Charles University in Prague

1st Faculty of Medicine

PhD thesis in Immunology

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Pathogenetic mechanisms of Inflammatory Bowel Diseases: participation of intestinal microflora and immunological factors

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This PhD thesis is based on the following papers :

Involvment of innate immunity in the development of inflammatory and autoimmune diseases.

Tlaskalova-Hogenova H, Tuckova L, Stepankova R, Hudcovic T, Palova-Jelinkova L, Kozakova H, Rossmann P, Sanchez D, Cinova J, Hrncir T, Kverka M, Frolova L, Uhlig H., Powrie F, Bland P

Ann NY Acad Sci 1051: 787-98, 2005

Oral administration of probiotic bacteria (E. coli Nissle, E. coli O83, Lactobacillus casei) influences the severity of dextran-sodium-sulfate-induced colitis.

Kokesova A, Frolova L, Kverka M, Sokol D, Rossmann P, Bartova J, Tlaskalova-Hogenova H

Folia Microbiol 51: 478-84, 2006

Expression of Toll-like Receptor 2 (TLR2), TLR4, and CD14 in Biopsy Samples of Patients With Inflammatory Bowel Diseases: Upregulated Expression of TLR2 in Terminal Ileum of Patients With Ulcerative Colitis.

Frolova L, Drastich P, Rossmann P, Klimesova K, Tlaskalova-Hogenova H

J Histochem Cytochem 56: 267-274, 2008

Detection of galectin-3 in patients with Inflammatory Bowel Diseases: New serum marker of active forms of IBD?

Froľová L, Smetana K Jr, Borovská D, Malíčková K, Janatková I, Lukáš M, Drastich P, Beneš Z, Kitanovičová A, Klimešová K, Tučková L, André S, Gabius H-J, Tlaskalová-Hogenová H

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Abbreviations

APC	antigen-presenting cell
ATG16L1	autophagy-related 16-like 1 gene
CARD15	caspase recruitment domain protein
CD	Crohn`s disease
CRP	C-reactive protein
dsRNA	double-stranded ribonucleic acid
DC	dendritic cell
IgA	immunoglobulin A
IELs	intestinal epithelium lymphocytes
IL-2	interleukin 2
FAE	follicle-associated epithelium
GALT	gut associated lymphatic tissue
GM-CSF	granulocyte macrophage colony stimulating factor
HLA	human leukocyte antigen
IBD	Imflammatory Bowel diseases
INF-γ	interferon gamma
LBP	lipopolysaccharide-binding protein
LFA-1	lymphocyte function-associated antigen-1
LPLs	lamina propria lymphocytes
LPS	lipopolysaccharide
NF-κB	nuclear faktor kappa B
MAMPs	microbe-associated molecular patterns
NALPs	nacht domain-leucin-rich repeat- and pyd-containing proteins

NODs	nucleotide-binding oligomerization domain containing proteins
PDGF	platelet-derived growth factor
PGN	peptidoglycan
PRRs	pattern recognition receptors
TGF-β	transforming growth factor
TLRs	toll-like receptors
TNF-α	tumor necrosis factor alpha
UC	ulcerative colitis

Contents:

1. Mucosal immune system

Surfaces of gastrointestinal, respiratory and urogenital tracts, conjuctivae and outlets of endocrine glands represent in human about 300 m^2. They are mostly covered by single-layered epithelium. The mucosal immune system has evolved mechanisms for discriminating between harmless (food and microflora) and dangerous antigens.

Anatomically, gut mucosa consist of one-layer epithelial cells and various cells including mucosal immune cells presented in the lamina propria. Basic function of mucosal immune system is protection against penetration of pathogenic microorganisms from mucosal surface into the internal environment of organism. Mucosal immune system has regulatory mechanisms, which help to eliminate harmful antigens or establish oral tolerance to commensal bacteria and food antigens. Is believed that Crohn`s disease (CD) and ulcerative colitis (UC) are developed as a result of a disregulated inflammatory response to components of the normal intestinal microbiota.

Pathogenetic mechanisms of Inflammatory Bowel diseases (IBDs) include both components of immune system, adaptive (mediated by B and T cells) and innate immunity.

1.1. Innate immunity components

Components of innate immunity are represented by epithelial cells, macrophages, dendritic cells, NK-cells, mast cells, fibroblasts, neutrophils, eosinophils and their humoral forms presented on mucosal surfaces (cytokines, chemokines, complement, lysozyme, lactoferrin, mannan-binding protein), important are also production of antimicrobial peptides (Tlaskalova-Hogenova et al., 2002; Městecký et al., 2005).

A characteristic feature of innate immunity is an ability of distinguishing between potentially pathogenic microbial components and harmless antigens by pattern recognition receptors (PRRs) (TLRs, NODs, NALPs etc.).

1.1.1. Toll-like receptors (TLRs)

TLRs share high homology in the signal transduction structures and pathways with receptors for IL-1 and IL-18. In mammals, TLRs are present on macrophages, neutrophils, epithelial cells, dendritic cells and others.

A lower expression of TLRs on intestinal epithelial cells was described in healthy controls (Cario at al., 2000; Frolova et al., 2008). They are example of molecules which enabling mammalian cells to recognize conserved characteristic molecules present on microorganisms and described as microbe-associated molecular patterns (MAMPs) (LPS, flagelin, lipoteichoic acid, PGN, dsRNA, CpG motifs etc.). It is known that commensal bacteria can express lower amount of MAMPs than pathogenic bacteria and produce molecules with immunosuppressive effects (Wilson et al., 2002). Recognition of microbes activates NF-κB and MAP kinases and triggers cytokine production, upregulation of costimulatory molecules on antigen presenting cells (APC) leading to activation of T-cells. The role of other molecules e.g. CD14, MD2, LBP, which are able to bind bacterial components and modulate activation of TLRs, is important in this process (Aderem et al., 2000; Kawai et al., 2006).

1.1.2. Epithelial cells

Epithelial cells of intestine are polarised and are replaced every 3-5 days. The main mechanical barrier of mucosal surfaces, which protect organism against pathogens, is

formed by a layer of epithelial cells covered with glycocalyx. Intestinal epithelial layer is reinforced by tight junctions (TJs) present in paracellular spaces of epithelial cells. TJs were found to act as dynamic and strictly regulated ports of entry that open and close in response to various signals (e.g. cytokines). TJs participate in preserving cellular polarity and are regarded as key elements in intestinal diffusion mechanisms. The molecules forming TJs are connected to the cytoskeleton of epithelial cells.

The integrity of the intestinal barrier in patients with CD and UC is known to be compromised (Fasano, 2001; Tlaskalová et al., 2005). These deffects are abetted by genetically determined alterations that enhance exposure of the mucosal immune system to microflora components (Strober, 2007).

