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## ***Chlamydiae* in Gastrointestinal Disease**

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### **1. Introduction**

Disorders of gut function are among the most prevalent problems presented to physicians practicing in gastroenterology as well as primary care physicians and irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorder. There is no biomarker of IBS. Instead, the diagnosis is based on symptom criteria and the absence of an organic cause for symptoms (Longstreth et al., 2006). Patients with IBS complain of recurrent abdominal pain, bloating after meals, and disturbed bowel habits. The population prevalence of IBS shows considerable variation between countries. In Asian countries the prevalence varies between 4.0%-22.1% (Makharia et al., 2011), in Europe 6.2%-12% (Hungin et al., 2003) and in North America 11.6%-12.1% (Thompson et al., 2002). In most studies females dominate over males with a factor of 1.4-1.9.

An intriguing finding is that 6%-17% of patients with IBS report that the onset of their disease followed from an acute infection (Spiller, 2007). So called post-infectious IBS (PI-IBS) has mainly been reported after bacterial gastroenteritis but over the years PI-IBS has been reported also after infection with viruses, worms, and protozoa. A study from our group showed that the infectious agent *per se* was not a risk factor for PI-IBS (Törnblom et al., 2007) and this led to the hypothesis that a host factor could be the determinant of PI-IBS.

Although IBS is not a life-threatening disease, the chronic nature of IBS tends to interfere with the normal daily life and IBS can have a strong impact on patients' quality of life (Amouretti et al., 2006; Drossman et al., 2009). IBS has been associated with fibromyalgia, chronic fatigue syndrome, temporo-mandibular joint disorder and chronic pelvic pain (Williams et al., 2004). Despite its abundance the cause of IBS has remained unclear. It has long been considered a psychosomatic disorder but a number of studies have reported signs of immune activation with increased numbers of lymphocytes, activated macrophages or mast cells in mucosa biopsies from the small bowel or the large bowel (Akiho et al., 2011). We investigated full-thickness biopsies from the jejunum of 10 patients with IBS and found increased numbers of mucosal lymphocytes in 4/10 patients (Törnblom et al., 2002). We also found low-grade inflammation of myenteric plexa in 9/10 patients and neuron degeneration in 7 patients. These findings were confirmed in a larger series of patients with severe IBS and the concomitant finding of enteric dysmotility (Lindberg et al., 2009). The driving force behind observed immune activation is not known but both innate and adaptive immune responses seem to be involved in IBS pathogenesis (Öhman & Simrén, 2010).

The gastrointestinal tract is also the largest endocrine organ in the body. Enteroendocrine cells (EEC), which are present in the mucosa of the stomach, small intestine, colon, and

rectum, are highly specialized cells that produce hormones and other signalling substances that are vital to the normal function of the gut. Due to their diffuse localisation EEC are difficult to study and so far, their role in the pathogenesis of bowel disorders has been only little explored. A well-characterised subset of EEC are the enterochromaffin cells, which are the main source of the biogenic amine serotonin (5-hydroxytryptamine, 5-HT) in the GI tract (Kim & Camilleri, 2000). The aminoacid tryptophan serves as a precursor for the production of serotonin, which is transported from the cytosol to large dense-core secretory vesicles (LDCV) by the vesicular monoamine transporter (VMAT1) in the membrane of LDCV (Jakobsen et al., 2001). Serotonin influences the intestinal homeostasis by altering gut motor activity and secretion and has been implicated in the pathophysiology of various GI disorders, including inflammatory bowel disease (IBD) and IBS (Sikander et al., 2009).

## 2. Hypothesis and aim of studies

Life style, genetic risk factors and the individual's microbiome can all act as host factors for disease. The latter includes the microbial flora of skin, airways, uro-genital organs and the gastrointestinal tract. Over the years we also become populated by a number of viruses, intracellular bacteria and parasites that have the ability to remain in our own cells. One typical example is *Varicella zoster virus* (VZV), which causes chickenpox in children or adults when first infected. After healing of the acute infection virus will remain in a persistent state in nerve cells and can later become reactivated causing shingles. Several bacteria including *Mycobacterium tuberculosis*, *Salmonella enterica* serovar Typhi, and *Chlamydia spp.* also have the ability to survive in a persistent state (Monack et al., 2004).

We hypothesized that a pre-existing persistent infection could be an important host factor for the development of IBS, most evident in so called post-infectious IBS. A candidate agent should be compatible with an asymptomatic carrier-ship, have a preference for female gender, and have the ability to become persistent and to live in bowel epithelium. There were several observations to support the idea that a persistent infection with *Chlamydia trachomatis* might constitute such a host factor. Trachoma related blindness is 2-4 times more likely to affect females compared to males (Courtright & West, 2004). It is known that IBS occurs in a high proportion (35-38 %) of females with chronic pelvic pain syndrome, which is often caused by a chronic infection with *C. trachomatis* (Williams et al., 2004; Zondervan et al., 2001). Previous experience from veterinary medicine has shown that *Chlamydia spp.* can infect the intestines of calves and sheep and also lead to persistent infections (Storz & Spears, 1979).

The aim of our studies was to find out if a persistent infection of the gut with the obligate intracellular pathogen *C. trachomatis* might have a role in the pathogenesis of IBS. At the beginning of our studies it was unknown whether *Chlamydia spp.* could reside in the human gut or not. It was also unknown if *Chlamydia* had any particular cell tropism that would be compatible with a long-term persistence in the gut.

The thought that *C. trachomatis* could be involved in IBS was not unique. Based on the frequent concomitance of IBS and pelvic inflammatory disease an attempt was made to link *C. trachomatis* to IBS using serum IgG antibodies but the study showed no significant difference between control subjects and patients with IBS (Francis et al., 1998). However, we think that IgG antibody patterns may be insufficient to rule out persistence of *Chlamydia* due to a dominating cellular immune response to infection (Witkin, 2002).

### 3. Chlamydial antigens in small bowel mucosa of patients with IBS

We investigated archived biopsies from the small bowel of 65 patients (61 females) with IBS (Dlugosz et al., 2010). IBS was defined according to the Rome-II criteria (Thompson et al., 1999). Full-thickness biopsies of the jejunum were available in 60 patients and mucosa biopsies from the jejunum or the duodenum in 24 patients. We recruited 32 (22 females) healthy controls, which underwent mucosa biopsy of the jejunum and we utilized archived full-thickness small bowel biopsies from another 10 (7 females) obese but otherwise healthy control subjects. In order to detect chlamydial antigens we used a genus-specific mouse monoclonal antibody to *Chlamydia* lipopolysaccharide (LPS) that was FITC conjugated with Evans blue (RDI-PROAC1FT, Fitzgerald Industries International, Concord, USA). We also used a mouse monoclonal antibody to *C. trachomatis* major outer membrane protein (MOMP) (Gene-Tex, San Antonio, USA) and a species-specific mouse monoclonal antibody to *C. pneumoniae* as primary antibodies with a polyclonal rabbit anti-mouse antibody-FITC conjugated (Dako, Glostrup, Denmark) as secondary antibody. In addition we used a number of cell-specific antibodies to identify different cell types. Enteroendocrine cells were identified using antibodies to chromogranin-A, mast cells using antibodies to CD117, macrophages using antibodies to CD68, and dendritic cells using antibodies to CD11c.

From each biopsy 6 new sections were taken up for immunofluorescence staining. Sections were investigated using a fluorescent microscope (Leica DMRXA, Leica Microsystems, Wetzlar, Germany). Positive staining for *Chlamydia* LPS was seen in triangular cells mainly at the crypt level (Figure 1A) but also in irregularly shaped cells in *lamina propria* (Figure 1B). Staining for *Chlamydia* LPS was positive in 58/65 patients with IBS and only 6/42 controls. The odds ratio, corrected for differences in age and gender distributions, for mucosal *Chlamydia* LPS being indicative for presence of IBS was 43.1 (95% CI: 13.2-140.7).

