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The immune response and genetic predisposition have been implicated in the pathogenesis of several complex diseases in which the identification of genetic determinants constitutes a major challenge. Infectious diseases such as those associated with Chlamydia trachomatis (Ct) infection and chronic inflammatory bowel diseases are complex diseases in which multiple genetic, environmental, and pathogenic factors are likely to contribute to pathogenesis.

The inflammatory bowel diseases Crohn’s disease and ulcerative colitis are complex and multifactorial diseases of which the exact aetiology is not yet known. Crohn’s disease may affect any part of the gastrointestinal tract from the oral cavity to the rectum, whereas ulcerative colitis is a disease of the large bowel only. Both are chronic diseases where environmental, immune-mediated and genetic factors determine the course. Several studies have failed to show the involvement of a specific bacterium in the aetiology of these diseases. The disease course might be asymptomatic at presentation or life-threatening. Patients may require lifelong immune suppressive treatment and in most cases surgical intervention. Often recurrences of intestinal inflammation have a severe impact on the patient’s quality of life. Extraintestinal manifestations of these diseases also play an important role in the quality of life and morbidity.

Chlamydia trachomatis is an obligate intracellular bacterium causing most cases of sexually transmitted infection with subsequent inflammation resulting in reproductive tract destruction and infertility. The exact aetiology needs to be elucidated.

A common factor between the gastrointestinal and urogenital tract is the mucosal surface that is continuously exposed to potential pathogens as well as beneficial commensal microorganisms, and forms the first station for the immune system. Both tracts are part of the mucosal immune system. The small intestine plays a dual role which consists of exclusion of pathogens while facilitating absorption of nutrients. Homeostasis and balance between immunity and tolerance challenges the immune system of the mucosa.

Remarkable interindividual variations have been observed in susceptibility to infection and inflammation, in the course of eradication of pathogens, and the development of complications. These variations might be due to differences in the genetic predisposition with regard to the immune regulation.

The advances in human genetics and in particular in relation to the control of the immune response offer new opportunities to investigate the role of various immune mediators in disease susceptibility and severity.
Introduction

General introduction

The relationship between the mammalian host and commensal microorganisms is the product of millions of years of co-evolution and is a fundamentally symbiotic one. The mucosal surfaces of the intestinal and urogenital tracts are constantly exposed to potential pathogens as well as beneficial commensal bacteria. Colonization of both the intestine and the urogenital tract by beneficial bacteria, through occupation of this environmental niche, competition for nutrients, and secretion of antimicrobial peptides, probably provides a degree of protection for the host against rapid colonization by pathogenic microorganisms. The required balance between tolerance and immunity represents a regulatory challenge to the mucosal immune system. The beneficial properties endowed by commensal bacteria on host physiology underlie the requirement for immune hyporesponsiveness to these microbial communities. Paradoxically, some degree of innate immune recognition of commensal bacteria is essential for normal development and function of the mucosal and peripheral immune system. These observations indicate that molecular mechanisms must exist to facilitate the recognition of commensal bacteria and allow for a basal level of immune activation in order to program gene-expression patterns that are required for the normal development and function of immune cells.

Mucosal epithelial cells form a crucial cell lineage for maintaining mucosal immune homeostasis by sampling the intestinal and urogenital microenvironment, discriminating pathogenic and commensal microbes and influencing the function of antigen-presenting cells and lymphocytes. Regulation of the immune responses to commensal bacteria is obligatory to prevent pathogenesis of numerous inflammatory and infectious conditions. Innate immunity involves phagocytic cells such as neutrophils, macrophages, and dendritic cells, also the natural killer cells, and molecules such as natural antibodies, complement proteins, and cytokines. Epithelial and dendritic cells are the host cells mostly interacting with luminal flora. These cells play a crucial role in innate and adaptive immunity and interaction occurs by means of their pattern recognition receptors (PRRs). These PRRs detect microbe-associated molecular pattern (MAMP) or pathogen-associated molecular patterns (PAMPs) that are located on bacterial surface molecules, and are not expressed by the host. Alterations in the acquisition or composition of commensal microbes may influence susceptibility to, or progression of, immune-mediated diseases as well as metabolic diseases. A defective immune regulation at mucosal sites may lead to the so-called “barrier diseases”. The concept of the mucosal immunity arose from observations based on immunoglobulin-containing cells in intestinal, respiratory, mammary, and genital tissues. The interaction of the microflora with the host gastrointestinal and urogenital tracts, and genetic polymorphisms together with environmental changes in lifestyle might have turned the genetic variability controlling the balance between beneficial and pathogenic bacteria into disease-causing mutations (fig 1).
Introduction