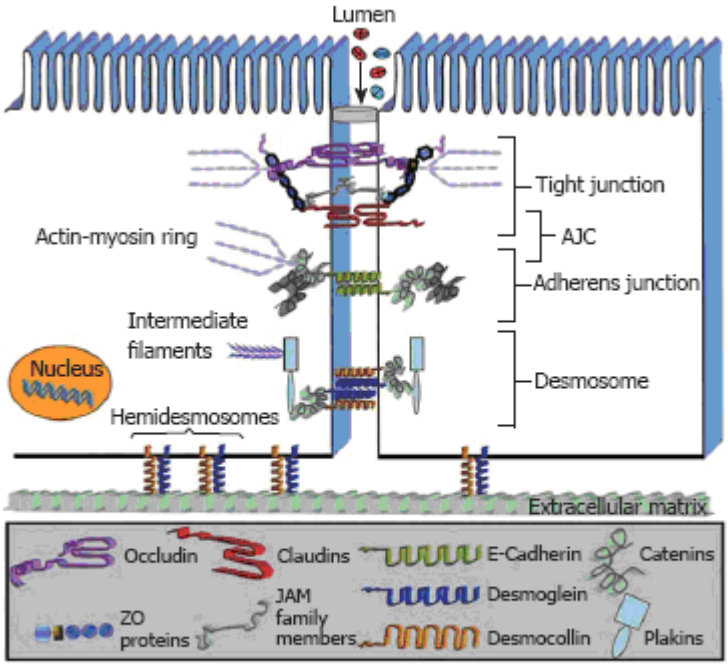

Fig. 1 Intestinal epithelial intercellular junction (Laukoetter et al., 2008)

Accept TJ there are adhesive molecules which are associated with membrane of the epithelial cells. These molecules participate on homotypic and heterotypic reactions.

Through E-catherin epithelial cells are able to bind to the integrins ($\alpha_E\beta_7$) on the surface of intraepithelial lymphocytes (Tlaskalová et al., 2003).

Various types of cells participate in mucosal barrier function: the main and most frequent are conventional enterocytes, of importance are goblet cells (produce mucus and trefoil peptides required for epithelial growth and repair), enteroendocrine cells (produce neuroendocrine molecules having a paracrine effect) and Panneth cells (secrete antibiotic peptides-defensins).

Recent studies provide evidence that reduced expression of Paneth cell defensins may be a key factor in the pathogenesis of ileal Crohn's disease (Bevins, 2006).

Epithelial cells are directly involved in various immune processes. Their capacity to transport secretory immunoglobulins (IgA, IgM) produced by plazma cells in lamina propria to the lumen was described (Brandtzaeg, 1995).

There is strong evidence that epithelial cells express numerous molecules that are involved in antigen presentation: transplantation antigen class I classic and non-classic (HLQ A-C, HLA E, CD1d, MICA/MICB), transplantation class II. Cells of epithelial lines were found to produce constitutively or by induction number of cytokines, chemokines and mediators (IL-1, IL-6, IL-7, IL-8, Il-10, PDGF, GM-CSF, TGF-β) (Standyk, 1994).

1.1.3. Antigen presenting cells (APCs)

Monocytes, macrophages and dendritic cells belong to group of (APCs). They play an important role in the maintenance of oral tolerance in the lamina propria. APC under physiological conditions express low level of surface antigens CD80 and CD86 which are costimulatory molecules for T-cells (Smith et al., 2005).

Intestinal macrophages and dendritic cells located in the lamina propria are the first phagocytic cells of the innate immunity system which protect host against foreign pathogens and regulate response to commensal bacteria. They process antigen and present it on the surface to other cells of the immune system, thus functioning as antigen-presenting cells.

Mononuclear phagocytes system

The representatives of this system are macrophages. Immature forms of macrophages are monocytes present in the blood stream. Monocytes are differentiated in bone marrow from hematopoietic stem cell precursors called monoblasts. They circulate in the bloodstream for about one to three days and then move into tissues throughout the body. In the tissues monocytes mature into different types of macrophages at different anatomical locations. They are able to recruit to the site of inflammation and produce proinflammatory cytokines and chemokines as an answer to microbial stimuli (Zidek et al., 1998). The gastrointestinal mucosa is the largest reservoir of macrophages. Under physiological conditions, resident macrophages are phenotypically and functionally distinct from blood monocytes: do not express receptors for LPS, IgA, IgG, CR3, CR4, growth factors IL-2 and IL-3, and the integrin LFA-1 (Smith et al., 2005).

Moreover, resident intestinal macrophages do not respond to inflammatory stimuli by production of proinflammatory chemokines and cytokines (IL-1, IL-6, TNF-α, IL-8, IL-10, IL-12 or TGF-β, compared to similarly treated monocytes (Smythies et al., 2005).

However, an increased expression of innate receptors (including CD14) on macrophages was detected in IBD, corresponding to potential loss or dysregulation of macrophages inflammatory anergy (Strober et al., 2002; Frolova et al. 2008). This leads to the

recognition of microflora componets through PRRs and activation of NF-κB pathway and trigger proinflammatory cytokine production. Participation of both innate and adaptive immunity is necesary in the induction of IBD.

Dendritic cells (DC)

Dendritic cells are derived from hemopoietic bone marrow progenitor cells $CD34^+$. Monocyte-derived dendritic cells can be generated in vitro from peripheral blood mononuclear cells (PBMCs). Treatment of these monocytes with IL-4 and granulocyte-macrophage colony stimulating factor (GM-CSF) leads to differentiation to immature DCs ($CD11^-$, $CD11^+CD14^+$). They are characterized by high fagocytic activity and low T-cell activation potential. Immature DCs recognize and respond to microbial antigens using PRRs including TLRs. After stimulation with antigen, they become activated into mature DCs and migrate to the lymph node. Simultaneously, they upregulate expression of their cell-surface receptors such as CD80, CD86, and CD40, CCR7 greatly enhance their ability to activate T-cells. Non-infection antigens are presented by immature DCs which induce Th2 or Th3 polarization and initiate oral tolerance. Pathogens are presented by differentiated DCs and induce Th1 immune response and lead to inflammation or resistance to intracellular infections (Tlaskalová-Hogenová, 2003).

Dendritic cells are present in small quantities in tissues that are in contact with the external environment. They can also be found in an immature stage in the blood. As activated, they migrate to the lymphoid tissues where they interact with T cells and B cells to initiate and shape the adaptive immune response.

2.1.4. Galectins

Galectins are family of animal lectins defined by shared consensus of amino acid sequences and affinity for β-galactose containing oligosacharides. Members of galectin family are expressed by variety of cell types and are present both intracellularly and extracellularly. By recognising carbohydrate ligands on cell surface, they can modulate processes such as apoptosis, cell adhesion, migration and cell proliferation (Rubinstein et al., 2004; Rabinovich et al., 2005). In this respect, they regulate different steps of inflammatory cascade (Sato et al., 1994; Sano et al., 2000) and thus may play an important role in modulation of chronic inflammatory disorders such as inflammatory bowel diseases (IBD) and other autoimmune diseases. Galectin-3 deserves special attention in this context.

Galectin-3 (Gal-3), chimera type galectin, is isolated as ~ 30 kDa monomer and is found in cytoplasm, nucleus, on the cell surface and in the extracellular compartment. Its localization depends on various factors e. g. cell type, proliferation status of the cell (Sato et al., 1994; Perillo et al., 1998). Gal-3 is expressed and secreted by a wide variety of cells especially monocytes, macrophages, mast cells and epithelial cells including gastrointestinal epithelium (Gabius, 1997; Hughes, 1999). The biological functions of Gal-3 has been described in several physiological and pathological processes, including its role in cell proliferation, apoptosis, pre-mRNA splicing, metastatic dissemination, cell-cell and cell-matrix interactions. In relationship to inflammatory processes, Gal-3 has been described as strong pro-inflammatory mediator (Rabinovich et al., 2002; Almkvist et al., 2004). It behaves as potent chemoatractant for monocytes and macrophages (Sano et al., 2000) is able to support macrophage phagocytosis (Sano et al., 2003) interact with bacterial LPS (Mey et al., 1996) can activate naive and primed neutrophils (Nieminen et al., 2005).