No LPS-positive cells were found in the deeper levels of the bowel wall, i.e. submucosa, muscle layers and the enteric nervous system. Staining for *C. trachomatis* MOMP (Figure 1C) was positive in 69% of LPS-positive biopsies, including 2/6 LPS-positive biopsies from control subjects, but none was positive in staining for *C. pneumoniae*.

Further characterization of LPS-positive cells was done using double staining with antibodies to LPS and cell specific markers. Double staining with antibodies to LPS and chromogranin-A showed that *Chlamydia* LPS was present in enteroendocrine cells of the epithelium (Figure 1D-F). Similarly, double staining with antibodies to LPS and CD117, CD11c and CD68 showed that in *lamina propria* the LPS-positive cells were macrophages (Figure 1G-I). In order to validate our findings of *Chlamydia* LPS we examined 20 slides (10 LPS-positive and 10 LPS-negative) using a different antibody to *Chlamydia* LPS, a polyclonal rabbit antibody (Fitzgerald Industries International, Concord, USA) and an immunoenzymatic assay with Streptavidin-Biotin Complex (Dako, Glostrup, Denmark). All 10 biopsies that were positive for *Chlamydia* LPS remained positive also in this experiment, whereas all LPS-negative biopsies remained negative. We took new biopsies from 4 patients in whom a previous biopsy had been positive for *Chlamydia* LPS. The new biopsies were also positive for *Chlamydia* LPS in immunofluorescence and the presence of LPS was confirmed using Western blot analysis. However, an attempt to confirm the presence of bacteria using real-time PCR after amplification of 23S ribosomal DNA (Everett et al., 1999) was negative in all 4 biopsies.

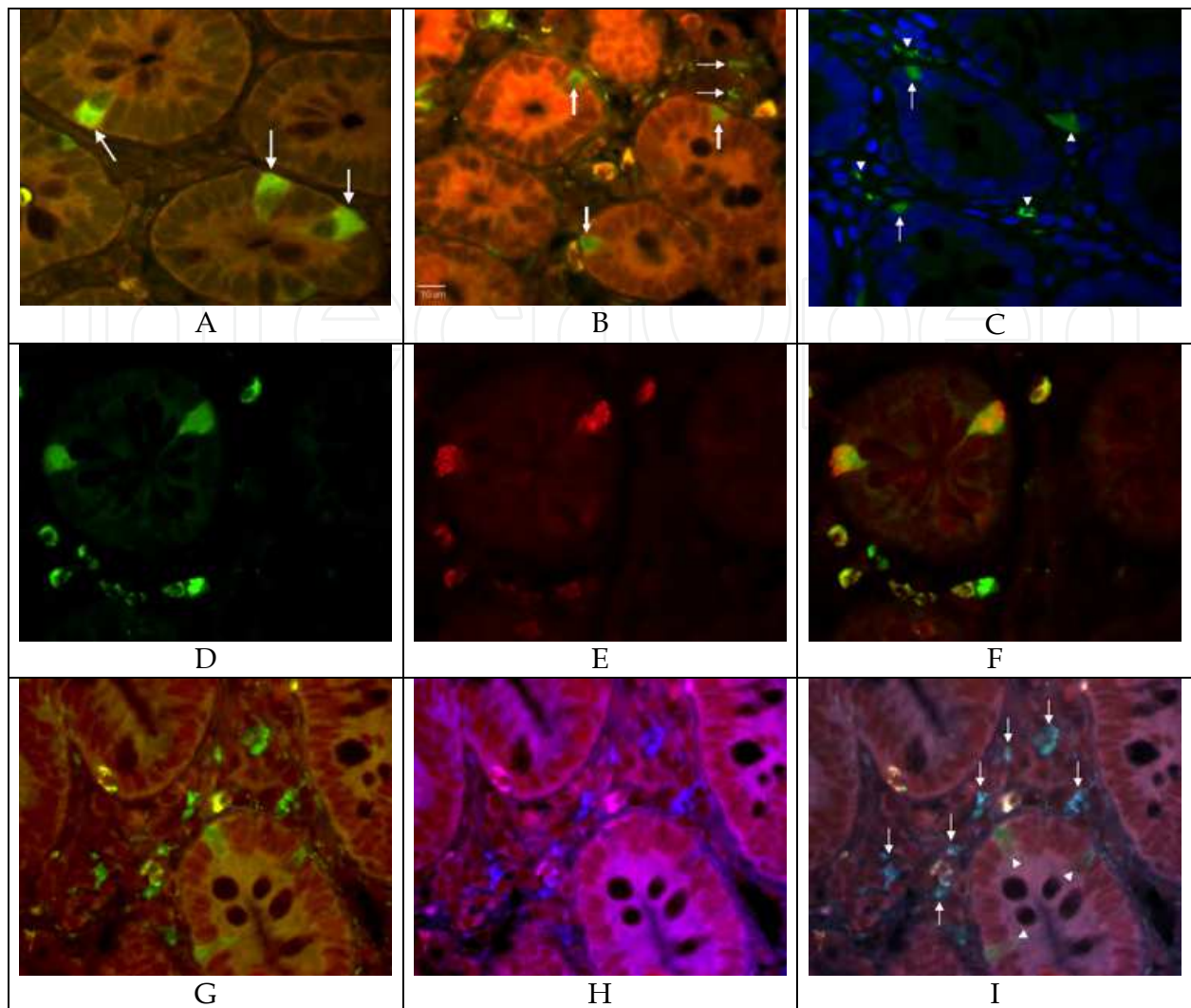


Fig. 1. Fluorescent microscope images of small bowel preparations from patients with IBS. A: *Chlamydia* LPS in EEC-like cells with apical nuclei and strong basal immunofluorescence (arrows); (Monoclonal FITC-conjugated antibody with Evans blue; original magnification  $\times 63$ ). B: *Chlamydia* LPS in a few cells within the epithelium (thick arrows) and lamina propria (thin arrows); (Monoclonal FITC-conjugated antibody with Evans blue; original magnification  $\times 63$ ). C: *Chlamydia trachomatis* MOMP-positive immunofluorescence within 2 EEC-like cells (arrows) and 4 cells within *l. propria* (arrowheads); Mouse MOMP-antibody and FITC-conjugated rabbit anti-mouse antibody; original magnification  $\times 63$ ; Hoechst (DAPI conjugated) for nuclear staining. D-F: Immunostainings for (D) *Chlamydia* LPS (FITC, green); (E) chromogranin A (Alexia 568, red); and (F) merged showing co-localisation of chromogranin A and *Chlamydia* LPS in enteroendocrine cells and *Chlamydia* LPS in lamina propria. G-I: Immunostainings for (G) *Chlamydia* LPS (FITC, green,); (H) CD68 (Alexia 350, blue); and (I) merged showing co-localisation of CD68 and *Chlamydia* LPS in macrophages (arrows). Three enteroendocrine cells are also positive for *Chlamydia* LPS (arrowheads).

Reproduced from Dlugosz et al., *BMC Gastroenterology* 2010, Vol. 10, p. 19 doi:10.1186/1471-230X-10-19.

Thus, we found chlamydial antigens in enteroendocrine cells and macrophages of the small bowel mucosa. Chlamydial antigens were present in biopsies from 89% of patients with severe IBS but in only 14% of controls. The odds ratio for mucosal *Chlamydia* LPS being indicative for presence of IBS is much higher than any previously described pathogenetic marker in IBS (Öhman & Simrén, 2007).

