimed to recruit 25 patients and 15 controls (quiescent UC patients having surveillance colonoscopy carried out). All 15 control patients will have been recruited by 3rd February 2009. A total of 8 patients with ASUC have been recruited. Three patients who met the criteria could not be recruited due to the unavailability of personnel whilst another 7 patients did not meet Truelove – Witts criteria. Three patients have subsequently been shown to have infective colitis. Whilst it was not anticipated that these patients would be recruited, they will be used as a very interesting and important additional control group.

Results

FACS staining has been carried out on all samples recruited thus far. Numbers are too small to carry out any meaningful analysis in terms of outcomes as yet, however a number of interesting insights into T-regulatory lymphocytes biology have already being gained, for example figure 1 may suggest there are changes in percentages of CD4CD25 populations in response to treatment. qPCR is currently under way to study these makers in the paired mucosal biopsies. The panel of markers being used has been expanded to include recent advances such as the Th-17 pathway.

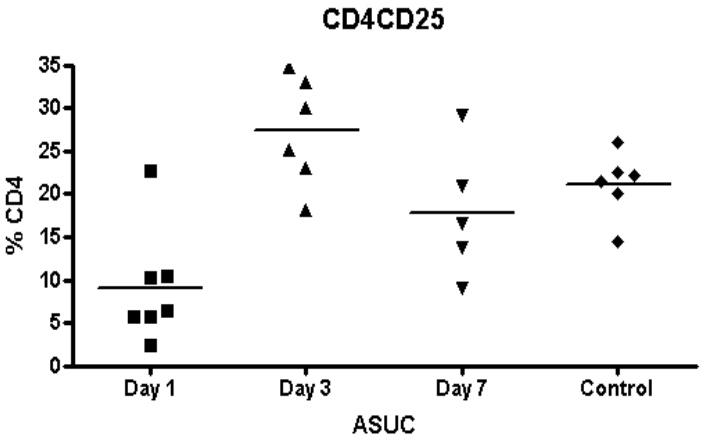

Figure 1 Percentage change in CD4CD25 lymphocytes over time in response to treatment for ASUC in peripheral blood.

Samples for proteomic analysis are being stored until study recruitment is complete when these will be processed as a batch.
Recruitment of ASUC patients is continuing and it is anticipated that the study will be successfully concluded within the next year.

Nikolaus Pedarnig (ECCO Grant 2008)

Title of Fellowship: European-wide Validation of the Web-based Documentation Standard IBDIS by Inter-observer Analysis
Institute: Universitätsklinik für Innere Medizin III, AKH, Vienna, Austria

SYNOPSIS:

Date	Content
03.07.08	Initial Call for participation (ECCO individuals and NatReps)
17.07.08–27.10.08	Confirmation for participation of 123 observers of 37 countries for INTERobserver Agreement analysis: Albania, Austria, Belgium, Bosnia & Herzegovina , Brazil , Bulgaria, Canada, China (PRC), Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece , Hungary , Ireland, Israel , Italy , Latvia , Lithuania , Netherlands, Poland, Portugal, Romania, Russia, Saudi Arabia, Serbia, Spain, Sweden, Switzerland, Taiwan, Tunisia, Turkey, Ukraine, United Kingdom, Uruguay
12.08.08	Final call for participation
09.01.09	Closing of data capture 57 observer entered at least one patientfile, 5 Users entered 1 Patientfile 5 Users entered 2 Patientfiles 2 Users entered 3 Patientfiles 0 Users entered 4 Patientfiles 2 Users entered 5 Patientfiles 0 Users entered 6 Patientfiles 0 Users entered 7 Patientfiles 0 Users entered 8 Patientfiles 0 Users entered 9 Patientfiles 0 Users entered 10 Patientfiles 0 Users entered 11 Patientfiles 0 Users entered 12 Patientfiles 1 Users entered 13 Patientfiles 1 Users entered 14 Patientfiles 41 Users entered 15 Patientfiles Number of Blocks entered by the 57 observers: BLOCK A Epidemiology: 996 BLOCK B Location: 8327 (i.e. Patient 1 had 6 examinations like Ileocolonoscopy or MRI) BLOCK C Phenotype: 902 BLOCK D Course of disease: 844 BLOCK E Complications: 810 BLOCK F Intestinal Surgery: 656 BLOCK G Comorb / Risk: 785 BLOCK H Pregnancy: 967 BLOCK I Therapy: 2251 41 observer entered all 15 patient patientfiles

