Molecular pathogenesis of Crohn's disease

Molekularne patomechanizmy choroby Leśniowskiego-Crohna

Paweł Kustosz[1], **Marek Durlik**[1,2]

[1]Department of Surgical Research and Transplantology, Mossakowski Medical Research Centre, Polish Academy of Science, Warsaw, Poland
[2]Central Clinical Hospital of the Ministry of Interior, Warsaw, Poland

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Address for correspondence: Paweł Kustosz MD, Department of Surgical Research and Transplantology, Mossakowski Medical Research Centre, Polish Academy of Science, 5 Pawińskiego St, 02-106 Warsaw, Poland, phone: +48 22 608 64 07, e-mail: panpkustosz@gmail.com

Abstract

Crohn's disease (CD) with ulcerative colitis comprises a group of inflammatory bowel diseases. It involves inflammation of the walls of the digestive tract and can affect every part from the mouth to the anus. It is a chronic condition with periods of remission and exacerbation and can often lead to hospitalization and the use of medications and surgery. Based on current literature, it is difficult to clearly determine the causes of CD. Several factors that are involved in molecular pathogenesis have been identified. These include bacterial antigens of the intestinal flora and abnormal immune response, processes responsible for antigen recognition and the balance between T cell subpopulations. As a result of disturbances in the interaction between the intestinal flora and the immune system, local control of inflammation is likely to be lost, which, together with a probable genetic substrate, determines the abnormal immune responses.

Streszczenie

Choroba Leśniowskiego-Crohna (ChLC) wraz z wrzodziejącym zapaleniem jelita grubego należy do nieswoistych chorób zapalnych jelit. Według obecnego stanu wiedzy jest to stan zapalny ściany przewodu pokarmowego i może dotyczyć każdego odcinka od jamy ustnej do odbytu. Ma charakter przewlekły, występują naprzemiennie okresy remisji i zaostrzeń prowadzące często do konieczności hospitalizacji oraz zastosowania leczenia farmakologicznego oraz chirurgicznego. Wraz ze zwiększeniem liczby zachorowań w ostatnich latach nastąpił znaczny postęp w badaniach nad tą chorobą. Na podstawie aktualnego piśmiennictwa nadal trudno jest jednoznacznie określić przyczyny ChLC. Zidentyfikowano kilka czynników, które biorą udział w molekularnym patomechanizmie. Należą do nich: antygeny bakteryjne flory jelitowej, nieprawidłowe reakcje układu immunologicznego, procesy odpowiedzialne za rozpoznawanie antygenów oraz równowaga między subpopulacjami limfocytów T. W wyniku zaburzeń w interakcjach pomiędzy florą jelitową i układem odpornościowym prawdopodobnie zostaje utracona lokalna kontrola rozwoju stanu zapalnego, co wraz z prawdopodobnym podłożem genetycznym determinuje nieprawidłowe reakcje immunologiczne.

Introduction

In 1769 Morgani described the case of a young man with chronic abdominal pain, frequent bloody diarrhoea, inflammatory infiltrates, ulcers, intestinal stenosis and mesenteric lymphadenopathy. The disease was diagnosed as granulomatous enteritis. Another report in 1903 came from the Polish surgeon Antoni Leśniowski, who described a patient with inflammation of the small intestine and colon and a fistula between them [1]. More precise characterization and naming of the disease did not occur until 1932, when Burrill Bernard Crohn and his colleagues described 14 cases of the disease and named it *ileitis regionalis* or *ileitis terminalis* [2]. These terms still exist today, but the common name is derived from the names of the researchers – in Poland it is called Leśniowski-Crohn's disease, and throughout the rest of the world it is known as Crohn's disease (CD). Together with ulcerative colitis (UC) it comprises a group of inflammatory bowel diseases (IBD) [3].

Crohn's disease

Current scientific opinion is that CD is the inflammation of the walls of the digestive tract and can affect every part from the mouth to the anus. It mainly affects young people between 15 and 25 years of age. It is a chronic condition with periods of remission and exacerbations and can often lead to hospitalization and the use of medications and surgery. Until recently, CD was considered to be a disease of unknown aetiology, and it was suspected that it might have been an autoimmune reaction or nonspecific inflammatory reactions [3].