In absence of DNA proof of bacterial presence several questions remain to consider. One is whether observed immunofluorescence findings represent true presence of bacterial antigens or if they are unspecific findings or artefacts. We think that unspecific or artefactual binding is unlikely in view of the difference between patients and controls. The genus-specific epitope for our anti-LPS antibody is not shared by other Gram-negative bacteria and monoclonal antibodies to this epitope do not bind to LPS from those organisms (Caldwell & Hitchcock, 1984).

Another question is if observed antigens represent an ongoing infection or remainders of a past infection. In 19 patients biopsies we had access to more than one biopsy that had been taken with a time interval of at least 1 year. *Chlamydia* LPS was present in biopsies with a median time difference of 5.2 (range 1-11) years. Such a long-term presence of chlamydial antigens is most likely attributable to replicating *Chlamydiae* residing in the diseased tissue (Beatty et al., 1994).

The finding of *C. trachomatis* antigens in EEC makes it tempting to suggest a novel pathogenetic mechanism in IBS. The enteroendocrine system is crucial in particular to the digestive functions of the gastrointestinal tract. Serotonin-producing EEC may present an ideal location for *Chlamydia* due to the abundance of tryptophan and tryptophan metabolites in these cells. Tryptophan is required for normal development in *Chlamydia* species and tryptophan metabolism has been implicated in *Chlamydia* persistence and tissue tropism (Akers & Tan, 2006). If infection of EEC leads to changes in their production or secretion of signalling substances, disturbances of gastrointestinal function are likely to occur.

#### 4. Infection of enteroendocrine cell lines

Our findings in the small bowel mucosa of patients with IBS suggested that infection of enteroendocrine cells (EEC) with *C. trachomatis* could be involved in the pathogenesis of their disease. In order to study this mechanism in more detail we set up an *in vitro* model using enteroendocrine cell lines (Dlugosz et al., 2011). We used two different human enteroendocrine cell lines: LCC-18 (a kind gift from K. Öberg, Uppsala University Hospital, Uppsala, Sweden), derived from a neuroendocrine colonic tumour and CNDT-2 (a kind gift from L. M. Ellis, University of Texas, Houston, USA), derived from a small intestinal carcinoid. We studied the influence of acute and persistent infection with *C. trachomatis* L2 strain 434 (ATCC) on gene expression and protein distribution of enteroendocrine markers.

Growth of *C. trachomatis*, manifested through inclusions containing both elementary bodies (EB) and reticulate bodies (RB), could be observed for both cell lines. Similar growth was observed in HeLa cells, which were used as positive control. Similar to previously described infection cycles in other cell types, *C. trachomatis* successfully infected and multiplied within the confinements of the inclusion in EEC and yielded productive EBs, which can therefore be considered an active infection (Figure 2A). In order to investigate whether the persistent life-cycle could be induced in the EEC, cells were infected with *C. trachomatis* and incubated

in the absence and presence of penicillin G (penG), which has been demonstrated previously to induce persistent growth of *Chlamydia* in HeLa cells. When EEC were treated with penG we observed enlarged, aberrant reticulate bodies reminiscent of persistent growth forms suggesting that persistence can be induced in both EEC tested (Figure 2B).

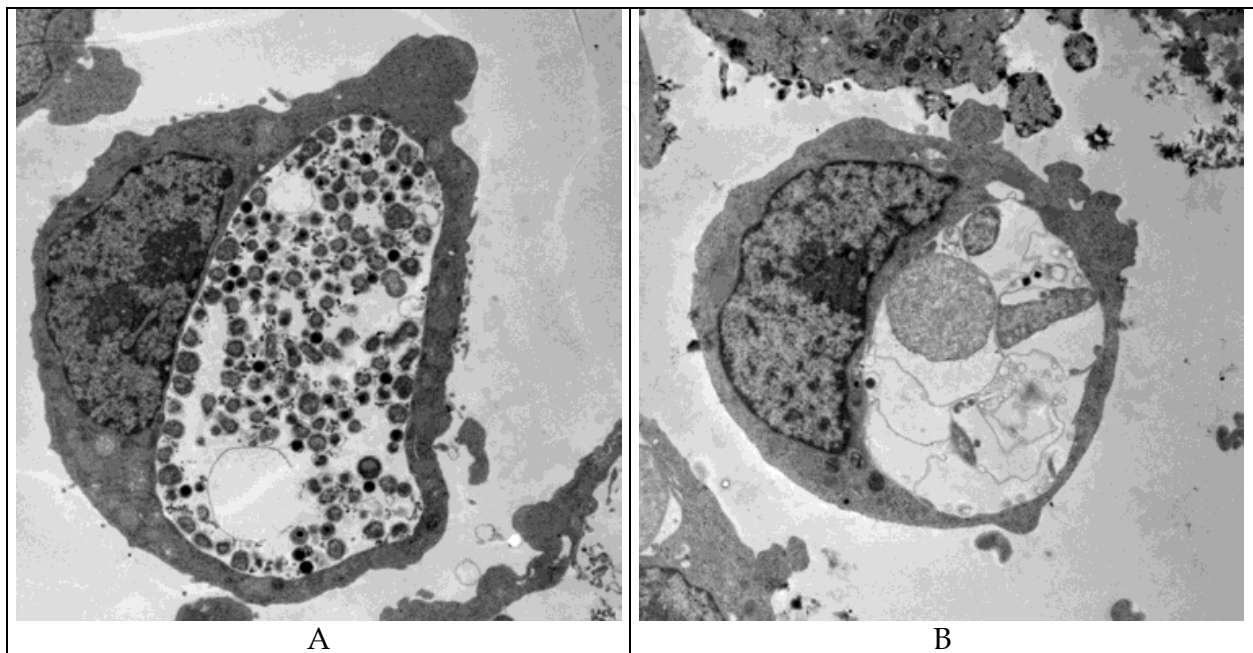


Fig. 2. Infection of enteroendocrine cells from cell line LCC-18 with *Chlamydia trachomatis*. A: Active infection. B: Persistent infection.

In order to investigate if infection with *C. trachomatis* affected the intracellular transport of signalling molecules we studied the distribution of immunoreactivity for serotonin and chromogranin-A (CgA) in infected and non-infected cells. Immunofluorescence demonstrated differences in the cellular distributions of serotonin and CgA between infected and non-infected cells (Figure 3). In infected cells serotonin and CgA were mainly localized within chlamydial inclusions, whereas in non-infected cells these markers predominantly exhibited a cytoplasmatic distribution. No serotonin or CgA was detected in infected or non-infected HeLa cells, which in this instance served as negative control.

In order to analyze the infection process at the molecular level, we investigated the expression of selected target genes using real time PCR. Genes coding for EEC protein markers (CHGA, VMAT1, mGluR4, TPH1, TRPA1), house-keeping proteins (TFCP2, GAPDH), environmental stress marker (HSPB1) as well as TLR4, which is believed to mediate the main line of response upon *Chlamydia* infection, were subjected to PCR analysis. Transcriptomes of *C. trachomatis* infected EEC (LCC-18 and CNDT-2) were analysed after 24h. The same time-point was investigated after induction of persistence using penicillin G. We found significant down-regulation of VMAT1 expression in persistent infection compared to non-infected cells ( $p < 0.05$ ) and up-regulation of TLR4 expression in active and persistent infection ( $p < 0.05$ ). Expression of CHGA, the gene coding for CgA, as well as TPH1 and TRPA1, genes coding for proteins associated with serotonin synthesis and release, were not changed upon infection with *Chlamydia*. Gene expression changes were associated with infection and did not appear in cells exposed to heat-treated bacteria.

