Research Portfolio
Neuro-immune group
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1-models for visceral hyper-perception; Irritable Bowel Syndrome (IBS)

Background.

IBS is a functional bowel disorder characterized by abdominal pain or discomfort associated with changes in bowel habit. Although increased sensitivity to rectal distension (so called visceral hypersensitivity) is considered a possible pathophysiological mechanism[1], the exact aetiology of IBS is not known.

The model.

Impaired parent-child interactions are considered a possible environmental trigger for IBS[2] and animal models reflecting this trigger may, although they can never fully reflect complex human parent-child interactions, contribute to a better understanding of this disorder. For this reason we use the **maternal separation model in rats** in which pups are separated from their mothers for 3 hours daily from postnatal days 2 to 14.[3] In contrast to nonhandled (NH)-rats, adult maternal separated (MS) rats display an IBS-like phenotype (intestinal barrier dysfunction and visceral hypersensitivity). Importantly, onset depends on rat strain; Long Evans rats only show hypersensitivity after an additional acute stress at adult age while this is not required in Wistar rats. In our group we prefer to work with Long Evans because also in IBS-patients acute stress is an important trigger for symptom generation.[4]

Results with this model.

Using this rat model we were able to show that stress-induced visceral hypersensitivity depends on the degranulation of mast cells.[5, 6] In a translational follow up study with the mast cell stabilizer and histamine-1-receptor (H1R) antagonist ketotifen we confirmed the possible relevance of these cells.[7] Ketotifen not only decreased abdominal pain and other IBS symptoms but also improved health related quality of life and increased the threshold of discomfort in hypersensitive patients. Investigations comparing pre- and post-therapy mediator release by submerged rectal biopsies suggested that H1R antagonism was the main molecular mode of action in this trial. Because ketotifen crosses the blood brain barrier we subsequently tested two highly selective and peripherally restricted H1R antagonists in our rat model. Results (unpublished) showed reversal of post stress hypersensitivity by both antagonists and we are now evaluating one of these antagonists in a new clinical trial.

Potential use for research partner.

We used the maternal separation model to **evaluate pharmacological interventions** capable of reversing post-stress visceral hypersensitivity.[5, 6, 8] Importantly, as shown by our mast cell data, **results obtained in this model can be translated to IBS**.[7] Others used it to **examine the effect of probiotic interventions**[9-12] and our own most recent data (unpublished) indicated that **other dietary interventions** might also proof beneficial. Further, we recently demonstrated that the enhanced susceptibility to stress-induced hypersensitivity observed in maternally separated (F1) rats can be transferred to next generation (F2) animals without further separation protocols (submitted for publication). This is highly relevant because IBS was shown to cluster in families in the absence of clear genetic components. Our data open up possibilities to **investigate treatment strategies that may interfere with transfer across generations** (e.g. treatment of mothers or children).

2-models for functional bowel and motility disorders; Postoperative Ileus (POI)

Background.

POI is characterized by dysmotility of the gastrointestinal tract that occurs after essentially every abdominal procedure and results in increased patient morbidity and prolonged hospitalisation.[13] The costs related to POI have been estimated to amount 1.47 billion dollars annually in the USA, illustrating its large socio-economical impact. Recent evidence obtained in mouse POI models indicated that ileus following bowel manipulation is a biphasic process.[13] An acute phase of generalized enteric hypomotility is due to activation of inhibitory neural reflexes.

The model.

Specific surgical intestinal manipulations are performed in a routine fashion. Graded severity of the manipulation is optimized (inspection, severe handling). *In vivo* motility is measured in life mice in real time.

Results with this model.

Preclinical investigations from our own group significantly demonstrated that a subsequent prolonged phase is mediated by mechanical manipulation-induced inflammation of the intestinal muscularis externa.[14-19] This inflammation results in hypomotility of the entire gastrointestinal tract. In our mouse model we showed that manipulation induced mast cell degranulation is an essential step in the induction of this inflammatory process[20, 21] and we were able to subsequently translate these results to the human setting.[22, 23] Recent results have demonstrated IL-1 activation and receptor triggering is a crucial contributor to inflammation-induced motility disturbance (Stoffels et al, Gastroenterology, under review 2012)

As demonstrated by our translational research in POI, this mouse model provides reliable methodology to assess

1) **mechanisms** relevant to this iatrogenic condition and

2)**pharmacological interventions** that may benefit patients.

Potential use for Research partner.

The demonstration of dietary components, or pre, or probiotics to relieve post-operative motility disturbances and enhance post-operative recovery in a clinically relevant model. Recently, the successful tests of dietary polyunsaturated fatty acids (PUFA) in this models model for mouse POI suggesting that **dietary intervention strategies** can also be evaluated in this model system.[24]

3-models for experimental Colitis and *in vitro* approaches on patient intestinal resection material

Background.