Possible causes of Crohn's disease

Currently, there is a perception that CD is the result of interactions between environmental factors (including the bacterial flora) and the innate immune response in genetically susceptible individuals [4]. Factors which significantly contribute to cases of CD include geographic location, tobacco smoking, appendectomy, the presence of genetic mutations, gene expression mechanism susceptibility to environmental factors other than physiological composition of the intestinal flora, ineffective intestinal immune system and the so-called "hygiene hypothesis". This hypothesis suggests that excessive protection of the body against bacteria, especially early on in life, increases the likelihood of CD in later life [3, 5]. The study of molecular mechanisms of the disease have contributed to the development of breakthrough therapies based on blocking the biological activity of tumour necrosis factor α (TNF-α). This success has led to further testing using molecular biological techniques.

Intestinal flora – disorders of immune tolerance

The intestinal flora is one of the key environmental factors affecting the development of CD. About 500 species of aerobic and anaerobic bacteria live in symbiosis with the host organism, of which 30-40 species are the main part of the intestinal flora [6, 7]. Physiological protective flora supports the mucosal immune response. It helps protect against pathogens involved in the digestion of metabolically active, short-chain fatty acids, carbohydrates, vitamins and bile acids, and plays an important role in maintaining the balance of the immune system, contributing to the development of immune tolerance [7, 8]. The organism does not usually begin defensive reactions against harmless bacteria and food antigens, and inhibits the so-called controlled inflammation by CD4+ T lymphocyte production. Anergy and apoptosis of T cells are also involved in this process [7].

The apoptosis level in normal mucosa is high, while in the case of CD it is significantly reduced. It is associated with excessive production of antiapoptotic cytokines (interleukins IL-2, IL-6, IL-15, IL-17 and IL-18). The confirmation of the apoptosis impact on CD also makes use of TNF-α inhibitor treatments. It was found that administration of infliximab, a drug with strong apoptotic potential, gives much better results than treatment with etanercept, which does not have apoptotic potential. Apoptotic activity is also important in other CD therapies such as the use of immunosuppressive drugs like azathioprine and sulfasalazine, which induce apoptosis of T cells [7].

An additional immune tolerance mechanism is the production of T lymphocyte subpopulations responsible for the release of various anti-inflammatory cytokines such as IL-10, transforming growth factor β (TGF-β) and IL-4 [7]. There are also secreting B cells and dendritic cells (DC) that release interferons α and β (IFN-α and IFN-β) and prostaglandin J2 [9].

Changes in the composition of the normal bacterial flora in the context of a genetic predisposition adversely affect the integrity of the epithelial and mucosal immune protection, even without the presence of pathogens.

Under conditions of abnormal interaction between the microbial flora and the mucous membrane, a loss of immunological tolerance and inflammatory bowel disease (including ulcerative colitis and CD) may occur. Recent studies have shown that the balance of physiological and pathogenic bacteria may be related to the presence of pro-inflammatory and anti-inflammatory cytokines, and different subpopulations of T cells [10].

Pattern recognition receptors

Over the last decade there has been substantial progress in the study of molecules of the innate immune system responsible for antigen recognition, so-called pattern recognition receptors (s). s recognize molecules associated with groups of pathogens called pathogen-associated molecular patterns (PAMPs) and molecules generated during cellular stress called damage-associated molecular patterns (DAMPs).

The s are expressed by cells of the innate immune system in different parts, for example Toll-like receptors (TLRs) on the surface membrane or NOD2/CARD15 protein (nucleotide oligomerization domain 2/Caspase recruitment domain family member 15) in the cytoplasm. The receptors of the intestinal immune system constitute the first line of defence in the interaction of mucosal bacteria [11].

