



**Karolinska  
Institutet**

**Institutionen för Medicin, Huddinge**

# Towards a bacterial origin of Irritable Bowel Syndrome

**AKADEMISK AVHANDLING**

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av

**Aldona Dlugosz**

Leg. läkare

*Huvudhandledare:*

Professor Greger Lindberg  
Karolinska Institutet  
Institutionen för Medicin  
Enheten för Gastroenterologi och Hepatologi

*Fakultetsopponent:*

Professor Peter Malfertheiner  
Otto-von-Guericke-Universität,  
Klinik für Gastroenterologie, Hepatologie und  
Infektiologie  
Magdeburg, Tyskland

*Betygsnämnd:*

Professor Hans-Gustaf Ljunggren  
Karolinska Institutet  
Centrum för infektionsmedicin (CIM)

Docent Martin Rottenberg  
Karolinska Institutet  
Institutionen för Mikrobiologi, Tumör- och  
Cellbiologi (MTC)

Professor Curt Tysk  
Örebro Universitet  
Hälsokademin

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## ABSTRACT

**Background:** The irritable bowel syndrome (IBS) is one of the most prevalent disorders and affects about 15% of the Swedish population. Patients with IBS suffer from abdominal pain and disturbed bowel function. Despite being so common, little is known about causality in IBS. Patients with IBS have been found to exhibit low-grade inflammation of nerve plexuses in the gut. The driving force for observed immune activation is yet unknown.

**Aim of the thesis:** To assess if bacteria may have a role in the pathogenesis of IBS. This was addressed using different models. The first was to investigate if *Chlamydia* antigens were present in the small bowel of patients with severe IBS. Then the interaction between *Chlamydia* and enteroendocrine cells (EEC) *in vitro* using enteroendocrine cell lines was studied and finally the composition of small bowel mucosa-associated microbiota was explored in patients with IBS and healthy controls using pyrotag sequencing.

**Material and methods:** In Study I full-thickness jejunum biopsies and mucosa biopsies from the duodenum and the jejunum from patients with severe IBS and healthy controls were investigated with immunofluorescence for chlamydial antigens. In studies II and III two different human enteroendocrine cell lines were studied: LCC-18 from a neuroendocrine colonic tumour and CNDT-2 from a small intestinal carcinoid. Cell lines were infected with *C. trachomatis* serovar LGV II strain 434. Penicillin G was used for inducing persistent infection. The ultrastructure of infected cells was studied using transmission electron microscopy and immunofluorescence and we used RT-PCR analysis and microarray analysis (Affymetrix GeneChip®) for studying changes in gene expression at different stages of infection. In Study IV capsule biopsies from the jejunum of patients with IBS and healthy volunteers were studied using barcoded 454-pyrosequencing to determine the composition of microbial communities in the upper small bowel.

**Results:** *Chlamydia* LPS was detected in enteroendocrine cells of the mucosa or subepithelial macrophages in 89% of patients with IBS, but in only 14% of healthy controls ( $p < 0.001$ ) and 79% of LPS-positive biopsies were also positive for *C. trachomatis* major outer membrane protein. The cell line experiments showed that both cell lines could be infected with *C. trachomatis* yielding productive infection and persistence could be induced using penicillin G. The cellular distribution of serotonin and chromogranin A was altered by infection from a cytoplasmatic distribution to a location mostly in chlamydial inclusions. Significant differences in the gene transcription levels between persistently infected and non-infected cells were found in 10 genes coding for different solute carrier transporters (SLC) and 5 genes related to endocrine function (GABARAPL1, GRIP1, DRD2, SYT5 and SYT7). Study IV showed no major difference in small bowel mucosa-associated microbiota between patients with IBS and healthy controls.

**Conclusions:** Study I introduced the novel concept that infection with *C. trachomatis* might be involved in the pathogenesis of IBS. *In vitro* studies confirmed that such an infection affects enteroendocrine cell function. The luminal flora of the small bowel was not identified as a host factor for developing IBS.

**Key-words:** Chlamydia, irritable bowel syndrome, enteroendocrine cells, immunofluorescence, microarray, pyrotag sequencing, small bowel microbiota