15.1.09	Nomination of 7 randomly elected INTRAobservers for IOA

Next steps:

Date	Content
31.03.09	First results of statistical analysis INTERobserver data
31.03.09	Closing of data capture for INTRAobservers
31.04.09	First results of statistical analysis INTRAobserver data
30.06.09	Validation report

Cesare Ruffolo (ECCO Travel Award 2008)

Host Institute: UZ Leuven, Leuven, Belgium
Supervisor: Prof. Freddy Penninckx

FINAL REPORT:
During my 3-month stay as a visiting fellow at the Department of Abdominal Surgery, University of Leuven, Belgium, under the supervision of Prof. Freddy Penninckx and Prof. André D'Hoore, I actively assisted in numerous surgical procedures of both Crohn's disease and ulcerative colitis which markedly contributed to my surgical training and experience. I came into contact and discussed IBD topics with the gastroenterological team (Prof. Rutgeerts and colleagues). I also reviewed the early and late results of surgery for rectovaginal fistulas in the era of biological therapies of 52 Crohn's disease patients. I prepared a manuscript regarding this subject that has been submitted for possible publication in an international peer reviewed journal. Furthermore, this subject was also included in my PhD thesis. Finally a protocol for a prospective study to be performed in collaboration with the University of Padova on local cytokine profile in patients with perianal Crohn's disease was prepared and is now being examinated by the ethical committees of these institutions.

Andrea Cassinotti (ECCO Travel Award 2008)

Host Institute: John Radcliffe Hospital, Oxford, United Kingdom
Supervisor: Dr. Simon Travis

FINAL REPORT:
Last April I started my visit in Oxford thanks to the first Travel Award program organized by ECCO. During my attendance, I joined the local gastroenterology staff, lead by dr Travis, in all clinical and educational activities. This was an opportunity to learn new ways to think about IBD. I mean not only concerning diagnosis and therapy of IBD (the ECCO guidelines are spreading worldwide), but also about the way to organize the everyday activity of each leading actor (from nurses to doctors to secretaries). This gave me the favourable impression of an excellent interactive staff, able to guarantee an effective clinical approach and relationship to the patient. I discovered the new figure of IBD specialist nurse, as

well as a particular interest to intestinal failure and its nutritional management, with very professional dieticians and nutritional nurses.
Very stimulating and didactic was the attendance of histology, X-ray and multidisciplinary meetings and teaching sessions for specialist registrars. The saying of the local Cairn's library sounding "Clinical Practice, Research, Education", reflects the aim of this hospital, where all the different aspects of medicine are taken in consideration.
The specific scientific purpose of my visit was also to undertake a project comparing IBD care in Oxford and Milan using the validated National UK IBD Audit tool. This was another interesting opportunity to acquire more awareness about my clinical practice, through the comparison of the achievement of well defined clinical parameters as a reflection of good IBD management. Preliminary results of the study will be presented as oral poster presentation during the incoming ECCO congress, while a full paper will be soonly submitted for publication.
The National UK IBD Audit tool is an electronic database created to improve the quality and safety of care for IBD patients by auditing individual patient care, service resources and organization against national standards. Briefly, in this study we compared the organization and process of IBD care between 2 IBD services in Oxford (UK) and Milan (Italy), using the National UK IBD Audit tool, as a pilot study to evaluate its application outside national boundaries. Clinical and demographic data of patients with CD and UC, consecutively admitted during a 2 month period, were collected and compared between the 2 centres, to each other and to the UK IBD standards obtained by previous audit analyses performed in Oxford in 2006. Twenty and 26 patients with UC were enrolled in Oxford and Milan, as well as 21 and 20 patients with CD, respectively. Most admissions in Milan were planned admissions for moderately active treatment-refractory disease. No patient died. Oxford had a higher surgery rate. Endoscopy for UC consisted mainly of colonoscopy in Milan and flexible sigmoidoscopy in Oxford. In CD, Oxford data revealed a higher use of immununomodulators and CT scan, compared with higher use of bowel ultrasound in Milan. CRP was the preferred biomarker of disease activity. The following areas did not reach the standards set for the 2006 UK IBD Audit: the lack in Milan of IBD specialist nurses and few dietitian visits, as well as little attention to heparin prophylaxis and abdominal radiography in UC. Both sites paid little attention to stool coltures and revealed a high rate of active smokers in CD and little attention to bone protection in steroids users. Since the 2006 audit in Oxford, changes include IBD specialist nurse visits, dietitian visits, number of active smokers, stool samples, prophylactic heparin, bone protection and nutritional assessment. In conclusion, procedural differences between Oxford and Milan are quite similar for audits of both UC and CD, indicating systematic differences that could be resolved by organisational change. The UK IBD Audit tool is an easy instrument to assess the processes and outcomes of care delivery in IBD and can be applied also outside UK. A further result of the active interaction between Milan and Oxford, established thanks to the travel grant, was the preparation, by dr Travis and I, of a review of the literature on one intriguing issue about biological therapy in IBD, in particular on the role of immunogenicity to infliximab. A full paper has been prepared and recently accepted for publication in Inflammatory Bowel Disease.
Dr Travis was also so kind to help me to organise a further 1 month –visit, until the middle of July, as a visitor of the laboratory of Immunology at the Sir John Dunn School of Pathology of the Oxford University, directed by prof. Fiona