Numerous studies have shown the increased expression of TLRs in the intestinal mucosa of CD patients [9]. TLR interaction with antigens initiates pro-inflammatory cytokine expression by the transcription factor NF-κB,

which connects the innate and acquired immune systems. Deficiency or malfunction of these receptors can lead to loss of control of the development of the inflammatory processes that lead to CD. Of all the 14 identified types of TLRs, the best described is TLR4, the most sensitive receptor to bacterial lipopolysaccharide (LPS) [7, 12]. This receptor expression is mediated by MD-2, the molecule involved in recognition of LPS [13]. MD-2 is difficult to measure in the normal colon mucosa, due to its low concentration, but the CD level is significantly elevated. Intestinal bacterial flora consists mainly of Gram-negative bacteria, which present LPS on their surface, indicating the probable contribution of TLRs in the pathogenesis of CD.

Different levels of TLR4 expression have been observed between small and large intestines, which are likely due to differences in the composition of the bacterial flora [7]. In patients with active CD immunohistochemical analysis of intestinal specimens showed a significant increase in expression of TLR4 in the terminal ileum. Enhanced expression of CD14 receptor was also observed in this same part of the intestine. Deregulated expression of TLRs, especially TLR4 and CD14, in different parts of the intestinal mucosa may be critical in the pathogenesis of CD patients [14]. This is related to the process of interaction of membrane protein CD14 with LPS, which leads to the activation of TLR4 and, with the involvement of two intermediate signalling pathways, results in the expression of NF-κB, thus inducing the expression of TNF-α and IL-6.

Another important receptor for pathogenesis is the protein CD NOD2/CARD15, which is present in the cytoplasm of peripheral blood monocytes, dendritic cells, and intestinal epithelial cells, such as Paneth cells. It recognizes pathogens by binding to muramyl dipeptide (MDP) located in the walls of the bacteria, Gram-positive and Gram-negative. After their diagnosis a pathway of reactions begins, leading to the activation of NF-κB and the expression of cytokines (such as TNF-α and IL-1β) and antimicrobial peptides (PAD). This process makes the NOD2/CARD15 protein an important factor in the development of the inflammatory response [15].

The correlation between NOD2/CARD15 gene mutation and the development of the disease has been demonstrated in CD patients [7]. It occurs in an area rich in leucine, which is responsible for identifying MDP, and thus for maintaining antigenic characteristics [16]. In the large intestine of patients with CD NOD2/CARD15 expression levels in epithelial cells are higher than in healthy subjects [17]. This observation is consistent with previous studies, which have shown that bacterial infection may play an inductive role in the development of CD [7].

In the Paneth cells, located in the lower parts of the intestinal crypts, the presence of the protein NOD2/CARD15 results in antimicrobial peptide expression [18]. The antimicrobial activity of the two main families of antimicrobial peptides, defensins and cathelicidin, is essential to the protection of the integrity of normal mucosa. In the small intestine, Paneth cells, secreting α-defensins, are able to regulate the type and number of commensal bacteria, whereas β-defensins and cathelicidin may cause an inflammatory response [16]. Any antimicrobial barrier dysfunction can lead to chronic inflammation. In mice with a mutant NOD2/CARD15 gene, Paneth cells produce significantly fewer antimicrobial peptides, weakening localized protection against bacteria in the intestinal tract [7, 19]. This is consistent with the increased adherence of bacteria in the terminal ileum in patients with CD, in contrast to the healthy population. A thorough understanding of the role of antimicrobial peptides may be the key to the development of new therapeutic methods [7].

Cytokines – in response to presence of antigens

Cytokines (from Lat. *citos* – cell and *kinesia* – movement) are the main transmitters in the intestines and the immune system and may also be responsible for disturbances in the normal intestinal inflammatory process [20, 21]. These molecules are glycoproteins and polypeptides consisting of 50-500 amino acids and prosthetic sugar groups produced primarily by immune cells. They facilitate intercellular communication, stimulate the proliferation of antigen-specific effector T cells and mediate inflammatory signalling pathways [20, 21]. Intercellular cytokine production affects the intracellular pathway of transcription factors including the signal transducers and activators of transcription (STAT). It plays a role in the secondary signalling receptors of various inflammatory cytokines, through activation by the cytokine family IL-6, IL-10, granulocyte colony stimulating factor (G-CSF) and hepatic growth factor (HGF). Experimental data show that STAT3 protein deficiency leads to functional disturbances of IL-10, and severe inflammation of the gastrointestinal tract [7].