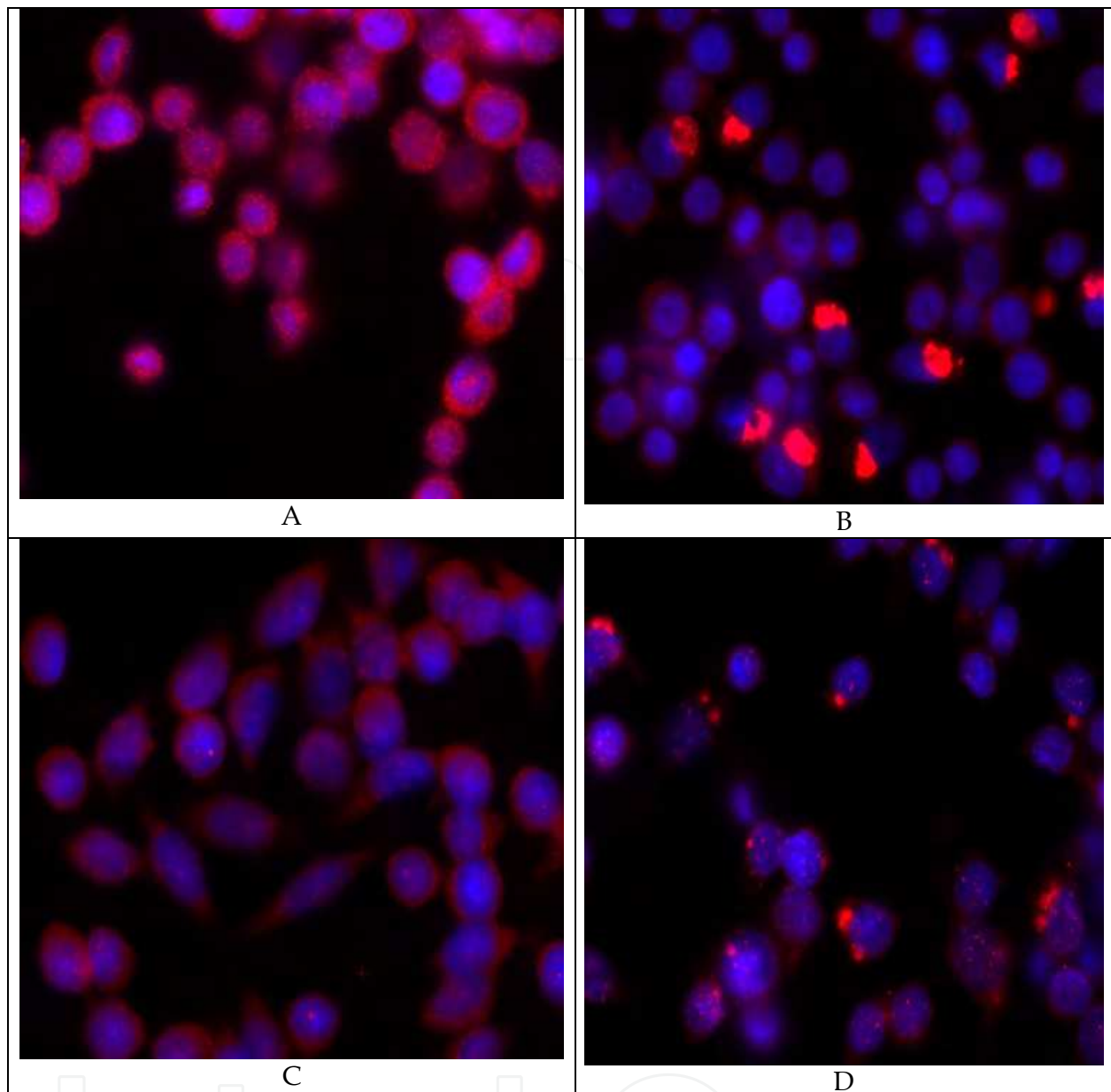


Fig. 3. Altered cellular distribution of serotonin and chromogranin A in non-infected and *Chlamydia trachomatis* L2-infected EEC. A: Cytoplasmatic distribution of chromogranin A (CgA) in non-infected LCC-18 cells (rabbit CgA antibody and Alexia 568-red and DAPI nucleus staining (blue), original magnification x63). B: Chromogranin A labeling in *Chlamydia* containing inclusions (arrows) in infected LCC-18 cells (rabbit CgA antibody and Alexia 568-red and DAPI nucleus staining (blue), original magnification x63). C: Cytoplasmatic distribution of serotonin in non-infected LCC-18 cells (rabbit serotonin antibody, Alexia 568-red and DAPI nucleus staining (blue), original magnification x63). D: Serotonin in *Chlamydia* containing inclusions (arrows) in persistently infected CNDT-2 cells (rabbit serotonin antibody and Alexia 568-red and DAPI nucleus staining (blue), original magnification x63).

Our study is the first to show that the presence of *C. trachomatis* alters the cellular distributions of serotonin and CgA in vitro. Both serotonin and CgA are important for



immune activation and gut inflammation *in vivo* and several serotonergic receptors have been characterized in lymphocytes, monocytes, macrophages and dendritic cells (Cloez-Tayarani & Changeux, 2007). Serotonin has been shown to activate immune cells, which are responsible for the production of proinflammatory mediators (Khan & Ghia, 2010). Consequently, a manipulation of the serotonin system could modulate responses to gut inflammation. CgA on the other hand has antimicrobial activity (Shooshtarizadeh et al., 2010) and exhibits both proinflammatory and anti-inflammatory functions (Khan & Ghia, 2010).

We think that altered protein distribution and down-regulation of the vesicular monoamine transporter VMAT1 suggest bacterial influence on vesicular transport. The expression of genes associated with serotonin synthesis (TPH1) and release (TRPA1) was not impaired. At this stage, it is unclear if the altered distribution of serotonin and CgA in *C. trachomatis* infected EEC is induced by the bacteria themselves or is part of an innate immunity response via up-regulation of toll-like receptors. Others have reported increased LPS-induced serotonin secretion in EEC derived from patients with Crohn's disease (Kidd et al., 2009). TLR4 stimulation with its agonist LPS also caused the release of human  $\beta$ -defensin-2 (HBD-2) from EEC (Palazzo et al., 2007) and elevated HBD-2 levels have recently been found in patients with IBS (Langhorst et al., 2009).

## 5. Innate immunity in IBS

The ability of intestinal mucosa to detect bacterial cellular components requires the expression of pattern recognition receptors (PRR) that recognize repetitive patterns present on Gram-positive and Gram-negative bacteria, fungi, viruses and parasites. Toll-like receptors (TLR) belong to the family of PRRs and are present on macrophages of the lamina propria, dendritic cells, paneth cells and intestinal epithelial cells. TLR4 is regarded as the PRR for lipopolysaccharide (LPS), the toxin of Gram-negative bacteria (including *Chlamydia*). Expression of TLR4 by intestinal cells is normally down-regulated to maintain immune tolerance to the luminal microorganisms but up-regulated in gut inflammation (Gribar et al., 2008).

A research group from Cork, Ireland recently investigated the potential involvement of TLRs in IBS (Brint et al., 2011). They studied biopsies from the sigmoid colon of 26 female patients with IBS, 19 female healthy controls and 29 disease controls (10 with ulcerative colitis and 19 with Crohn's disease) and applied quantitative real-time RT-PCR for RNA gene expression. They found that the expression of TLR4 was significantly up-regulated (5-fold) in patients with IBS compared to healthy controls. They also found a small but statistically significant up-regulation of TLR5 (1.7-fold) and down-regulation of TLR7 and TLR8 (2-fold). The increase in the expression of TLR 4 was 15-fold in patients with ulcerative colitis and 8-fold in Crohn's disease.