Powrie. Both the director and the site were guarantor of excellent opportunity to be updated about current IBD basic research. I get familiar with their projects on the role of the IL23/IL17 pathways, T-regulatory cells and innate immunity in IBD. This was a very stimulating period, absolutely original compared with my routine practice in Milan, and it was the source for new ideas and future projects. In conclusion, I can only thank ECCO and the IBD experts which I had the honour to meet, for the great experience I made and that I strongly advise to all youngs with special interest in IBD. I do not have specific considerations to make to improve the program, a part of the fact that applicants have to be aware that additional funds are necessary for every day life, especially in expensive countries like England, as well as the frequent need to have a good umbrella…to be repaired from the traditional sun of this nice country!

2007

Konstantinos Karmiris (ECCO Fellowship 2007)

Title of Fellowship: ECCO fellowship: a fascinating scientific and social adventure
Host institute: Leuven, Belgium

FINAL REPORT:
Mission: research fellow in the Laboratory of inflammatory bowel diseases in the University Hospital in Leuven under the auspices of Professor Paul Rutgeerts. February 2007: first days in Leuven are quite peculiar. I had to adapt to a new working environment with many new colleagues, get used to my new home and neighborhood, realize that I had moved from South to North Europe (never got used to the climate, always seeking the sun and the blue sky of my place and always checking if the umbrella is in my bag) and above all fight the homesick feeling for my wife and 2 year old daughter…major change. The fascinating triad: Paul Rutgeerts is a great Professor always very friendly despite being a leading expert. His advices and guiding comments during the lab meetings were always valuable and time sparing for my project. Severine Vermeire is a unique scientist, woman and recently mother. In other words "the queen of IBD". Gert Van Assche is an excellent combination of a scientist, a doctor, a teacher, a family leader and a friend. Severine, Gert, I owe you a lot for my successful work in Leuven. I will try to use the way you combine science with clinical work and also your modest character as my inspiration for the future. The lab: Maja, Marc, Liesbet, Marko, Fabian, Herma, Kristine, Roger, Isabelle, Ingrid, Marie, Natalie, Vicky, Lieselot, Els, Karolien, Vera, Tamara, Nele, Sofie, Isolde, An, Karolien. So many different personalities. Thank you all for the fruitful collaboration. I enjoyed also our social events and company. I even had the chance with some of you to visit places in Belgium and taste so many different beers and chocolates. June 2008: the end of an interesting journey. What Leuven meant to me? Well, I viewed Gastroenterology from a very educative perspective, became a better scientist, experienced a different culture, lived in a very beautiful city, met some interesting people and made some good friends. From my side, I tried to impart a little of the Mediterranean temperament and the lifestyle. But again, isn't this a

major aim of ECCO when calling for fellowships? To bring people from different European countries and cultures together? If this is the case, mission was successfully accomplished.