In the pathogenesis of CD, of particular importance is the imbalance between pro-inflammatory and anti-inflammatory cytokines associated with antigen-presenting cells (APCs), lymphocytes Th1, Th2, Treg, Th17, dendritic cells (DCs) and macrophages [20, 23]. These cytokines include, among others, TNF-α, IL-1, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, IL-18, IL-21, IL-23, IL-27 and IFN-γ and TGF-β [7, 20]. These proteins are jointly involved in many reactions of the immune response in IBD such as the regulation of production of inflammatory

factors, reactive oxygen metabolites, nitric oxide, leukotrienes, platelet activating factor, and prostaglandins, activation of transcription of nuclear factor κB, or inhibition apoptosis. However, they may act in different ways in the various forms of the disease [24]. For example, the secretion of cytokines by APCs leads to the start and differentiation of T cells activating specific immune responses.

Crohn disease abnormalities in the activation of specific immune responses leads to its deregulation or overproduction of effector T cells involved in the development and exacerbation of the disease [20, 23]. In such cases there is a dominance by CD4+ T cells of the Th1 phenotype responsible, among others, for the increased secretion of TNF-α, IL-6 and IFN-γ, which inhibit the function and proliferation of Th2 cells [7].

A common phenomenon in the pathogenesis of CD is an increased number of macrophages and dendritic cells in the lamina propria mucosa. T cell activation may be due to the release of these cytokines, such as IL-12, IL-23, IL-18, IFN-γ and TNF-α in response to an encountered antigen secreted from the intestinal lumen [25]. Interleukin-23 regulates the differentiation of Th1 cells in combination with IL-15 and IL-18, and IL-21 affects the stability of the process (the immune polarization) [20, 24]. Tumour necrosis factor-α increases the production of IL-1, IL-6 and IFN-γ, probably affecting the maintenance of chronic inflammation.

Once you start the process of T cell activation of IFN-γ keeps your concentration on the trails through the action of intracellular STAT1-4, and the transcription factor T-bet [7]. Interleukin-1, TNF-α and LPS block the activation of transcription factors by IL-6 and IL-10, but not by IFN-γ in the case of immature macrophages.

Interleukin-6 indirectly affects many immunological reactions involved in the development of IBD.

Another important factor in the pathogenesis of CD patients is anti-TGF-β, which is regarded as a key regulator of immune system homeostasis. It inhibits the production of other pro-inflammatory cytokines and the response associated with Th1 CD4+ cells and CD4+ Th2 cells. *In vitro* studies have demonstrated that cytokines produced in the neutralization of Th1 cells can be caused by increased secretion of IFN-γ, TNF-α, IL-1 and LPS. At the same time, it is noted that in the case of patients with CD, the presence of high levels of IL-12 and IFN-γ may have an impact on the activation of cells producing TGF-β [20, 24]. The experimental conditions in an animal model of TGF-β deficiency causes severe enteritis and death. The signal transduction of TGF-β is mediated by the protein Smad2. The level of the natural inhibitor Smad7 is increased in IBD, which also has pro-inflammatory effects. This is interpreted as an explanation of increased TGF-β levels in IBD, but the signal transduction is blocked so there is no anti-inflammatory effect [7].

Current state of knowledge about treatment options

Advanced techniques have provided genetic research capabilities of both the molecular mechanisms involved in the immune response of the digestive system as well as the genes responsible for them. This gives hope for improving our knowledge and the introduction of new, more effective diagnostic and therapeutic methods.

Therapy which blocks T-cell functions, disposes subpopulations of T cells, secretes proinflammatory cytokines, or induces apoptosis of specific types of T cells, is the solution currently proposed by leading centres around the world [26]. Research is also conducted into the use of alternative methods of anti-inflammatory therapy in IBD, including ω-3 fatty acids and leukocyte apheresis therapy (LCAP). Gastrointestinal parasites were used in a pilot study of CD treatment. After colonization of a CD patient's gastrointestinal tract by eggs of *Trichuris suis*, alleviation of symptoms of the disease was observed [27]. Given the gut flora imbalance between the protective bacteria and harmful species (e.g. *Escherichia coli* may have an impact on the development of inflammatory bowel disease), attempts at probiotic treatment are being made. Research into innovative therapies based on autologous stem cell transplantation is led by Professor Chris Hawkey, a gastroenterologist at the University of Nottingham [28].