The authors believed that disruption of the intestinal epithelial barrier in inflammatory bowel disease, allowing translocation of commensal bacteria to the underlying submucosa, could explain the up-regulation of TLR expression observed in ulcerative colitis and Crohn's disease (Brint et al., 2011). They speculated that a similar but less pronounced disruption of the intestinal epithelial barrier could exist also in patients with IBS. The authors concluded that the differences observed in TLR expression might indicate an appropriate immune response to a pathogen or to alterations in the host microbiota.

Another recent study from the same group investigated cytokine and cortisol levels in plasma and peripheral TLR activity after stimulation of cultured whole blood with TLR agonists in 30 patients with IBS and 30 healthy controls (McKernan et al., 2011). Patients with IBS had elevated plasma levels of IL-6, IL-8 and cortisol. Release of IL-1 $\beta$ , IL-6, IL-8 and TNF- $\alpha$  was measured after stimulation with agonists for TLR1-8. Patients with IBS exhibited elevated responses to TLR2 (TNF- $\alpha$ ), TLR3 (IL-8), TLR4 (IL-1 $\beta$  and TNF- $\alpha$ ), TLR5 (IL-1 $\beta$  and TNF- $\alpha$ ), TLR7 (IL-8), TLR8 (IL-1 $\beta$ , IL-6, IL-8 and TNF- $\alpha$ ). No difference between patients and controls was seen in cytokine release after stimulation of TLR1, 6 and 9.

The authors concluded that patients with IBS demonstrate elevated cytokine levels, a finding that supports a previous observation from the same group (Dinan et al., 2006), and enhanced TLR activity in the periphery, which indicates that patients with IBS have some immune dysregulation (McKernan et al., 2011). They speculated that there might be a link between stress (increased plasma levels of cortisol), TLR activation and cytokine profiles in patients with IBS, similar to that observed in mice (Zhang et al., 2008).

We analysed endoscopic biopsies from the right and the left colon in 10 patients with inflammatory bowel disease (5 with Crohn's colitis and 5 with ulcerative colitis) and 10 patients with IBS (5 with diarrhoea-predominant and 5 with constipation-predominant IBS) and biopsies from the left colon in 5 healthy controls. We used rabbit polyclonal antibodies to TLR4 (Abcam, UK) and tyramide signal amplification (Invitrogen, USA) technology for immunohistochemistry and Nikon NIS-Elements (Nikon, Japan) for image analysis. TLR4 expression was calculated as a percentage of lamina propria area occupied by TLR4 positive cells.

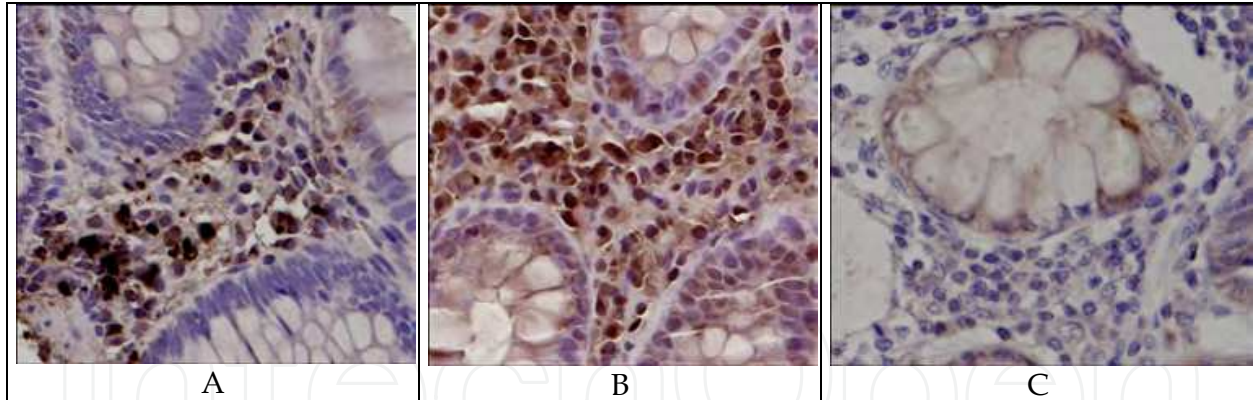


Fig. 4. Immunohistochemical expression of TLR4 in colon mucosa. A: Patient with IBS (original magnification x40). B: Patient with inflammatory bowel disease (original magnification x40). C: Healthy control (original magnification x40). Immunohistochemistry using a rabbit polyclonal antibody to TLR4 and tyramide signal amplification.

Immunohistochemical expression of TLR4 was increased in both inflammatory bowel disease and IBS ( $p=0.04$ ) compared to healthy individuals. In IBS and inflammatory bowel disease the increase of TLR4 expression was mainly in cells of *l. propria* (Figure 4). Our findings support the previous observation that TLR4 gene expression is up-regulated in IBS and inflammatory bowel disease (Brint et al., 2011). Our data again suggest the involvement of Gram-negative pathogens or dysregulation of the intestinal immune response to the commensal flora as possible pathogenetic mechanisms in IBS.

## 6. Discussion

Chlamydial antigens were found in enteroendocrine cells and macrophages of the small bowel mucosa in 89% of patients with IBS but in only 14% of controls. Even though we were unable to prove the presence of viable *Chlamydia*, lack of positive PCR being the major obstacle, the evidence for an intracellular organism or at least a protein structure with an antigen in common with *Chlamydia* in patients with IBS is strong. Given the crucial role of enteroendocrine cells in digestive function, our results suggest that the presence of chlamydial antigens in these cells may be involved in the pathogenesis in IBS. Chlamydial antigens have not been described in human enteroendocrine cells before. If this finding can be corroborated by DNA evidence of bacterial presence, it represents a previously unknown cell tropism of *Chlamydia*.

In an *in vitro* model we found that enteroendocrine cells from both the small bowel and the large bowel could be infected with *C. trachomatis* yielding productive infections and that persistence could be induced using penicillin G. Immunofluorescence showed different cellular distributions of serotonin and chromogranin A in non-infected (cytoplasmatic distribution) compared with infected cells (serotonin and chromogranin A mostly in chlamydial inclusions). In line with the microscopical findings, we found a significant down-regulation of the gene coding for the vesicular monoamine transporter (VMAT1) in infected compared with non-infected EEC ( $P < 0.05$ ). Altered protein distributions together with down-regulation of VMAT1 suggest that chlamydial infection may influence vesicular transport. It is therefore possible that such an infection *in vivo* could lead to disturbances in the regulation of gut functions.

Up-regulation of TLR4 suggests the involvement of Gram-negative pathogens or dysregulation of the intestinal immune response to the commensal flora as possible pathogenetic mechanisms in IBS. This finding also lends support to the idea that a persistent infection of the gastrointestinal tract with *C. trachomatis* is part of the pathogenesis in IBS.

## 7. Speculations

The symptom profile of patients with IBS has long been a challenge to the medical community. The main symptoms are abdominal pain, diarrhoea or constipation or both, and a temporal relation between worsening or onset of pain and change in bowel frequency or stool consistency (Longstreth et al., 2006). However, patients with IBS may have a number of other symptoms from the gastrointestinal tract including dysphagia, dyspepsia, nausea, vomiting, anorexia, post-prandial distension, flatulence, borborygmia (noisy bowel sounds), mucus in stools, and feeling of incomplete evacuation. There is considerable overlap between IBS and other prevalent functional gastrointestinal disorders such as functional dyspepsia and patients often change their symptom profile from one to the other with time (Agréus et al., 2001). If a persistent infection of enteroendocrine cells is important in the pathogenesis of IBS, then it is reasonable to assume that this infection can affect different populations of enteroendocrine cells and lead to correspondingly different symptom profiles. Such a mechanism could explain the variability of presenting symptoms among patients with functional gastrointestinal disorders and perhaps there is no meaningful difference between IBS, functional constipation, functional diarrhoea, functional bloating,

functional abdominal pain and the majority of other functional gastrointestinal syndromes (Longstreth et al., 2006).