1.2. Adaptive immunity

GALT (Gut Associated Lymphatic Tissue) consists of T and B cells which formed lymphatic follicles (Peyer`s patches, appendix) or are freely dispersed in intestinal epithelium (IELs) and lamina propria (LPLs).

1.2.1. T lymphocytes

T cells evolve in thymus from lymphoid precursors, enter the circulation as naïve T lymphocytes and migrate to the peripheral lymphoid organs. Here they are activated by antigen presented on APC. After exposure to an antigen they form effector and memory T lymphocytes. T lymphocytes circulate in blood and tissues can be divided into helper T cells (Th) ($CD3^+CD4^+$) and cytotoxic T cells (Tc) ($CD3^+CD8^+$). 95% of lymphocytes carry αβTCR and 5% γδTCR. Minority subpopulation recognizes non-peptid character antigens not presented on APC (Buc, 2001).

Important role in pathogenesis of CD and UC play $CD4^+$ T-lymphocytes. In Crohn's disease there is predominantly a T-helper cell type 1 (Th1) response that develop in the genetically susceptible host and is driven by antigens from the intestinal mucosa with exaggerated production of interleukin 2 (IL-2) and interferon-gamma (IFN-γ). There is intestinal inflammation mediated by macrophage activation and the release of proinflammatory cytokines such as IL-1, IL-6 and tumor necrosis factor-alpha (TNF-α) (Duchmann and Zeitz, 2004). In ulcerative colitis there is Th2 response characterized by production of IL-4, IL-5, IL-10 and IL-13. Th2 cells also support a humoral immune response (Hanauer et al., 2006; Young et al., 2006). Despite these differences, downstream inflammatory events are the same in both conditions. In Crohn’s disease and ulcerative colitis mucosa, IL-1γ, IL-6, IL-8 and tumor necrosis factor-alpha (TNF-α) are produced in

excess, and the production of free radicals accompanying the influx of nonspecific inflammatory cells into the mucosa is above the normal range (MacDonald et al., 2000).

1.2.2. B lymphocytes

Differenciated B cells are present in germinal centres of lymphoid follicles. After antigen recognition they change into plazma cells and produce antibodies. Plazma cells in the lamina propria produce large amount of IgA (2-5g daily), in less degree IgM and IgG. B lymphocytes are able work as APC (Tlaskalová-Hogenová, 2003).

1.2.3. Organised lymphatic tissue

Organised lymphatic tissue (Peyer`s patches and lymphatic follicles) represents an inductive site of the mucosal immune system. Germinal centres of lymphatic follicles consists mainly of differentiating B cells, T cells occupy interfollicular space preferably around venules with high endothelium. Organised lymphatic tissue is covered with an epithelial layer (follicle-associated epithelium) FAE containing special type of epithelial cells called M cells. M cells are most effective in absorbing particular antigens and transporting them from lumen to follicular environment, where antigens activate T cells and induce thus mucosal immunity (Městecký et al; 2004).

1.2.4. Diffused lymphocytes

Diffused lymphocytes represent an efficient effector component of the mucosal immune system. Lymphocytes presented in the epithelium on the basolateral side of enterocytes are called intraepithelial lymphocytes (IELs). They are divided into two populations: with αβ T cell receptor (TCR) and γδTCR. Most of them are $CD3^+CD8^+$, with $CD45RO^+$

phenotype, adhesive molecules (integrin α4β7) and cytoplasmatic granules containing perforin (Městecký et al; 2004).

Diffused lymphocytes of lamina propria (LPLs) are the most numerous and the most active mucosal effector cells. Most of them are $CD4^+$, carried αβTCR and according cytokine production they are divided into Th1 (IL-2, INF-γ, lymphotoxin), Th2 (IL-4, IL-5, IL-6, Il-9, IL-13), Th3 (Il-10, TGF-β).

Unique group is created by T regulatory cells (Treg) ($CD4^+CD25^+$), Th3 and Tr1 which have supressor effect and help to maintain oral tolerance. Treg cells are effective in the prevention and down-regulation of IBD animal models (Singh et al. 2001). $CD4^+CD25$ (high) and $FOXP3^+$ Treg cells are increased during remission of IBD but decreased during active disease (Maul et al. 2005).

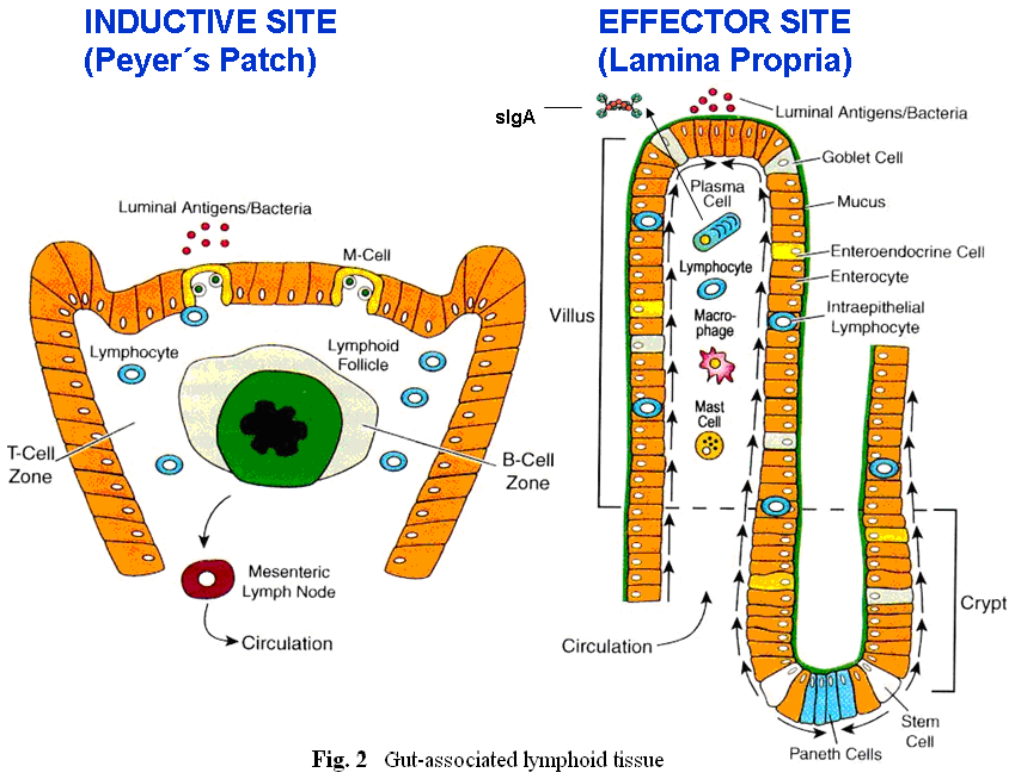

Fig. 2 Gut-associated lymphoid tissue (Tlaskalova, 2005)

2. Inflammatory Bowel Diseases (IBDs)

Crohn`s disease (CD) and ulcerative colitis (UC) are two major forms of idiopathic IBD and have been recognized as distinct disease entities for over a century. They have a devastating impact on a quality life and require long-standing medical care. Despite long-lasting efforts, the etiology and pathogenesis of IBD remain unclear. Is believed that interactions of genetic, immune and environmental factors are involved. New investigative techniques and experimental models of IBD help to increase the understanding of the major pathophysiologic processes of these diseases and develop an effective therapy.