The Department of Gastroenterology and Hepatology of the Academic Medical Center in Amsterdam is an expert and referral centre for IBD and has a longstanding tradition of excellent patient care combined with both clinical and fundamental research activities. Since several years now its fundamental IBD-research is bundled in the Tytgat Institute where it is part of the research theme of 3 different principle investigators (GR van den Brink, AA te Velde and WJ de Jonge). The **close collaboration between the Tytgat Institute and clinicians of the Gastroenterology department** is an important strength of our approach.

The models.

Routinely used models in our lab are:
-DSS colitis
-TNBS colitis
-CD45RB^{high} Tcell transfer colitis (Powrie model)

Results with this model

In the Netherlands, but also internationally, the AMC fulfils a leading role in the treatment of IBD. In the past we introduced the concept to treat patients with Crohn's disease with antibodies against tumor necrosis factor (anti-TNF),[25] a breakthrough in the therapy of this disabling disease. Over the years, other novel insights in the pathogenesis and genetics of IBD have been introduced, including the importance of IL-10 as potential treatment. The latter has led to the generation of genetically manipulated lactobacillae producing IL-10 (tested in patients with Crohn´s disease[26]). At present the clinical IBD group is headed by Prof G. D'Haens who plays a crucial and coordinating role in phase I and II clinical trials evaluating the therapeutic potential of new biologicals. His internationally acclaimed expertise ensures **timely 'bench to bedside' translation** of results obtained in the Tytgat Institute.

At the Tytgat Institute **state of the art *in vitro* assays and *in vivo* models** (used on a routine basis) allow us to investigate the role of pathways relevant to the development of IBD. In a large number of publications we not only used conventional mouse models like DSS- and TNBS-colitis but also the CD45RB T cell transfer model that most closely reflects human IBD. These models were used to establish **basic mechanisms in IBD as well as pharmacological and dietary intervention strategies** (example publications[27-32]).

Since we have **broad access to patient tissues and isolated cells** we also validate our experimental data by immunological phenotyping and activation of mucosal lymphocytes, dendritic cells and macrophages. Data obtained in these *in vitro* investigations can be **correlated with clinical phenotype and genetic profile**.

Potential use for Research partner.

These models are optimized for testing of pharmaca, biological, or nutritional interventions to relieve the severity of colitis. An *in vivo* mouse endoscope has been recently set up to be able to perform life imaging of the colitic lesions, and the recovery due to experimental treatment.
Human resection material can be processed in MLII environments. All ethical approval is in place for the use of human material in test setting in our lab.

4-primary epithelial cultures and epithelial cells lines

Background.

A clear disadvantage of the use of clonal epithelial cell cultures (such as the commonly used Caco-2 or HT29 cell lines) is the fact that those are usually tumor cell derived and may not represent the full spectrum of specialized epithelial cells. In order to assay the true impact of shifts in metabolic activity of bacteria we have adapted the crypt-organoid culture as described earlier.[33-35]

The model.

To allow study of the relevance of for instance **Paneth cell function** and antimicrobial peptide secretion, as well as epithelial responses to a changed probiotic or prebiotic influence and the metabolic consequences thereof, we have set up the matrigel-based culture system for intestinal crypts in our laboratory (see Fig 1). In this culture system containing EGF, the Wnt agonist R-spondin 1 and the BMP inhibitor noggin, single crypt stem cells autonomously grow into crypt-like structures with de novo generated stem cells and Paneth cells at their bottom.

Results with this model

The Paneth cells amount up to 10% of the epithelial culture-all other relevant specialized epithelial cell types are represented such as neuroendocrine cells (marked by their expression of ChAT *in vivo* (A) as well as in organoid culture (B). An example of such an organoid culture is given in Fig. 1. As described earlier, Paneth cells are clearly distinguishable in these cultures (indicated by arrows in the figure). We have confirmed the presence of transcript and protein expression of relevant anti-microbial peptides in these organoid-derived cells in our own lab. Next to *in situ* hybridization studies (Fig1D), we have also interesting RNA-sequencing and microarray data available on the cytokine induced changes in **epithelial retinoic acid** metabolism that directly relate to the role of microbial influences on mucosal immune tolerance.

Clearly, these crypt cell based organoid cultures have as advantage that all **relevant epithelial cell types** (i.e. Paneth cells) are fully represented in the culture. Our group at AMC, in collaboration with the Gastroenterology Department, is further exploring the possibility to adapt the human crypt cell cultures as described earlier(2011l) at different levels and there is a clear opportunity for Research partner probiotic products to be tested in this system.