One of the most common measurable abnormalities in patients with IBS is visceral hypersensitivity (Akbar et al., 2009). The mechanisms behind visceral hypersensitivity have remained unproven but glial cells and TLR4-activation seem important both in the CNS (Tanga et al., 2005) and in the spinal cord (Saito et al., 2010). In light of our findings of chlamydial antigens and TLR4 up-regulation in IBS it is reasonable to hypothesize that visceral hypersensitivity can arise from TLR4 activation either of enteroglial cells or of glial cells in the spinal cord or CNS.

The most commonly noted overlap syndrome with IBS is fibromyalgia (Sivri et al., 1996; Sperber et al., 1999; Triadafilopoulos et al., 1991). It has therefore been suggested that IBS and fibromyalgia may have a common pathogenesis (Veale et al., 1991). Fibromyalgia is a chronic form of diffuse musculoskeletal pain with tenderness at specific locations, often associated with persistent fatigue, cognitive and mood disorders, joint stiffness, and insomnia that occurs mainly in females (Solitar, 2010). It has long been speculated that the driving force for fibromyalgia might be an infection and similar to IBS many patients associate the onset of their condition with an acute illness or have noticed that infections may worsen their symptoms (Bennett et al., 2007). An interesting similarity between IBS and fibromyalgia is that both groups seem to have increased peripheral levels of the cytokine IL-8 (McKernan et al., 2011; Wang et al., 2009). We therefore hypothesize that the driving force for fibromyalgia and possibly also other chronic inflammatory conditions is a persistent infection in the gastrointestinal tract. In the case of fibromyalgia the overlap with IBS supports the view of a common aetiology. The gastrointestinal tract is our largest interface to the world of microbes and the most likely location of a driving force for other chronic inflammatory disorders would therefore also be the gastrointestinal tract (Scheinecker & Smolen, 2011).

## 8. Conclusions

The above series of studies collectively indicate a role for *C. trachomatis* in the pathogenesis of IBS. Our data suggests that patients with IBS may have a persistent infection with *C. trachomatis* of enteroendocrine cells and macrophages. So far only the small bowel has been studied and it remains to find out if also the mucosa of the large bowel exhibits the same findings. Cell line experiments indicate that enteroendocrine cells can indeed be infected by *C. trachomatis* and that persistent infection of such cells leads to profound changes of the intracellular distribution of serotonin and chromogranin A.

Patients with IBS exhibit a pronounced up-regulation of TLR4. The level of up-regulation is similar to that found in inflammatory bowel disease. It is possible that observed changes in the innate immune system reflect stimulation from chlamydial LPS. It is yet unclear if the mechanisms behind up-regulation of TLR4 are the same in IBS and inflammatory bowel disease.

Further studies are needed to confirm or refute the hypothesis that live bacteria are present in the mucosa of the small bowel in patients with IBS. It is tempting to suggest a therapeutic trial aimed at eradicating a persistent infection with *C. trachomatis*. However, it is uncertain if eradication of a persistent chlamydial infection is at all possible (Gieffers et al., 2001).

## 9. Acknowledgments

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## 10. References

- Agréus, L.; Svärdsudd, K.; Talley, N. J.; Jones, M. P. & Tibblin, G. (2001). Natural history of gastroesophageal reflux disease and functional abdominal disorders: a population-based study. *The American Journal of Gastroenterology*, Vol. 96, No. 10, (October 2001), pp. 2905-2914, ISSN 0002-9270
- Akbar, A.; Walters, J. R. & Ghosh, S. (2009). Review article: visceral hypersensitivity in irritable bowel syndrome: molecular mechanisms and therapeutic agents. *Alimentary Pharmacology & Therapeutics*, Vol. 30, No. 5, (September 2009), pp. 423-435, ISSN 0269-2813
- Akers, J. C. & Tan, M. (2006). Molecular mechanism of tryptophan-dependent transcriptional regulation in *Chlamydia trachomatis*. *Journal of Bacteriology*, Vol. 188, No. 12, (June 2006), pp. 4236-4243, ISSN 0021-9193
- Akiho, H.; Ihara, E. & Nakamura, K. (2011). Low-grade inflammation plays a pivotal role in gastrointestinal dysfunction in irritable bowel syndrome. *World Journal of Gastrointestinal Pathophysiology*, Vol. 1, No. 3, (August 2011), pp. 97-105, ISSN 2150-5330
- Amouretti, M.; Le Pen, C.; Gaudin, A. F.; Bommelaer, G.; Frexinos, J.; Ruzsniowski, P.; Poynard, T.; Maurel, F.; Priol, G. & El Hasnaoui, A. (2006). Impact of irritable bowel syndrome (IBS) on health-related quality of life (HRQOL). *Gastroentérologie Clinique et Biologique*, Vol. 30, No. 2, (February 2006), pp. 241-246, ISSN 0399-8320
- Beatty, W. L.; Morrison, R. P. & Byrne, G. I. (1994). Persistent *Chlamydiae*: from cell culture to a paradigm for chlamydial pathogenesis. *Microbiological Reviews*, Vol. 58, No. 4, (December 1994), pp. 686-699, ISSN 0146-0749
- Bennett, R. M.; Jones, J.; Turk, D. C.; Russell, I. J. & Matallana, L. (2007). An internet survey of 2,596 people with fibromyalgia. *BMC Musculoskeletal Disorders*, Vol. 8, No., (March 2007), pp. 27, ISSN 1471-2474
- Brint, E. K.; MacSharry, J.; Fanning, A.; Shanahan, F. & Quigley, E. M. (2011). Differential expression of toll-like receptors in patients with irritable bowel syndrome. *The American Journal of Gastroenterology*, Vol. 106, No. 2, (February 2011), pp. 329-336, ISSN 0002-9270
- Caldwell, H. D. & Hitchcock, P. J. (1984). Monoclonal antibody against a genus-specific antigen of *Chlamydia* species: location of the epitope on chlamydial lipopolysaccharide. *Infection and Immunity*, Vol. 44, No. 2, (May 1984), pp. 306-314, ISSN 0019-9567
- Cloez-Tayarani, I. & Changeux, J. P. (2007). Nicotine and serotonin in immune regulation and inflammatory processes: a perspective. *Journal of Leukocyte Biology*, Vol. 81, No. 3, (March 2007), pp. 599-606, ISSN 0741-5400
- Courtright, P. & West, S. K. (2004). Contribution of sex-linked biology and gender roles to disparities with trachoma. *Emerging Infectious Diseases*, Vol. 10, No. 11, (Nov 2004), pp. 2012-2016, ISSN 1080-6040