Figure 1: The interaction of microbial factors, environmental triggers, the host immune response, and the host genetic susceptibility contributes to the clinical course of IBD and thus to the differences observed in disease progression. Figure from Sartor et al.\textsuperscript{12}

**Immunogenetics: host genetics and the immune system**

Conventionally, research in (Chlamydial) infectious diseases concentrated on environmental and microbial factors to implement the risk of infection and transmission. Specific strains with potential differences in virulence were analysed. However, the analysis of environmental and microbial factors alone, could not explain the observed differences in the course of infection. Later, specific genes linked to extreme phenotypes of disease were identified. Recent studies have provided information on the role of host genetics in disease aetiopathogenesis, and a prominent role for genes encoding immune mediators has been established. The innate immune system is the immediate host defence system in response to foreign bacteria or viruses by sensing and recognizing the pathogens by PRRs, as fundamental components initiating the innate immune responses to infection. PRRs serve as a link between innate and adaptive immunity, and help defend against infection by an inflammatory response.

Some of the elements of innate immunity are preformed (e.g. natural antibodies, the alternative complement system) while others are mobilized following cellular activation via receptors which recognize MAMPs or PAMPs, lipopolysaccharide (LPS) on Gram-negative bacteria (endotoxins), flagella, peptidoglycans, outer membrane lipopeptides, CpG motifs in bacterial DNA, etc.).\textsuperscript{13-17} Cell stimulation through these receptors results in upregulation of antimicrobial killing mechanisms and pro-inflammatory mediator release.
PRRs are germ line-encoded receptors consisting of two major classes. The first includes transmembrane Toll-like receptors (TLRs) which monitor the extracellular environment and phagolysosomal compartments, and recognize PAMPs. The second consists of soluble cytosolic nucleotide oligomerisation domain (NOD) like receptors (NLRs). These NLR proteins contain leucine-rich repeats (LRRs), a membrane spanning motif and a Toll/Interleukin-1 receptor domain (TIR) required for signal transduction. NLRs complement host defence by providing surveillance of the cytosol. The NOD containing proteins recognize cell wall fragments from both gram-negative and gram-positive bacteria. Activation of PRRs by specific PAMPs results in genomic responses in host cells involving activation of transcription factors, the induction of antimicrobial molecules, chemokines, cytokines, and costimulatory molecules, and the induction or expression of immune regulatory genes.

For a proper host response to microbial challenge, a tightly controlled TLR signalling is obligatory. An impaired signalling leads to susceptibility to infection whereas excessive signalling can lead to septic shock and possibly autoimmune disease and allergies. The role of TLRs has been studied in several diseases, and the genetic variations in these immunologically important TLR genes play a pivotal role in the course and outcome of infectious and inflammatory diseases. Single nucleotide polymorphisms (SNPs) are genetic variations composed of one substituted, inserted, or deleted nucleotide, and occur within coding sequences of genes, non-coding regions, and the intergenic regions. Genetic variations may have direct or indirect biological consequences. Some of the direct biological consequences consist of up or down regulation of protein translation, or translation of aberrant proteins. SNPs within individual TLRs and within their adaptor molecules and downstream kinases used in the TLR signalling cascades have been identified. Some are linked with disease or disease progression highlighting the role of genetics in the function of TLRs in a variety of diseases and infections. It has been shown that functional polymorphisms often occur with different frequencies in diverse ethnic populations and that these polymorphisms can differently influence aetiopathogenesis in these populations. Based on these data, it can be assumed that the host (immuno-) genetic background, combined with environmental and microbial factors contribute to the course and outcome of infection.