IBD affect approximately 0.2% of the human population and the peak age of onset for IBD is 15 to 30 years old. The second, smaller peak occurring in individuals ages 50 to 70 years. UC and CD are most prevalent in developed, industrialized regions, including the United States, United Kingdom and Scandinavia (Hanauer et al., 2006). In past decades, it was thought that occurred less frequently in ethnic or racial minority groups compared with whites. This gap has been closing; an increase incidence in African Americans and south Asians who have migrated to developed countries has been shown (Carr et al., 1999; Loftus et al., 2002).

2.1. Etiology of IBD

Remarkably growing incidence of UC and CD in second half of 20 century is result of using better diagnostic technique and increased number of factors, which cause formation and development of IBD.

2.1.1. Environmental factors

It has been shown that northern geographic location and high social and economic status increase the risk of IBD. This finding is in agreement with "the hygiene hypothesis" for allergic and autoimmune diseases, which has the association with "westernization" of lifestyle, such as changes in diet, high degree of sanitation, vaccination, sedentary indoor work, stress etc. (Loftus et al., 2002; Kemp et al., 2003).

2.1.2. Genetic factors

It has been shown that genetic factors play an important role in etiology of IBD. Significant HLA associations have been identified with specific extraintestinal manifestations of IBD; ankylosing spondylitis has been associated with HLA-B27 genotype in both UC and CD patients (Tiwana et al., 1997).

A disease susceptibility gene linked to the innate immune response has been identified as caspase recruitment domain protein 15 (CARD 15) genes. It is located at chromosome 16 and encodes NOD2 (nucleotide-binding oligomerization domain 2). A mutant form of NOD2 (three specific mutations has known) is present in some 10%-20% of Caucasian patients with CD. Homozygous patients for these mutations have a 20- to 40-fold increased risk for disease development. Heterozygotes have only a 2- to 4-fold increased risk (Hugot et al., 2001; Ogura et al., 2001).

Other genes associated with CD were detected: interleukin 23 receptor (IL-23R) gene and autophagy-related 16-like 1 (ATG16L1) gene. The link between IL-23R and CD is consistent with the role of IL-23 in mucosal inlfammation and intestinal barrier function. The gene is located on chromosome 1. ATG16L1 gene is localized on chromosome 2 and is connected with autophagy (Mathew, 2008).

2.1.3. Mucosal microbiota

The number of indigenous bacteria colonising mucosal surfaces and skin (10^{14}) exceeds the number of cells forming the human body (10^{13}). The commensal bacteria exhibit enormous diversity (minimum of 1000 species) gram-negative and gram-positive mostly anaerobic and non-cultivable. The highest numbers of bacteria displaying enormous diversity are found in the colon (Savage et al., 2004; Tlaskalova-Hogenova et al., 2004).

The commensal microbiota has been proposed to play a major role in the development and progression of IBD. It is widely believed that loss of tolerance towards intestinal microbiota resulting in an inappropriate immune response in the mucosa which is manifested by the chronic inflammatory process typical of CD and UC and eventually also to cancer (Duchmann et al., 1995, 1999; Tlaskalova et al., 1998; Vitek et al., 2005; Rescigno, 2008). Under normal circumstances there is an intimate interaction between commensal intestinal bacteria and the immune system. Why tolerance is brokendown and an abnormal response to normal gut bacteria develops in IBD is still not entirely clear.

Recent studies from animal`s models of IBD indicates that the normal gut microflora is needed to develop experimental colitis. Gut inflammation only arises in aninals reared in conventional but not in germ-free enviroment (Taurog et al., 1994; Hudcovic et al., 2001). The paradigm “no bacterial, no colitis” is also supported by clinical observations in IBD patients (Swidsinski et al., 2002).

Moreover, based on the results from studies in both animal models and clinical trials, there is an evidence that oral application of live probiotic bacteria assist in reduction of inflammation and has protective effect in development of UC (Steidler et al., 2000; Bibiloni et al., 2005; Kokesova et al., 2006).

2.1.4. Immunological factors

Both, innate and adaptive immune system is involved in the pathogenesis of IBD. More detailed mechanisms were mentioned in chapter “Mucosal immune system”. Briefly, it was suggested that crucial role in the pathogenesis of IBD play intestinal microbiota (Duchmann et al., 1995; Hudcovic et al., 2001). Intestinal macrophages and dendritic cells located below the epithelium in the lamina propria are the first phagocytic cells of the innate immune system which interact with micro-organisms and their products through for example Toll-like receptors (TLRs) (Tlaskalova et al., 2005). Recognition of microbes activates NF-κB signaling pathway, triggering production of cytokines and other inflammatory mediators (Aderem et al., 2000; Medzhitov et al., 2000; Akira et al., 2003). $CD4^+$ T-lymphocytes also participate in the pathogenesis of CD and UC. In Crohn's disease there is predominantly a T-helper cell type 1 (Th1) response and in ulcerative colitis there is Th2 response develop in the genetically susceptible host (Hanauer et al., 2006; Young et al., 2006).

2.2. Crohn`s disease

Crohn`s disease is chronic and relapsing disease, which can involve any part of the gastrointestinal tract from the oropharynx to the perianal area, most commonly in the distal ileum. The inflammation in CD extends deep into the affected tissue (transmural) and is asymmetrical and segmental.

According to Montreal classification CD allows extent to be defined into four subgroups: *ileal, colonic, ileocolonic, upper* (Satsangi et al., 2005).

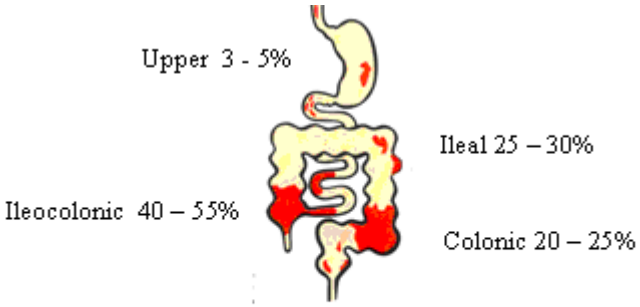

Fig. 3 Localization (subgroups) of Crohn`s disease (Roth, 2003)

Etiology

Incidence of CD is high in the countries with developed economic and in the Czech Republic is comparable with Western European countries. Prevalence rates are between 20 – 40 cases /100,000 inhabitants and incidence rates is of 2 – 6 cases/100,000 persons per year.

Clinical symptoms

Patients report gastrointestinal symptoms of right lower abdominal pain, irregular bowel movements containing mucus and blood, diarrhea. Rectal bleeding which is less frequently seen in CD like in UC. Systemic symptoms of weight loss (malabsorbtion), anemia, fever and fatigue are characteristic (Strober et al., 2007). Fistulas, internal or external, may complicate long-standing cases.