- Dinan, T. G.; Quigley, E. M.; Ahmed, S. M.; Scully, P.; O'Brien, S.; O'Mahony, L.; O'Mahony, S.; Shanahan, F. & Keeling, P. W. (2006). Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? *Gastroenterology*, Vol. 130, No. 2, (February 2006), pp. 304-311, ISSN 0016-5085
- Dlugosz, A.; Törnblom, H.; Mohammadian, G.; Morgan, G.; Veress, B.; Edvinsson, B.; Sandström, G. & Lindberg, G. (2010). *Chlamydia trachomatis* antigens in enteroendocrine cells and macrophages of the small bowel in patients with severe irritable bowel syndrome. *BMC Gastroenterology*, Vol. 10, (February 2010), pp. 19, ISSN 1471-230X
- Dlugosz, A.; Zakikhany, K.; Muschiol, S.; Hultenby, K. & Lindberg, G. (2011). Infection of human enteroendocrine cells with *Chlamydia trachomatis*: a possible model for pathogenesis in irritable bowel syndrome. *Neurogastroenterology & Motility*, Vol. 23, No. 10, (October 2011), pp. 928-934, ISSN 1350-1925
- Drossman, D. A.; Morris, C. B.; Schneck, S.; Hu, Y. J.; Norton, N. J.; Norton, W. F.; Weiland, S. R.; Dalton, C.; Leserman, J. & Bangdiwala, S. I. (2009). International survey of patients with IBS: symptom features and their severity, health status, treatments, and risk taking to achieve clinical benefit. *Journal of Clinical Gastroenterology*, Vol. 43, No. 6, (July 2009), pp. 541-550, ISSN 0192-0790
- Everett, K. D.; Hornung, L. J. & Andersen, A. A. (1999). Rapid detection of the *Chlamydiaceae* and other families in the order *Chlamydiales*: three PCR tests. *Journal of Clinical Microbiology*, Vol. 37, No. 3, (March 1999), pp. 575-580, ISSN 0095-1137
- Francis, C.; Prior, A.; Whorwell, P. J. & Morris, J. (1998). *Chlamydia trachomatis* infection: Is it relevant in irritable bowel syndrome? *Digestion*, Vol. 59, No. 2, (May 1998), pp. 157-159, ISSN 0012-2823
- Gieffers, J.; Fullgraf, H.; Jahn, J.; Klinger, M.; Dalhoff, K.; Katus, H. A.; Solbach, W. & Maass, M. (2001). *Chlamydia pneumoniae* infection in circulating human monocytes is refractory to antibiotic treatment. *Circulation*, Vol. 103, No. 3, (January 2001), pp. 351-356, ISSN 0009-7322
- Gribar, S. C.; Anand, R. J.; Sodhi, C. P. & Hackam, D. J. (2008). The role of epithelial Toll-like receptor signaling in the pathogenesis of intestinal inflammation. *Journal of Leukocyte Biology*, Vol. 83, No. 3, (March 2008), pp. 493-498, ISSN 0741-5400
- Hungin, A. P.; Whorwell, P. J.; Tack, J. & Mearin, F. (2003). The prevalence, patterns and impact of irritable bowel syndrome: an international survey of 40,000 subjects. *Alimentary Pharmacology & Therapeutics*, Vol. 17, No. 5, (March 2003), pp. 643-650, ISSN 0269-2813
- Jakobsen, A. M.; Andersson, P.; Saglik, G.; Andersson, E.; Kölby, L.; Erickson, J. D.; Forssell-Aronsson, E.; Wängberg, B.; Ahlman, H. & Nilsson, O. (2001). Differential expression of vesicular monoamine transporter (VMAT) 1 and 2 in gastrointestinal endocrine tumours. *The Journal of Pathology*, Vol. 195, No. 4, (November 2001), pp. 463-472, ISSN 0022-3417
- Khan, W. I. & Ghia, J. E. (2010). Gut hormones: emerging role in immune activation and inflammation. *Clinical & Experimental Immunology*, Vol. 161, No. 1, (July 2010), pp. 19-27, ISSN 1365-2249

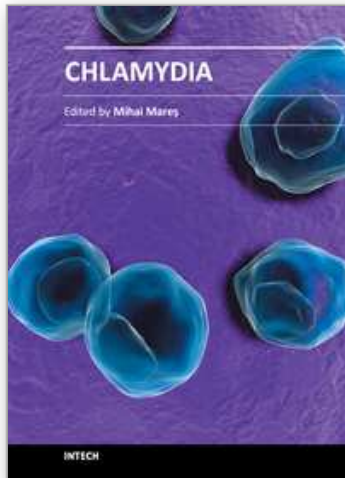
- Kidd, M.; Gustafsson, B. I.; Drozdov, I. & Modlin, I. M. (2009). IL1beta- and LPS-induced serotonin secretion is increased in EC cells derived from Crohn's disease. *Neurogastroenterology & Motility*, Vol. 21, No. 4, (April 2009), pp. 439-450, ISSN 1365-2982
- Kim, D. Y. & Camilleri, M. (2000). Serotonin: a mediator of the brain-gut connection. *The American Journal of Gastroenterology*, Vol. 95, No. 10, (October 2000), pp. 2698-2709, ISSN 0002-9270
- Langhorst, J.; Junge, A.; Rueffer, A.; Wehkamp, J.; Foell, D.; Michalsen, A.; Musial, F. & Dobos, G. J. (2009). Elevated human beta-defensin-2 levels indicate an activation of the innate immune system in patients with irritable bowel syndrome. *The American Journal of Gastroenterology*, Vol. 104, No. 2, (Feb 2009), pp. 404-410, ISSN 0002-9270
- Lindberg, G.; Törnblom, H.; Iwarzon, M.; Nyberg, B.; Martin, J. E. & Veress, B. (2009). Full-thickness biopsy findings in chronic intestinal pseudo-obstruction and enteric dysmotility. *Gut*, Vol. 58, No. 8, (August 2009), pp. 1084-1090, ISSN 0017-5749
- Longstreth, G. F.; Thompson, W. G.; Chey, W. D.; Houghton, L. A.; Mearin, F. & Spiller, R. C. (2006). Functional bowel disorders. *Gastroenterology*, Vol. 130, No. 5, (April 2006), pp. 1480-1491, ISSN 0016-5085
- Makharia, G. K.; Verma, A. K.; Amarchand, R.; Goswami, A.; Singh, P.; Agnihotri, A.; Suhail, F. & Krishnan, A. (2011). Prevalence of irritable bowel syndrome: a community based study from northern India. *Journal of Neurogastroenterology and Motility*, Vol. 17, No. 1, (January 2011), pp. 82-87, ISSN 2093-0879
- McKernan, D. P.; Gaszner, G.; Quigley, E. M.; Cryan, J. F. & Dinan, T. G. (2011). Altered peripheral toll-like receptor responses in the irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics*, Vol. 33, No. 9, (May 2011), pp. 1045-1052, ISSN 0269-2813
- Monack, D. M.; Mueller, A. & Falkow, S. (2004). Persistent bacterial infections: the interface of the pathogen and the host immune system. *Nature Reviews Microbiology*, Vol. 2, No. 9, (September 2004), pp. 747-765, ISSN 1740-1526
- Öhman, L. & Simrén, M. (2007). New insights into the pathogenesis and pathophysiology of irritable bowel syndrome. *Digestive and Liver Disease*, Vol. 39, No. 3, (March 2007), pp. 201-215, ISSN 1590-8658
- Öhman, L. & Simrén, M. (2010). Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. *Nature Reviews Gastroenterology & Hepatology*, Vol. 7, No. 3, (March 2010), pp. 163-173, ISSN 1759-5045
- Palazzo, M.; Balsari, A.; Rossini, A.; Selleri, S.; Calcaterra, C.; Gariboldi, S.; Zanobbio, L.; Arnaboldi, F.; Shirai, Y. F.; Serrao, G. & Rumio, C. (2007). Activation of enteroendocrine cells via TLRs induces hormone, chemokine, and defensin secretion. *The Journal of Immunology*, Vol. 178, No. 7, (April 2007), pp. 4296-4303, ISSN 0022-1767
- Saito, O.; Svensson, C. I.; Buczynski, M. W.; Wegner, K.; Hua, X. Y.; Codeluppi, S.; Schaloske, R. H.; Deems, R. A.; Dennis, E. A. & Yaksh, T. L. (2010). Spinal glial TLR4-mediated nociception and production of prostaglandin E(2) and TNF. *British Journal of Pharmacology*, Vol. 160, No. 7, (August 2010), pp. 1754-1764, ISSN 0007-1188
- Scheinecker, C. & Smolen, J. S. (2011). Rheumatoid arthritis in 2010: from the gut to the joint. *Nature Reviews Rheumatology*, Vol. 7, No. 2, (February 2011), pp. 73-75, ISSN 1759-4790
- Shooshtarizadeh, P.; Zhang, D.; Chich, J. F.; Gasnier, C.; Schneider, F.; Haikel, Y.; Aunis, D. & Metz-Boutigue, M. H. (2010). The antimicrobial peptides derived from