**Candidate gene approach**

The very first and critical step in the candidate gene approach was that of selecting the candidate gene that may suitable play a relevant role in the pathogenesis of common and complex diseases. For example, when studying infectious diseases, genes encoding pathogen recognition and genes acting in various immune regulatory pathways are candidates to study. Subsequently, gene variants that for instance result in proteins with altered functions that might influence the trait are identified and studied between cases-controls.
About 90% of variants are single nucleotide polymorphisms (SNPs). More than 30 million SNPs (dbSNP; build 132) are publicly available.\textsuperscript{29}

Association studies using the candidate gene approach may provide large statistical power over linkage to detect genetic effects underlying diseases.\textsuperscript{30}

These studies can be performed in a direct or indirect approach:

The direct approach is based on availability of a list of functional variants that are tested for association with the disease in cases and controls.

The indirect approach is the analysis of a series of genomic variants for association with the disease, depending on the assumption that the studied variant is in linkage disequilibrium with the causative variant. Linkage disequilibrium in different parts of the genome seems to be highly variable.\textsuperscript{31}

Haplotypes consist of chromosomal segments with varying lengths which were conserved over history with a consequence that certain alleles are inherited together more often than would be expected under random inheritance. A few common haplotypes so far represent the majority of individuals in a population the haplotype diversity is general, whereas the frequencies do vary.\textsuperscript{31, 32}

This results in a great advantage since to study a specific region with high linkage disequilibrium, only few variants that each tag a haplotype are needed to cover the region (i.e. so called ‘haplotypes tagging SNPs’ or ‘tag SNPs’).

In October 2002 the HapMap project started, that was expected to take about three years to identify genetic sequences in chromosomal regions where genetic variants are shared. The HapMap originally comprised of two phases; the complete data obtained in Phase I was published on 27 October 2005, and the data of the second Phase were published in 2007. Subsequently, Phase III data were released in 2008, and the second draft of Phase III was released in 2009.

In this map SNPs were selected with a minor allele frequency \(\geq 0.05\). The principle is that analyses of local LD patterns in a specific population identify the haplotype block and all related regions.

When statistically significant associations are found, further studies, such as expression profiling of mRNA and protein levels, are initiated to identify the exact biological mechanisms through which the genetic polymorphisms influence the disease pathogenesis.

Accumulation of gene polymorphisms may increasingly influence the specific gene’s transcription, protein expression, or protein function. For example, several mutations in the \textit{TLR4} gene were studied. In this case, the single SNP association analysis revealed that there were no statistically significant associations between each individual polymorphism and the prevalence of meningococcal sepsis. Interestingly, the prevalence of sepsis was higher when the increasing number of unfavourable rare mutations in the \textit{TLR4} gene were combined in the systemic meningococcal infected patients.\textsuperscript{33} Combinations and accumulation of multiple
polymorphisms across multiple genes may influence pathogenesis, suggesting that the combined information about these variations is useful to assess the risk of disease.

In IBD, the mucosal epithelial cells are important in the recognition of microorganisms and/or substances by the TLRs and NOD proteins and produce chemokines and cytokines. CD, for example, seems to result from a defective function of the nucleotide-binding oligomerization domain 2 (NOD2) protein. Ogura et al. found that the 3020 frameshift mutation in NOD2 was associated with CD. It has been shown that NOD2 mutations cause loss of negative regulatory effects on TLR signalling. This concept is very relevant to understand the complexity of chronic inflammatory diseases where multiple genes are involved in predisposition and prognosis.

**Genetic variation of the immune system and its mediators**

Several key immune mediators have been studied in relation to gastro-intestinal and urogenital diseases. These regulatory cytokines and bacteria sensing receptors are involved in the activation of the NF-κB pathway, which is of major importance in the regulation of the inflammatory response. As patient-related immunomodulating factors, TLRs are closely involved in pathogen recognition and host defence in infection and inflammation. Both commensal and pathogenic bacteria express a variety of ligands that could serve as potential TLR ligands. A normal immune response to these microbes is based on the adequate pathogen recognition by TLRs and NLRs in the epithelial cells of the intestinal and urogenital tract. Genetic variation in the immune system in general, and more specific in TLR genes, may result in deviant receptors in the cell membrane or inside the cell, leading to altered recognition of pathogens and induction of signalling cascades that escalate a molecular response against detected pathogens.