There no specific blood tests available for diagnosis of IBD. Elevated leukocyte and trombocyte counts as well as C-reactive protein (CRP) are indicative of extensive, active intestinal inflammation (Nikolaus et al., 2006).

Endoscopic finding

The earliest endoscopic change of CD is the presence of small oedematous areas obscuring the normal vascular pattern. Well characterized are aphthoid ulcers surrounded by hyperaemic flare in this stage of disease too. Small non-ulcerated hyperaemic spots are occasionally encountered. Advanced and chronic disease shows loss of vascular pattern, map-shaped ulcers with raised red border, cobblestone mucosa (polyps), stenosis and fistulae (Schiller et al., 2002).

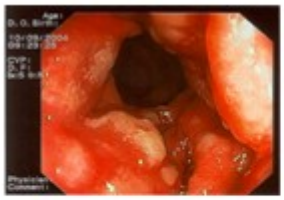

Fig. 4 Endoscopic changes – rectum of patient with CD

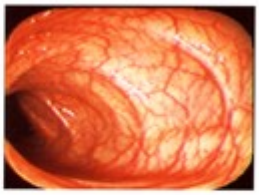

Fig. 5 Endoscopic finding of healthy gut

(Roth, 2003)

Histologic finding

One of the main criteria for diagnosing CD is the presence of granuloma. Irregular ulceration with T cells and monocytes are seen. Crypt abscess are not as conspicuous as in UC. Fistulas may complicate long-standing cases ((Fine et al., 1985; Nikolaus et al., 2006).

Extraintestinal symptoms

IBD symptoms may occur not only in the bowel but also at other sites in the organism. Extraintestinal symptoms affect more than 50% patients with UC and CD and support contention that IBD belong to systemic diseases. The most frequent extraintestinal

manifestations are enteropatic arthritis, osteoporosis, erythema nodosum, pyoderma gangraenosum, iridocyclitis, aphthae (Lukas et al., 1998; Scholmerich et al., 2000).

Medical treatment

The medical treatment approach for CD is individualized based on the severity of symptoms and degree and site of intestinal involvement. Therapy relays on classic anti-inflammatory and immunosuppressant drugs: corticosteroids, mesalamine compounds, antibiotics, azathioprine, biological drugs such as anti-TNF-α antibodies. Other extraintestinal manifestations are the consequence of malabsorbtion such as vitamins and mineral deficiencies. Nutritional intervention is often used to control disease activity (Hendrickson et al., 2002).

2.3. Ulcerative colitis

Ulcerative colitis is chronic and relapsing disease of gastrointestinal tract, which primarily affects the large bowel. The inflammation extends superficial tissue layers of colon and rectum and is symetrical and uninterrupted.

According to Montreal classification UC is categorised into three subgroups: *proctitis* (involvement limited to the rectum), *left side colitis* (involvement limited to the left flexure), *pancolitis* (entire colon) (Satsangi et al., 2006).

Pancolitis 18% Left side colitis 28% Proctitis 54%

Fig. 6 Localization (subgroups) of ulcerative colitis (Roth, 2003)

Etiology

Incidence of UC is high in the countries with developed economic and in the Czech Republic is comparable with Western European countries. Prevalence rates is around 40 cases /100,000 inhabitants and incidence rates is of 8 – 20 cases/100,000 persons per year.

Clinical symptoms

The symptoms are similar to CD, although fistula development does not occur; lower left abdominal pain, irregular bowel movements containing mucus and blood, diarrhea, and rectal bleeding as well as systemic symptoms of weight loss (lack of appetite), anemia, fever and fatigue (Strober et al., 2007).

Patients with long-standing UC have an increased risk of developing colorectal cancer (Vitek, 2006; Xie et al., 2008).

Elevated leukocyte and trombocyte counts as well as C-reactive protein (CRP) are indicative of extensive, active intestinal inflammation also in UC patients.

Endoscopic finding

The earliest endoscopic manifestation of UC is loss of vascular pattern, patchy erythema, and granulation. The inflamed mucosa is often covered with purulent exudate when disease is more advanced or chronic. Pseudopolyps are frequent and vary in size and shape. In acute progressing cases the entire colon is extremely fragile and bleeds freely. Shortening and narrowing of the colon is caused by the fact that muscle is becoming thick and rigid (Fine et al., 1985).

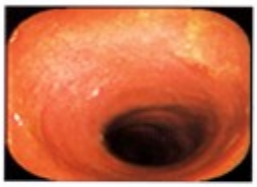

Fig. 7 Endoscopic changes – rectum of pacient with UC

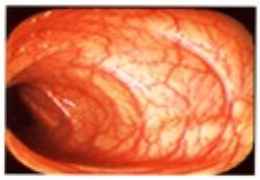

Fig. 8 Endoscopic finding of healthy gut

(Roth, 2003)

Histologic finding

Microscopic finding is characterized by crypt infiltration, particularly with neutrophils. Mucous depletion from the goblet cells, cryptitis with crypt abscesses, and disturbed crypt architecture are typical of UC. The abscesses tend to coalesce to form enlarging ulcers (Fine et al., 1985; Nikolaus et al., 2007).

Extraintestinal symptoms

IBD symptoms may occur not only in the bowel but also at other sites in the organism. Extraintestinal symptoms affect more than 50% patients with UC and CD and support contention that IBD belong to systemic diseases. The symptoms are similar to CD,

although primary sclerosing cholangitis is more common in patients with UC (Lukas et al., 1998; Scholmerich et al., 2000). In children, extraintestinal complication severely retarded growth and development (Kirsner and Shorter, 1995).

Medical treatment

The medical treatment approach is similar to CD and also based on the severity of symptoms and degree and site of intestinal involvement. Classic anti-inflammatory and immunosuppressant drugs are used. Recurrence in UC can be effectively blocked by the administration of probiotics (*E. coli Niessle* and various *lactobacilli*). Special diet is not required (Hendrickson et al., 2002).

3. Aims

1. To characterize the expression of Toll-like receptor 2 (TLR2), TLR4 and their transmembrane coreceptor CD14 in the intestinal mucosa obtained from different parts of the intestine from patients with ulcerative colitis (UC) and Crohn` s disease (CD), and compare it with controls.

2. To determine whether galectin-3 (Gal-3) as an indicator of the association with disease manifestation can be detected in serum of patients. Moreover, to introduce reverse lectin histochemistry to visualize binding sites for this lectin on *E. coli* O83 in inflamed intestinal mucosa (biopsies) from patients with Inflammatory Bowel Diseases (IBD). These lines of our study are aimed at defining clinical relevance of this tissue lectin for the disease.

3. To analyze whether manipulation of intestine microflora by probiotics could affect experimentaly induced intestinal inflammation by examining whether repeated preventive oral administration of live probiotic strains *Escherichia coli* O83: K24: H31, *E. coli Nissle* O6: K24: H31 and *Lactobacillus casei* DN 114001 could protect mice against DSS-induced colitis.

4. Results

Participation of both innate and adaptive immunity is necessary in the induction of IBD. Intestinal epithelial cells form the first line of contact between the host and the normal microbiota and provide the connection with immune system via cells in the lamina propria. PRRs, which are situated also on epithelial cells, participate in recognition of various characteristic bacterial components. Correct recognition and adequate response against commensal bacteria are essential for healthy function and maintenance of the mucosal barrier (Tlaskalová-Hogenová et al., 2002).