- chromogranin/secretogranin family, new actors of innate immunity. *Regulatory Peptides*, Vol. 165, No. 1, (November 2010), pp. 102-110, ISSN 0167-0115
- Sikander, A.; Rana, S. V. & Prasad, K. K. (2009). Role of serotonin in gastrointestinal motility and irritable bowel syndrome. *Clinica Chimica Acta*, Vol. 403, No. 1-2, (May 2009), pp. 47-55, ISSN 0009-8981
- Sivri, A.; Cindas, A.; Dincer, F. & Sivri, B. (1996). Bowel dysfunction and irritable bowel syndrome in fibromyalgia patients. *Clinical Rheumatology*, Vol. 15, No. 3, (May 1996), pp. 283-286, ISSN 0770-3198
- Solitar, B. M. (2010). Fibromyalgia: knowns, unknowns, and current treatment. *Bulletin of the NYU Hospital for Joint Diseases*, Vol. 68, No. 3, (October 2010), pp. 157-161, ISSN 1936-9719
- Sperber, A. D.; Atzmon, Y.; Neumann, L.; Weisberg, I.; Shalit, Y.; Abu-Shakrah, M.; Fich, A. & Buskila, D. (1999). Fibromyalgia in the irritable bowel syndrome: studies of prevalence and clinical implications. *The American Journal of Gastroenterology*, Vol. 94, No. 12, (December 1999), pp. 3541-3546, ISSN 0002-9270
- Spiller, R. C. (2007). Role of infection in irritable bowel syndrome. *Journal of Gastroenterology*, Vol. 42, No. Suppl 17, (January 2007), pp. 41-47, ISSN 0944-1174
- Storz, J. & Spears, P. (1979). Pathogenesis of chlamydial polyarthritis in domestic animals: characteristics of causative agent. *Annals of the Rheumatic Diseases*, Vol. 38, No. Suppl 1, (January 1979), pp. 111-115, ISSN 0003-4967
- Tanga, F. Y.; Natile-McMenemy, N. & DeLeo, J. A. (2005). The CNS role of Toll-like receptor 4 in innate neuroimmunity and painful neuropathy. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 102, No. 16, (April 2005), pp. 5856-5861, ISSN 0027-8424
- Thompson, W. G.; Irvine, E. J.; Pare, P.; Ferrazzi, S. & Rance, L. (2002). Functional gastrointestinal disorders in Canada: first population-based survey using Rome II criteria with suggestions for improving the questionnaire. *Digestive Diseases and Sciences*, Vol. 47, No. 1, (January 2002), pp. 225-235, ISSN 0163-2116
- Thompson, W. G.; Longstreth, G. F.; Drossman, D. A.; Heaton, K. W.; Irvine, E. J. & Muller-Lissner, S. A. (1999). Functional bowel disorders and functional abdominal pain. *Gut*, Vol. 45, No. Suppl 2, (September 1999), pp. II43-47, ISSN 0017-5749
- Törnblom, H.; Holmvall, P.; Svenungsson, B. & Lindberg, G. (2007). Gastrointestinal symptoms after infectious diarrhea: a five-year follow-up in a Swedish cohort of adults. *Clinical Gastroenterology and Hepatology*, Vol. 5, No. 4, (April 2007), pp. 461-464, ISSN 1542-3565
- Törnblom, H.; Lindberg, G.; Nyberg, B. & Veress, B. (2002). Full-thickness biopsy of the jejunum reveals inflammation and enteric neuropathy in irritable bowel syndrome. *Gastroenterology*, Vol. 123, No. 6, (December 2002), pp. 1972-1979, ISSN 0016-5085
- Triadafilopoulos, G.; Simms, R. W. & Goldenberg, D. L. (1991). Bowel dysfunction in fibromyalgia syndrome. *Digestive Diseases and Sciences*, Vol. 36, No. 1, (January 1991), pp. 59-64, ISSN 0163-2116
- Veale, D.; Kavanagh, G.; Fielding, J. F. & Fitzgerald, O. (1991). Primary fibromyalgia and the irritable bowel syndrome: different expressions of a common pathogenetic process. *British Journal of Rheumatology*, Vol. 30, No. 3, (June 1991), pp. 220-222, ISSN 0263-7103



- Wang, H.; Buchner, M.; Moser, M. T.; Daniel, V. & Schiltenwolf, M. (2009). The role of IL-8 in patients with fibromyalgia: a prospective longitudinal study of 6 months. *The Clinical Journal of Pain*, Vol. 25, No. 1, (January 2009), pp. 1-4, ISSN 0749-8047
- Williams, R. E.; Hartmann, K. E.; Sandler, R. S.; Miller, W. C. & Steege, J. F. (2004). Prevalence and characteristics of irritable bowel syndrome among women with chronic pelvic pain. *Obstetrics & Gynecology*, Vol. 104, No. 3, (September 2004), pp. 452-458, ISSN 0029-7844
- Witkin, S. S. (2002). Immunological aspects of genital chlamydia infections. *Best Practice & Research Clinical Obstetrics & Gynaecology*, Vol. 16, No. 6, (December 2002), pp. 865-874, ISSN 1521-6934
- Zhang, Y.; Zhang, Y.; Miao, J.; Hanley, G.; Stuart, C.; Sun, X.; Chen, T. & Yin, D. (2008). Chronic restraint stress promotes immune suppression through toll-like receptor 4-mediated phosphoinositide 3-kinase signaling. *Journal of Neuroimmunology*, Vol. 204, No. 1-2, (November 2008), pp. 13-19, ISSN 0165-5728
- Zondervan, K. T.; Yudkin, P. L.; Vessey, M. P.; Jenkinson, C. P.; Dawes, M. G.; Barlow, D. H. & Kennedy, S. H. (2001). Chronic pelvic pain in the community--symptoms, investigations, and diagnoses. *American Journal of Obstetrics & Gynecology*, Vol. 184, No. 6, (May 2001), pp. 1149-1155, ISSN 0002-9378

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## **Chlamydia**

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Nowadays, Chlamydia still represents a redoubtable pathogen. Among its consequences, the blindness in children and severe impairment of reproductive health in adults are the most mutilating. Worldwide, it is estimated that six million of people suffer from post-trachoma blindness and almost 90 million become sexually infected each year. Due to its silent evolution and sexually transmission, the chlamydial infection can occur in anyone. The book "Chlamydia - A Multifaceted Pathogen" contains an updated review of all-important issues concerning the chlamydial infection. It comprises 18 chapters grouped in four major parts dealing with etiology and pathogenicity, clinical aspects, diagnosis and prevention. The new molecular data about the pathogenicity and the exhaustive presentation of clinical findings bring novelty to the book and improve our knowledge about Chlamydia induced diseases.

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