**Toll-like receptor family**

PAMP receptors of the TLR family have been identified as fundamental components of the innate immune response to bacterial pathogens. Each of ten different family members has its own specific ligand (figure 2). TLRs recognize several microbial products, including bacterial cell wall components and DNA. The TLRs use different signalling cascades generally resulting in activation of transcription factors NF-κB and activating protein-1 (AP-1) in adaptor myeloid differentiation primary response protein (MyD)88 dependent pathways.
**Figure 2:** Binding of microbial adjuvants to extracellular and intracellular PRRs. TLRs on the cell membrane selectively bind to bacterial, viral or fungal components and activate signalling pathways followed by activation of the NFκB and mitogen-activated protein kinases.

**Chlamydia trachomatis infections of the urogenital tract**

Ct is the most important bacterial cause of sexually transmitted infections and may cause considerable reproductive morbidity with the highest rates in adolescent women. The incidence of Ct is increasing since the 1990s\(^{41,42}\) with about 89 million new cases worldwide each year.\(^{41}\) For the Netherlands, the estimated incidence of Ct encompasses 60,000 of the 110,000 reported sexually transmitted infections (STI).

*Ct* is an obligate intracellular pathogen with a biphasic life cycle, namely the elementary body (EB) and the reticulate body (RB). Ct requires the host cell for replication.\(^{43}\) The EB is infectious, but metabolically inactive, enters the mucosal epithelial cells where it is surrounded by an endosomal membrane to form an inclusion where replication of Ct takes place. Inside the inclusion, the EB transforms into the metabolically active RB that is non-infectious and replicates by binary fission. Within 40–48 hours, the RB transforms back into an infective EB, and is then released from the inclusion vacuole to infect neighbouring cells\(^{44}\) (figure 3). Three biovars of *Ct* are known (Table 1). In women, Ct infects the single-cell columnar layer of the epithelium in the endocervix. Within the epithelial cells, Ct undergoes a
developmental cycle and produces infective EB. EB can infect neighbouring epithelial cells, causing an intense inflammation leading to mucopurulent cervicitis. In men, Ct infects the urethra causing an intense inflammation of the epithelial layer and thereby leading to non-gonococcal urethritis.\textsuperscript{45}

![Figure 3: *C. trachomatis* developmental cycle (adapted from Brunham, *Nature Reviews Immunology* 2005)](image)

Infection often remains asymptomatic and provides a huge reservoir for transmission of the disease. It is generally accepted that 70-80% of female Ct genital tract infections (GTI) are asymptomatic and without severe sequelae.\textsuperscript{46} Approximately 50% of infected males are asymptomatic. Repeated infections seem associated with severe upper genital tract pathology which includes pelvic inflammatory disease (PID), ectopic pregnancy, and tubal infertility in decreasing order of occurrence.\textsuperscript{47} Due to limited early detection of Ct infection leading to unrecognized PID, patients may stay untreated. This causes a deteriorated reproductive health of the society and entails significant expenditures for the treatment of this pathology.

Chronic Ct infection has also been associated with foetal death, pre-term labour due to premature rupture of the membranes, low birth weight, and late-onset puerperal fever.\textsuperscript{48} Furthermore, infection may proceed to the abdominal cavity and cause ‘sterile’ peritonitis with ascites. Acute perihepatitis (Fitz-Hugh-Curtis syndrome) is observed in about 5-10% of patients with PID. In perihepatitis, an inflammation of the liver capsule is seen together with adjacent peritoneal structures. The liver tissue itself is not inflamed. This leads to
'violin-string' adhesions. Periappendicitis and perisplenitis have also been reported. Based on this variety of disease conditions causing significant morbidity worldwide, Chlamydiaceae are important and frequent human pathogens. The degree and mechanisms of such genetic control may have important implications for understanding the immunopathogenesis of Ct infection, therapeutic strategies and vaccine development, all of which are necessary to effectively treat and prevent Ct infection.

The clinical course of Ct infection shows remarkable interindividual differences in transmission, symptomatic course, persistence or clearance of infection, and development of late complications. Transmission of the infection from the index patient to the partner is observed in 45-65% of cases. However, exact percentages are lacking since the asymptomatic part is not exactly known. Percentages reported range from 60-80% in women and 30-50% in men. Some late complications of infection occur only in a subset of women. Some patients clear the infection spontaneously, while in others the infections recur despite treatment of.