In the present study our attention was concentrated on innate immunity and its role in pathogenesis of IBD. We investigated expression of TLR2, TLR4 and CD14 in biopsy samples from patients with IBD and non-inflamed gut mucosa from controls.

A total of 155 intestinal tissue samples from 59 different patients were obtained for our study. Control samples were taken from patients who underwent colonoscopy because of colon cancer screening examination. All of them had normal endoscopic findings without macroscopic and microscopic evidence of inflammatory or neoplastic disease. Biopsies were collected from patients with active IBD and patients who were in remission and had no signs of active disease at the time of sampling. Fresh tissue samples were frozen in liquid nitrogen and cryostat sections were processed by immunohistochemistry. Degree of expression was evaluated and graded into five points.

TLR2-positive epithelial cells were accumulated on the mucosal surface rather than in the crypts in biopsies from patients with IBD. The lamina propria remained negative or rarely displayed sporadic single cells. Noteworthy, statistically significant upregulation of TLR2 protein expression was observed in the ileal epithelium from UC patients with inactive (0.88 ± 0.35) and active disease (0.94 ± 0.30) as compared to the normal intestine

(0.41 ± 0.46). The expression of TLR4 was more apparent in the surface epithelium mainly in samples with weaker positivity and in cases with strong positivity the crypts were equally or more involved. Statistically significant difference were found in the terminal ileum (1.06 ± 0.68) and rectum (1.75 ± 0.60) of UC patients in remission and in the terminal ileum (1.05 ± 0.60) of CD patients with active disease as compared to controls (0.38 ± 0.43; 0.94 ±0.63). Mononuclear cells in the lamina propria displayed strong membranous expression of CD14 in both UC and CD patients while the epithelial cells remained negative. Significant upregulation of CD14 expression was identified in the terminal ileum of CD patients in remission (1.64 ± 0.74) and also those with active disease (1.50 ± 0.35) as compared to controls (1.04 ± 0.37). Moreover, significant upregulation was found in the caecum of UC patients in remission (1.63 ± 0.44) and with active disease (1.95 ± 0.42) and in the rectum of UC patients with active disease (1.85 ± 0.58) as compared to non-IBD tissues (1.11 ± 0.40; 1.08 ±0.56).

Based on the results of our study, we can suggest that epithelial cells are the predominant site of TLR2 and TLR4 expression in intestinal mucosa. Interestingly, in case of UC marker of increased immune activity (upregulation of TLR2) is distributed not only in the inflamed parts of the intestine but also in the non-inflamed part (terminal ileum). It seems that dysregulation of TLR2, TLR4 and CD14 expression in different parts of the intestinal mucosa may be crucial in pathogenetic mechanisms of IBD. Further studies are required to reach a more defined elucidation of the mechanisms involved in these complex processes.

Expression of Toll-like Receptor 2 (TLR2), TLR4, and CD14 in Biopsy Samples of Patients With Inflammatory Bowel Diseases: Upregulated Expression of TLR2 in Terminal Ileum of Patients With Ulcerative Colitis.

Frolova L, Drastich P, Rossmann P, Klimesova K, Tlaskalova-Hogenova H. J Histochem Cytochem 56: 267-274, 2008.

Galectin-3 (Gal-3), the endogenous lectin, is a pluripotent mediator in diverse immune responses. It is able to play an important role in modulation of chronic inflammatory disorders such as IBD and other autoimmune diseases (Gabius, 1997; Almkvist et al., 2004; Dimic et al., 2006). Recent studies suggested that cell surface glycosylation is hardware for biochemical signaling and endogenous lectins work as effectors translating the signals into cellular responses (Gabius, 2006; Villalobo et al., 2006).

The aim of this pilot study was to determine whether Gal-3 can be detected in serum of IBD patients in active and remission stage. And using lectin histochemistry to find whether Gal-3 will be present in inflamed intestine mucosa and participate in binding of bacteria.

A total of 197 blood samples were obtained from patients with IBD or non-IBD controls. Serum was stored until assayed. Western blot analysis showed that Gal-3 is present in serum and its concentration in sera from IBD patients appeared to be increased relative to controls. For quantitative measurement of Gal-3 Elisa kit was used. UC patients with active disease (mean, 6.13 ng/ml) and in remission (mean, 4.40 ng/ml) presented a significantly higher concentration of Gal-3 in the serum than seen in control samples (mean, 2.38 ng/ml). Moreover, in the group of CD patients, a statistically significant difference was found in patients with active disease (mean, 5.65 ng/ml) and in patients in remission (mean, 3.42 ng/ml) as compared to controls (mean, 2.38 ng/ml). Furthermore, serum levels of Gal-3 in patients suffering from IBD may well be used to monitor the efficacy of the therapy. The treatment with 5-aminosalicylic acid (ASA), corticosteroids (CS) or azathioprine (AZA) alone and with a combination of two drugs showed lower

effect than the combination of all three substances (ASA+AZA+CS). Increased serum level of Gal-3 has potential to signal insufficient therapy or relapse of the inflammatory process.

Biopsies were collected from patients with IBD and non-IBD controls. Cryostat sections were processed by immunohistochemistry. Strong expression of Gal-3 was determined on $CD14^+$ cells in biopsies from IBD patients, while positive enterocytes were minimal. Moreover, colocalization between cells interacting with FITC-labeled bacteria and expressing Gal-3 was detected. Gal-3 expression in biopsies from patients with remission or active disease yielded no notable difference. Tissue samples from non-IBD controls revealed no Gal-3 presence on $CD14^+$ cells but on enterocytes. Binding sites for bacteria were seen on $CD14^+$ cells in IBD patients. On the other hand, no $CD14^+$ cells were found in non-IBD controls. Furthermore, biopsy samples from non-IBD controls failed to be reactive with FITC-labeled bacteria.

This pilot study demonstrates that Gal-3 has a potential to become a therapeutic target. Significantly increased concentration of Gal-3 was determined in sera from patients with active and remission stage of UC and CD as compared to healthy controls. It also extend the evidence that Gal-3^+ cells are active in bacterial adhesion in tissue samples from IBD patients.

Detection of galectin-3 in patients with Inflammatory Bowel Diseases: New serum marker of active forms of IBD?

Froľová L, Smetana K Jr, Borovská D, Malíčková K, Janatková I, Lukáš M, Drastich P, Beneš Z, Kitanovičová A, Klimešová K, Tučková L, André S, Gabius H-J, Tlaskalová-Hogenová H. (under rewiew in journal Exp Clin Immunol)

The pathogenesis of IBD represents the outcomes of three interactive cofactors: host susceptibility, enteric microflora and mucosal immunity (Shanahan, 2001). The finding

that there is an abnormal T-cell responsiveness to normal intestinal microflora in human and experimental models awakened interest in the possibility those commensals may initiate and maintain IBD lessions. Normal microflora profoundly influences the host mucosal structure and function as well as the development of the whole immune systems (Tlaskalová-Hogenová, 1997).

In our study we examined whether repeated prevented oral administration of live probiotic bacterial strains *E. coli* O83:K24:H31, *E. coli* Nissle 1917 and *Lactobacillus casei* DN 114001 can protect mice against DSS-induced colitis.