Biological treatment

Biological therapy is based on the use of a new generation of drugs, which are specifically tailored to the mediators of the disease, such as by blocking their activity. It can be used in the treatment of diseases of unknown aetiology, in which important molecules involved in the pathogenesis were identified. Such a molecule with increased activity in CD patients is TNF-α, one of the major pro-inflammatory cytokines. It plays an important role in initiating and maintaining the inflammatory process [3].

Biological therapies directed against TNF-α (anti-TNF) have been used in the treatment of CD patients for several years, mostly in patients where standard therapy has not helped. In Poland, this has been available since 2001, and until now two anti-TNF drugs have been registered: infliximab and adalimumab. These drugs are IgG1 monoclonal antibodies, which bind, with high affinity, and inhibit the biological activity of human TNF-α.

With this mechanism, the drug is highly anti-inflammatory and probably induces apoptosis of inflammatory cells, which may also cause side effects, favour infection

and increase the risk of lymphatic system cancer. Therapy is effective for about half of the patients with CD who have rapidly induced and maintained remission and closure of a fistula.

Some patients may not tolerate treatment with infliximab; over time the body may also develop resistance to its effects. In both cases it is possible to continue treatment using the newer biological drugs. In the first instance other anti-TNF preparations, such as adalimumab and certolizumab pegol are used [3, 29]. When they do not give the desired effect, natalizumab – an antibody against integrin $\alpha 4$ – which inhibits inflammation by blocking subsequent recruitment of inflammatory cells, is then used [29].

Recent studies also show high efficacy of therapy that combines biological treatment with anti-TNF (infliximab mostly) and azathioprine, a drug with strong immunosuppressive and cytotoxic activity, the effect of which includes the stimulation of apoptosis of T cells [29].

Surgical treatment

Advances in drug therapy have reduced the proportion of CD patients sent for operations, but it is still predicted that most of them will require intervention during the course of the disease [30]. Surgical procedures are performed in addition to biological and immunosuppressive therapy, and often prevent the occurrence of systemic infection (sepsis) [3, 30].

Until recently, most operations were performed urgently when the goal was to save the patient's life. In many cases, bowel resection was a natural choice in the treatment of CD. Surgical treatment has changed significantly over the past decade thanks to the development of medical therapy and endoscopic techniques. There is evidence that extensive bowel resection is not necessary, or even potentially harmful.

The trend towards economised surgery has now been widely accepted, resulting in a change in operational procedures.

Extensive resection was replaced by limited resection in macroscopically altered tissues. Laparoscopic surgery has introduced an increasingly precise technique, which results in faster wound healing and the return of gastrointestinal function to the patient [30].

In 2010, the bringing together of world-class experts in the field of IBD (European Crohn's and Colitis Organization, ECCO) resulted in the publishing of the latest consensus on definitions, diagnosis and clinical management of CD.

In the case of ileocaecal localisation of the inflammatory lesion with partial or total blockage of the gastrointestinal tract. This operation can be performed safely using laparoscopic methods [30].

Summary

Based on current literature it is difficult to clearly determine the causes of CD. Nevertheless, the importance of genetic factors, especially innate immunity, appears to be important in IBD, particularly CD. As a result of disturbances in the interaction between the intestinal flora and the immune system, local control of inflammation is likely to be lost, which, together with a probable genetic substrate, determines the abnormal immune responses.

Several factors that are involved in molecular pathogenesis have been identified. These include bacterial antigens of the intestinal flora and abnormal immune response, processes responsible for antigen recognition and the balance between T cell subpopulations. The impact on these factors (by chemotherapy, biological therapy or probiotics) may lead to the prevention and effective treatment of inflammatory bowel diseases.

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