The reason for this heterogeneity in susceptibility to Chlamydia infection and disease progression following a rather uniform bacterial exposure remains incompletely understood. Some studies have shown relationships between Ct serovars and the clinical course of infection. Differences in infection variables between serovars also have been described. Nevertheless, no clear single bacterial virulence factor related to the mentioned differences or that can explain the heterogeneity has been identified.

In general, the described differences in clinical course could be explained by the interaction between the host (host factors such as interindividual genetic differences) and the pathogen (virulence factors). This interaction is on its turn influenced by environmental factors such as co-infections. The remarkable major interindividual differences in the susceptibility to and severity of infectious diseases were shown in earlier studies. The best known example is malaria, which is caused by the Plasmodium spp. Individuals heterozygous for haemoglobin S (HbS) are protected against infection with Plasmodium falciparum, whereas those with homozygous mutants for HbS have sickle cell anaemia. Twin studies have advanced the efforts to identify susceptibility genes to infectious diseases. Comparison of concordance rates in monozygotic and dizygotic twins provides an estimate of the size of the genetic component of susceptibility, and for many infectious diseases this is substantial.

The most relevant study in the field of Chlamydia immunogenetics was presented in 1998 during the ninth international Chlamydia symposium in San Francisco, USA. This study estimated the relative contribution of host genetics to the total variation in lymphoproliferative responses to Ct antigen by analyzing these responses in 64 Gambian pairs of twins from trachoma-endemic areas. Proliferative responses to serovar A Elementary Bodies antigens were estimated in monozygotic and dizygotic twin pairs. A stronger correlation and lower
within-pair variability in these responses was apparent in monozygotic compared with dizygotic twin pairs. The heritability estimate was 0.39, suggesting that host genetic factors contributed almost 40% of the variation.

**Inflammatory bowel disease**

Inflammatory bowel disease (IBD) is a chronic relapsing idiopathic inflammation of the gastrointestinal mucosa, that encompass the two main diseases Crohn’s disease (CD) and ulcerative colitis (UC). Generally, in a small group of patients UC and CD cannot be distinguished and the condition is called intermediate colitis. The prevalence of IBD is higher in developed countries and in north-European descendents. CD may affect any part of the gastrointestinal tract, but the terminal ileum, caecum, and colon are the commonest affected areas. Peri-anal fistulas are more common than internal or external fistulas. The affected regions may contain patches with normal bowel which is referred to as skip-lesions. Inflammation affects all layers of the bowel wall and causes fissuring fistulas and submucosal fibrosis. In UC, the disease may affect the whole colon, although in some cases it is restricted to the rectum and is referred to as proctitis. Inflammation affects the superficial mucosal layers, causing ulceration and crypt abscesses. It is thought that IBD results from inappropriate and ongoing activation of the mucosal immune system driven by the presence of normal luminal flora. The deviant response might be caused by a defective barrier function of the epithelial layer of the intestines and the activation of the mucosal immune system. Despite the advances in current understanding of IBD, the exact immunopathology and genetic basis of the disease remains to be elucidated. Evidence for genetically determined susceptibility to IBD started with clinical observation based on both animal and human studies, showing wide variations in the incidence and prevalence of the disease among different populations, especially familial aggregation with a high risk for first-degree relatives."64, 65 Increased rates are reported in the Ashkenazi Jews for example. A significantly higher rate of disease concordance in monozygotic twins than in dizygotic twins has been found, especially in those with CD.65 Podolsky describes a 10 to 25-fold increased risk in relatives of CD patients.66 Familial aggregation of IBD is observed in some populations. Thus, different individual genetic aspects contribute importantly to the susceptibility and resistance for IBD. These differences offer the opportunity to identify genes that contribute to occurrence of disease. Genome-wide and follow-up replicating candidate gene case-control association studies have identified 18 susceptibility loci for UC explaining about 11% of the heritability for this disease.67-71 In 2001 linkage analysis and positional cloning led to the identification of the first CD susceptibility gene, NOD2, later followed by a locus at chromosome 5q31 and less consistent replication at other loci (as reviewed by Mathew et al.72). A meta-analysis73 of early genome-wide association studies (GWAS) scans (up to mid-2008)74-76 implicated 32 susceptibility loci accounting for approximately 20% of the genetic contribution to CD risk.
Extraintestinal manifestations of IBD