Cultivated bacterial strains were harvested and diluted in PBS to final concentration 10^9 CFU/ml. BALB/c mice (n = 60) were fed daily for 14 d by gastric lavage with 100 μl of live bacterial suspension (n = 20 in the group for 1 bacterial strain) and control mice (n = 15) were fed with 100 μl of PBS. Colitis was induced by 5% DSS in drinking water for 7 d to 10 mice in every bacterial group and to 10 mice in the control group. 5 control mice and 10 mice in each bacterial group drank water without DSS.

Relative to animals from PBS and DSS groups, colitic mice fed with probiotic strains exhibited a decrease in the symptom score (was constructed attributing 1 point to each of the following events: rectal prolapse, rectal bleeding, colonic bleeding and death) Ec O83 (1.44 ± 0.33), Ec Nis (0.85 ± 0.54), Lc (0.65 ± 0.32) as compared to PBS control (2.20 ± 0.18) and had significantly longer colon at the end of the experiment. There was significant decrease in mass loss of colitic Lc mice. While severe inflammation, crypt destruction and ulceration of the mucosa were observed in the PBS-fed group by histology evaluation (1.23 ± 0.17), significantly less inflammation was observed in the Lc-fed group (0.86 ± 0.23). Mice exposed to both strain of *E. coli* had the inflammation score comparable to PBS

control group (1.23 ± 0.17). Mice drinking water instead of DSS had no colonic inflammation.

ELISA was used to evaluate concentration of specific antibodies against probiotic bacteria. There was a significant difference in IgA Ab content between Ec Nis (18 ± 3.4) and Lc (20 ± 2.0) pretreatment in DSS colitic mice as compared to healthy colonized mice (11.5 ± 3.4; 15 ± 1.5) while Ec O83 stimulated intestinal IgA production in healthy mice. No difference in specific IgM Ab production between mice with DSS-induced colitis and control animals was found. There was a significant difference in specific IgG Ab production in Lc group with DSS-induced colitis (50 ± 2.7) and healthy Lc-colonized mice (45 ± 3.7).

Our data corroborate the evidence that nonpathogenic luminal bacteria participate in the pathogenesis of chronic intestinal inflammation. Administration of probiotic bacteria influence the composition of intestinal microflora may be relevant to the development of novel strategy in the prevention and management of IBD patients.

Oral administration of probiotic bacteria (E. coli Nissle, E. coli O83, and Lactobacillus casei) influences the severity of dextran-sodium-sulfate-induced colitis. Kokesova A, Frolova L**,** Kverka M, Sokol D, Rossmann P, Bartova J, Tlaskalova-Hogenova H. Folia Microbiol 51: 478-84, 2006

Some of our results were published in review which described important role of mucosal immune system in the development of whole immune system of human body. Basic functions of mucosal immune system are protection against penetration of pathogenic microorganisms and induction of oral tolerance against antigens present on mucosal surfaces (Brandtzaeg et al., 2004; Mestecky et al., 2005). Components of innate immunity

protect organism immediately after birth when adaptive immunity is not yet completely develops. A characteristic feature of innate immunity is ability to distinguish between pathogenic microbial components and harmless antigens by pattern recognition receptors (PRRs). Example of these molecules is Toll-like receptors (TLRs) that recognize characteristic molecules pathogen-associated molecular patterns (PAMPs) present on microorganisms. Recognition of microbes activates NF-κB signaling pathway, triggering production of cytokines and other inflammatory mediators (Aderem et al., 2000; Medzhitov et al., 2000; Akira et al., 2004). Commensal bacteria are in close proximity to a large population of rapidly renewing epithelial cells. After birth is mucosal surface colonized with microorganisms (~ $1x10^{14}$). Most of microbes living in the gut are not cultivable that is why identification of bacterial strains are not yet complete and is the subject of many studies. Experiments performed in gnotobiotic models suggest that composition of gut microbiota plays decisive role in the pathogenic mechanisms of intestinal inflammation (Taurog et al., 1994; Madsen et al., 2000; Hudcovic et al., 2001).

The solution of the question which bacteria and what mechanisms are involved in induction and maintenance of chronic intestinal inflammation could bring new approaches to the therapy and prevention of several diseases such as ulcerative colitis, Crohn`s disease and celiac disease.

Involvment of innate immunity in the development of inflammatory and autoimmune diseases.

Tlaskalova-Hogenova H, Tuckova L, Stepankova R, Hudcovic T, Palova-Jelinkova L, Kozakova H, Rossmann P, Sanchez D, Cinova J, Hrncir T, Kverka M, Frolova L, Uhlig H., Powrie F, Bland P. Ann NY Acad Sci 1051: 787-98, 2005

5. Discussion

Inflammatory bowel diseases such as Crohn`s disease and ulcerative colitis are severe chronic disorders with periods of remission and relapse which affect gastrointestinal tract. There is increasing evidence that IBD results from an abnormal immune response to normal intestinal microflora that develops in the genetically susceptible host. IBD appear to involve interactions between immune, environmental and genetic factors; the combination of these factors results in induction of inflammation, subsequent mucosal lesions and their repair. The etiology and pathogenesis of IBD remain unclear despite intense study. It is known that $CD4^+$ T-lymphocytes participate in the pathogenesis of CD and UC. In Crohn's disease there is predominantly a T-helper cell type 1 (Th1) response and in ulcerative colitis there is Th2 response (Hanauer et al., 2006; Young et al., 2006). Microbial components, which are recognized by APCs and presented on their surface, activate specific $CD4^+$ T-lymphocytes; this reaction is joined by production of proinflammatory cytokines and chemokines. Now, components of innate immunity are the subjects of intensive study in many scientific groups. These studies are concentrated on activation of macrophages, dendritic cells and intestinal epithelial cells, expression of pattern recognition receptors (TLRs, NODs, etc) that recognize bacterial antigens, diversity and changes in intestine microflora composition, etc. (Cario et al., 2000; Hausmann et al., 2000; Smith et al., 2005; Bibiloni et al., 2005).

In our study we are interested in the pathogenesis of IBD in terms of innate immunity. Controversial data of other authors (Cario et al., 2000; Cario et al., 2002; Hausmann et al., 2002; Melmed et al., 2003) about the expression of TLR2 and TLR4 on mRNA and protein level obtained from intestinal biopses (usually only colon) or intestinal epithelial cell (IEC) lines led us to characterize the expression of TLR2, TLR4 and their transmembrane

coreceptor CD14 in the intestinal mucosa obtained from different parts of intestine including terminal ileum from patients with UC and CD.