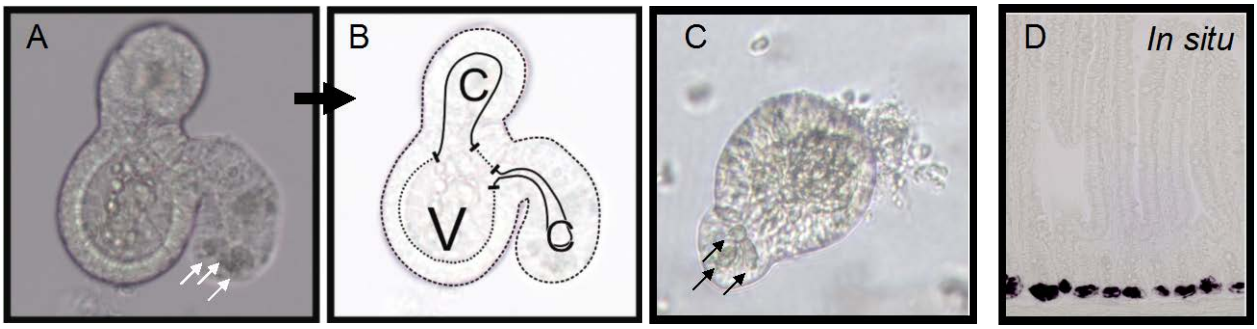

Fig 1. An example of a 5-day organoid culture of crypt cells grown in matrigel*. Panel A an B show clearly distinguishable Paneth cells in the crypt areas (arrows (A), in the crypt area (indicated by C)), and the villus dome (V). On the right panel C an earlier stage organoid is shown with autonomously proliferating crypt cells giving rise to Paneth cells indicated with arrows. Panel D shows tissue Paneth cells in the villus crypt using in situ hybridization for lysozyme transcript. WJ de Jonge and GR van der Brink; Unpublished, Tytgat Institute 2011.*

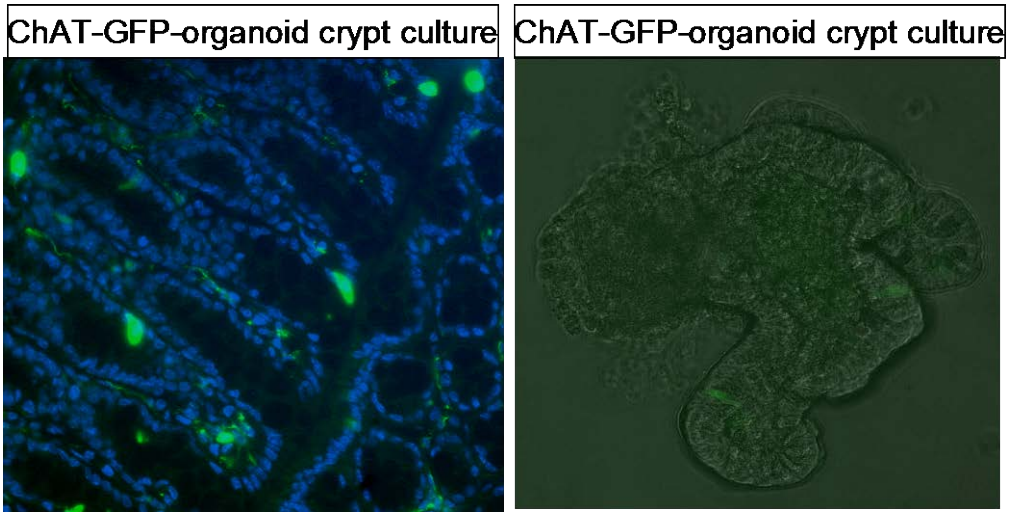

Fig.2 epithelial cells express enzymes for the production of acetylcholine (ChAT- shown as green signal in left) that can be found back in organoid systems (right).

Potential use for Research partner.

This culture setup allows us to study the direct effects of pre- and probiotics but also microbial metabolic factors that regulate epithelial and Paneth cell peptide expression and secretion of antimicrobial peptides in an easily manipulated *ex vivo* setting. Further, we can study the effect of inflammatory cytokines that intervene in transcriptional regulation of antimicrobial peptide expression and secretion.

Parameter measured include protein analyses but also transcriptome analyses and Q-PCR of epithelial cells. Antimicrobial peptide secretion (b-defensin A1-4, Lysozyme) can be analyzed by IHC and *in situ* hybridization that we do routinely in the laboratory (see for instance Fig 1D). furthermore human organoid systems are currently set up and will be operational shortly. Lentiviral shRNA strategies are in place to study specific gene regulation of epithelial responses.

5-further relevant assays for collaboration.

-Next to routine lab analyses we run multiple assays in our lab including a wide variety of ELISAs, Multiplex analyses, and state-of-the art FACS-sorting facility.

-The AMC houses an image suite with 2 confocal (new generation R8), life imaging and multiple fluorescence microscopy options

-RNA-seq and ChIP Sequencing is done in house at the laboratory of Genome sequencing (prof F Baas).

-Human material is routinely processed and banked in a Biobank (being part of the "string of pearls" the AMC has large biobanking availability). Importantly, we run state of the art isolations of specific cell types from human intestinal tissue.

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