Although IBD mainly affects the gastrointestinal tract, it is often associated with a variety of chronic inflammatory diseases that affect other organ systems. These diseases are called extraintestinal manifestations (EIM) because of their common prevalence among IBD patients (Table 2). The frequency of EIM varies from 6-47%, with a novel finding that asthma is the most common comorbidity increasing in CD patients compared with the general population. The organs most frequently involved are joints, eyes, skin, biliary tract, and lungs. Some symptoms, such as oral lesions, gallstones, pancreatitis, nephrolithiasis, and amyloidosis, seem to be more associated with CD than with UC. It seems that patients with perianal CD have a higher risk to develop extra-intestinal manifestations (EIM) than other IBD patients. In some cases EIM may be the first presenting symptom of IBD, and may cause higher morbidity than the initial disease. EIMs have the tendency to follow the clinical course of IBD, and may therefore have a high impact on the quality of life and morbidity in these patients. Furthermore, the presence of one EIM seems to increase the susceptibility to develop other EIMs.

Arthralgia and spondyloarthopathy (SpA) of the peripheral and the axial joints are most common EIM with a prevalence of 10–40%. The relation between SpA and gut inflammation has been confirmed by several studies. A high concordance has been described in EIM in siblings and first degree relatives with IBD. This suggests, like in IBD, a common genetic factor supporting the clinical and histological overlap between gut inflammation in IBD and SpA. Involvement of the immune system has been observed in clinically overt IBD and in subclinically inflamed bowel mucosa from SpA patients. In this respect, microbes may play an important role, possibly by molecular mimicry. Bacterial gut infections such as Salmonella typhimurium, Yersinia enterocolitica, Shigella, Campylobacter jejuni may induce reactive peripheral arthritis and 20% of these patients may develop chronic SpA.
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Table 2: Extraintestinal manifestations of IBD

<table>
<thead>
<tr>
<th>Type</th>
<th>Manifestations</th>
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<tbody>
<tr>
<td>Rheumatic</td>
<td>Peripheral arthritis</td>
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<tr>
<td></td>
<td>Axial arthritis</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Osteopenia/osteoporosis</td>
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<tr>
<td></td>
<td>Osteomalacia</td>
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<tr>
<td>Dermatologic</td>
<td>Erythema nodosum</td>
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<td></td>
<td>Pyoderma gangrenosum</td>
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<tr>
<td></td>
<td>Aphtous stomatitis</td>
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<tr>
<td></td>
<td>Sweet’s syndrome</td>
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<tr>
<td>Ophthalmologic</td>
<td>Uveitis</td>
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<tr>
<td></td>
<td>Episcleritis</td>
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<tr>
<td></td>
<td>Scleritis</td>
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<td>Hepatobiliary</td>
<td>Primary sclerosing cholangitis</td>
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<td></td>
<td>cholelithias</td>
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<tr>
<td>Hematologic</td>
<td>Anemia</td>
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<tr>
<td>Thromboembolic</td>
<td>Hyperhomocysteinemia</td>
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<tr>
<td>Urinary tract</td>
<td>Nephrourolithias</td>
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<tr>
<td>Pulmonary</td>
<td>Chronic bronchitis</td>
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<tr>
<td></td>
<td>Bronchiectasis</td>
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<tr>
<td>Pancreatic</td>
<td>Pancreatitis</td>
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</tbody>
</table>