We focused our interest on three different parts of the bowel, which can play different roles in the development of the disease and expression of TLRs and CD14 in patients and control individuals. Biopsies from terminal ileum, cecum and rectum from CD and UC patients and controls were examined by immunohistochemistry. We showed a significant increase in TLR2 expression on the mucosal surface in the terminal ileum of patients with inactive and active UC against controls, even if macroscopic inflammation or back-wash ileitis was lacking. Interestingly, the TLR2 and TLR4 positive IECs were found in the terminal ileum in the area of the gut which is not usually affected by inflammation in these patients. Moreover, TLR2 expression is significantly increased regardless of whether assessed in active or remission stage of UC. Possible explanation of this unexpected finding could be that this upregulation reflects the activation of innate immunity cells by as yet unknown bacterial components present also in the proximal part of the gut of UC patients. Another explanation is that the immunosuppression might influence bacterial colonization or bowel peristalsis in the terminal ileum. Thus, it seems that the terminal ileum can sensitively react to „potentially pathogenic“ but still undiscovered components of microbiota by immunological mechanisms impairing the homeostasis in the distant part of the gut, i.e. colonic tissues. Recent studies (Cario et al., 2000; Abreu et al. 2002; Hausmann et al. 2002) have described low TLR4 expression by IECs in healthy human biopsies and significantly increased expression of TLR4 in patients with IBD. These results corroborated our data; significant upregulation of TLR4 expression relative to controls was found in the terminal ileum and rectum of UC patients in remission and in the terminal ileum of CD patients with active disease. According to Duchmann (1995) and

Lange (1996) increased TLR4 expression in IBD patients could be the consequence of impaired host tolerance towards luminal LPS antigens. On the other hand, large quantities of luminal LPS are usually well tolerated within the healthy intestine. The immunohistochemical findings have been confirmed by studies measuring TLRs expression on mRNA level (Hausmann et al. 2000; Abreu et al. 2002; Melmed et al. 2003; Furrie et al. 2005). In our study, significant upregulation of CD14 expression on macrophages was identified in areas affected by IBD (terminal ileum of active CD patients and CD patients in remission, caecum of UC patients in active and remission stage and rectum of UC patients with active disease) as compared to non-IBD samples. Other authors in compliance with us showed that nearly a third of lamina propria macrophages in the inflamed mucosa of patients with IBD express CD14 (Rugtveit et al. 1994; Grimm et al. 1995; Rogler et al. 1997). We did not observe CD14 in IECs, which is in agreement with previous studies; human enterocytes in vivo do not express membrane CD14 at a level detectable by immunohistochemistry (Grimm et al. 1995, Pugin et al. 1993, Schumann et al. 1994).

At the beginig of our next report was a suggestion that Gal-3 is able to play an important role in modulation of chronic inflammatory disorders such as inflammatory bowel diseases (IBD) and other autoimmune diseases (Mey et al., 1996; Sano et al., 2000; Rabinovich et al., 2002; Sano et al., 2003; Almkvist et al., 2004; Nieminen et al., 2005), and evidence that enterocytes are the source of tissue Gal-3 and, in IBD, Gal-3 is downregulated in these cells (Jensen-Jarolim et al., 2002; Muller et al., 2006; Shiobara et al., 2007). In this study we address the question whether Gal-3 as an indicator of an association with disease manifestation can be detected in serum of patients. Moreover, we introduced galectin and

reverse lectin histochemistry to visualize for example the binding sites for this lectin on *E. coli* O83 in inflamed intestine mucosa (biopsies) from patients with IBD.

Serum samples from IBD patients (active and remission stage) and controls were processed by Western blot analyses and ELISA tests, tissue samples were examined by reverse lectin histochemistry. Western blotting showed that Gal-3 is present in the serum and quantification by ELISA revealed that this parameter was significantly upregulated in the serum of patients with UC (active and remission) and CD (active and remission) as compared to healthy controls. Gut microbiota are assumed to elicit dysregulation in intestinal immune responses hereby may thus become a factor in pathogenetic mechanisms of IBD (Tlaskalova et al., 2004; Elson et al., 2005; Swidsinski et al., 2005; Sydora et al., 2006; Stepankova et al., 2007). Applying multiple labeling at the single-cell level, binding sites for labeled bacteria were visualized on $CD14^+$ cells (monocytes/macrophages) in the lamina propria of IBD patients. No binding sites for bacteria were found in tissue samples from non-IBD patients. We observed CD14-positive cells with Gal-3 in the biopsy samples from IBD patients, while biopsies from healthy controls presented positivity in enterocytes. In consequence, intestinal macrophages and dendritic cells located below the epithelium in the lamina propria are the first phagocytic cells of the innate immune system that interact with microorganisms and their products (Tlaskalova et al., 2005). Human intestinal macrophages in normal mucosa do not express innate immunity receptors including CD14 (Smith et al., 2001). However, monocytes recruited to the inflamed mucosa do not undergo such stringent downregulation and retain their proinflammatory potential and functional profile (Rogler et al., 1997; Smith et al., 2005). These results implicate Gal-3-positive cells as being active in bacterial adhesion and Gal-3 then could be involved in the pathogenesis of IBD. Food-derived antigens and changes in microbiota

composition could be initial signals for the onset of development of a chronic inflammatory process involving CD14-positive cells (McDonald et al., 2005; Muller et al., 2006; McDermott et al., 2007).

The involvement of gut microbiota in pathogenetic mechanisms led us to examine if preventive oral administration of live bacterial strains *E. coli* O83, *E. coli* Nissle and *Lactobacillus casei* can protect mice against DSS-induced colitis. Our experiments were supported by previous studies where authors suggested a potential role of probiotic preparation in the treatment of IBD (Schultz et al., 2003) or demonstrated beneficial effect of *E. coli* Nissle treatment in the maintenance of remission of UC (Rembacken et al., 1999; Kruis et al., 2001).

Balb/c mice were used in our study, colonic samples were obtained for histopathology and serum and enteral fluid samples were processed by ELISA test. We shoved that manipulation of intestinal microflora could affect DSS-induced intestinal inflammation. *E. coli* O83, *E. coli* Nissle and *Lactobacillus casei* (Lc) ameliorate the clinical signs of intestinal inflammation, as was demonstrated by a lower symptom score, healthier clinical appearance of the animals and partial restoration of normal colon length. Lc-precolonized mice had also a significantly lower mass loss at the end of the experiment and lower histological inflammation score in comparison with untreated mice with DSS-induced colitis. A significant difference between probiotic-colonized mice with DSS-induced colitis and healthy probiotic-colonized mice was found in the level of specific IgA against bacterial strains in enteral contents. Moreover, a significant difference in total specific IgG in serum between DSS-colitic and healthy mice was determined in Lc-pretreated mice. The mechanism by which DSS induces colitis is not well defined, but it seems to result from an alteration of colonic epithelial cells and be dependent on the intestinal microflora

(Dieleman et al., 1994; Rath et al., 2001). Sartor (2004) has suggested some mechanisms that could explain the protective effect of probiotic in intestinal inflammation; suppression of growth or epithelial adhesion of pathogenic bacteria, improved epithelial barrier function or immunoregulatory activities.

6. Conclusion

In our study we contributed to clarifying the important role of innate immunity components and commensal bacteria in the pathogenesis of IBD. We have shown that upregulated expression of TLR2, TLR4 and CD14 in patients with CD and UC reflects the consequences of the interaction of intestinal epithelial cells and macrophages in the lamina propria with intestinal microbiota. Moreover, the pilot study focused on Gal-3 demonstrated a significantly increased concentration of Gal-3 in sera from patients with active and remission stage of UC and CD as compared to healthy persons. It also extended the evidence for upregulation of Gal-3 expressed by $CD14^+$ cells in intestinal tissue samples of IBD patients, pointing to the possibility to view Gal-3 as a therapeutic target. Moreover, we showed that administration of probiotic bacteria influences the development of experimentally induced intestinal inflammation and may be relevant in the development of a novel strategy in the prevention and management of IBD patients. Further studies are required to reach a more defined elucidation of the complex mechanisms involved in processes underlying inflammatory bowel diseases.

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