Larsen et al. 2010

Thromboembolism

Vascular diseases are serious EIM of IBD. The most important of these complications are arterial and venous thromboembolisms including life threatening pulmonary embolism. Venous thromboembolism of the leg has been associated with a short-term mortality rate of 6% and in case of pulmonary embolism of the short-term mortality rate increases up to 20%. These represent a significant cause of morbidity and mortality in IBD patients, especially those with active disease. Alterations in coagulation and fibrinolysis have been demonstrated in both CD and UC. Thromboembolism has been described as a disease-specific EIM of IBD with a three-fold increased risk. This EIM may result from multiple interactions between acquired and genetic risk factors. Disbalance of procoagulant, anticoagulant, and fibrinolytic factors predisposes IBD patients to thrombosis. Clinical studies describe an incidence of thromboembolism in IBD patients of 1-7.7%, and post-mortem studies of 39-41%. The cause for the strong association between IBD and thromboembolism remains to be elucidated. We studied genetic variation in two genes encoding proteins that may be involved in the pathogenesis of this complication in IBD: JAK2 and FXIII.
Immunomodulatory aspects of probiotics

The gastrointestinal and the urogenital tract constitute an important interface between host and environment. The gastrointestinal tract is colonized with a huge amount of microbes, with an increasing density of colonization from the stomach to the distal colon. Commensal bacteria are of immense importance to human health as they, for instance, contribute to food digestion as well as development and optimal function of the immune system. The principal role of the gastrointestinal tract is to facilitate the absorption of nutrients, and in the mean time exclude pathogens. Some commensal bacteria participate in the absorption of nutrients like complex carbohydrates. Other commensal bacteria inhibit colonization and overgrowth of potentially pathogenic bacteria, by competition for nutrients and adhesion sites.

Since the discovery of lactic acid bacteria and early observations of their benefits, they have been used for therapeutic relief of the intestinal disorders in the beginning of last century by Tissier. Elie Metchnikoff (1845-1916), a Russian Noble prize laureate was the first to propose the therapeutic use of high concentrations lactic acid bacteria for health and longevity in humans ever since a wealth of experiments have described the use of selected microorganisms, mainly belonging to the lactic acid bacteria family, for the prevention or treatment of a variety of pathological conditions. Growing interest in the beneficial effects of the human flora over the past centuries has resulted in the selection of microbes belonging to the natural human flora, with low or no pathogenicity and exhibiting health promoting capacities that are acknowledged for the treatment of specific diseases in which the flora itself or its function is disturbed. Probiotics are defined as live microbial feed supplements which benefit the host by improving its intestinal microbial balance by modifying the intestinal microflora and which upon ingestion in certain numbers induce health benefits beyond inherent basic nutrition by suppressing the inflammatory responses.

Probiotics are currently subject of intense and widespread research as functional foods since they are known to induce health benefits, may be used as pharmaceutical preparations, and have achieved a “generally recognized as safe” (GRAS) status. Lactobacillus strains can also be genetically engineered for use in oral immunotherapeutic applications, such as vaccination and delivery of immunoregulatory substances.

To understand the beneficial effects of probiotics, two different concepts have been proposed. The first was based on effects of one particular strain that is added as a food supplement (e.g. L plantarum and L Casei Shirota). The second concept is based on the use of a cocktail of different strains such as lactic acid bacteria and bifidobacteria.

The mechanisms of action by which probiotics exert their beneficial effects to human health were explained by direct contact with the epithelial cells with cross-talk between probiotics and the antigen presenting cells. This cross talk between bacteria and the epithelial cells plays an important role in the inhibition of colonization and overgrowth of pathogenic bacteria by
competition for nutrients and adhesion sites and thereby modulation of the endogenous flora. A second mechanism is the enhancement of the intestinal epithelial barrier by modulation of different signalling pathways that may cause the induction of mucus following *Lactobacillus* strains and production of defensins, enhancement of tight junction functioning, and prevention of apoptosis. A third mechanism is the host immune modulation that may result in both local and systemic modulated host immune responses, resulting in both local and systemic effects. Clinical applications of probiotics include prevention and treatment of infections of the gastrointestinal tract, IBD, and allergic diseases.
References

49. van Valkengoed IG. Asymptomatic Chlamydia trachomatis infections: should we screen? Thesis 2001, VUmc, Amsterdam, The Netherlands
Introduction


Mathew CG. New links to the pathogenesis of Crohn disease provided by genome-wide association scans. Nat Rev Genet. 2008;9:9-14


Consortium WTCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447:661-678


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103. Sloan WP, Jr., Bargen JA, Gage RP. Life histories of patients with chronic ulcerative colitis: a review of 2,000 cases. Gastroenterology. 1950;16:25-38


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