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Review article

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NUCLEAR FACTOR-κB - IMPORTANCE, INDUCTION OF INFLAMMATION, AND EFFECTS OF PHARMACOLOGICAL MODULATORS IN CROHN'S DISEASE

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Crohn's disease (CD) is a chronic inflammatory disease of unknown etiology that covers the entire digestive tract and occurs with periods of remission and clinical exacerbation. CD is most common in North America and Europe, but its incidence is rising rapidly in Asian countries. The pathogenesis of CD is unclear, while genetic predisposition, immune imbalance, and host-intestinal microbiota interactions are taken into account. Incorrect activation of κB nuclear factor (NF-κB) signaling pathways is associated with CD initiation and progression. NF-κB leads to excessive production of pro-inflammatory cytokines that cause a chronic inflammatory process of the intestines. It is currently believed that the NF-κB pathway plays a key role in the pathogenesis of CD, hence current treatments aim to block this pathway. Studies have shown that activation of NF-κB is reduced by treatment with, among others, mesalazine and glucocorticoids. This review presents epidemiology and pathogenesis of CD, the participation of NF-κB in this disease, as well as modern methods of treatment aimed at inhibiting NF-κB activation.

Key words: *inflammatory bowel disease, nuclear factor-κB, Crohn's disease, inflammatory bowel diseases, proinflammatory cytokines, ulcerative colitis, chemokines, pharmacotherapy, probiotics*

INTRODUCTION

Crohn's disease (CD) belongs to inflammatory bowel diseases (IBD) and is a chronic inflammatory disorder caused by granulomatous infiltration of the entire thickness of the intestinal wall (1). The disease can affect any part of the digestive tract, from the mouth to the anus (1). The clinical course consists of two phases: silencing symptoms, *i.e*. remission, and exacerbation, for which the Crohn's Disease Activity Index (CDAI) is used (1, 2). CDAI takes into account seven parameters of the patient's clinical condition and makes it possible to assess the stage of the disease of the patient (1, 2). According to the consensus of the European Crohn's and Colitis Organization (ECCO), exacerbation of the disease is defined as a condition in which the CDAI index exceeds 150 points, and is divided into mild (150 – 20 points), moderate (221 – 50 points) and severe (over 450 points) disease (1-3). CDAI is not an ideal indicator of CD activity. Monitoring of disease and guide therapeutic decisions are not only based on clinical activity indexes, which correlate poorly with endoscopic activity and remission of symptoms and may not indicate remission of CD (4). Mucosal healing of the inflammatory process in the intestine is a strong predictor of disease-related outcomes and has become a new therapeutic goal in IBD. The laboratory tests can detect endoscopic disease activity earlier before any clinical symptoms (4). Fecal calprotectin is used as noninvasive evaluation for monitoring the endoscopic activity of IBD, and the test could predict clinical relapse (4). According to Borren *et al*. the proteomic, metabolomic, and microbial biomarkers can identify a pro-inflammatory state in quiescent IBD that predisposes to clinical relapse (5).

The main symptoms of exacerbation of the disease are abdominal pain, diarrhea, weight loss, sometimes fever, anemia, absorption disorders, and related vitamin and mineral deficiencies that affect the patient's deteriorating clinical condition (6). Exacerbation period significantly reduces the quality of life and impairs patients' performance. The poor quality of sleep was reported in IBD patients, both in CD and UC, in strong relation to disease activity (7).

IBD affect patients of all ages, although the reproductive period seems to be the most difficult (8). During pregnancy hormonal, immunological, and microbial changes interact with the pathophysiology of IBD, and have the potential effect on the course of diseases (8). Optimal control of IBD is important not only during pregnancy but also prior to procreation. The vast majority of therapies are safe during pregnancy, however, some medication may be harmful for the child, and may affect the further complications related to fertility (8, 9). CD manifests in patients in the form of 3 phenotypes: inflammatory with infiltration of the intestinal wall, penetrating with fistula formation, and scarring with narrowing of the intestinal lumen and often intestinal obstruction (3, 6).

Therapeutic options in CD includes smoking cessation, dietary interventions, which can be related to vitamin D supplementation, the deficiency of which is a risk factor for delay of the induction of disease remission (10, 11), and the use of a number of drugs, either alone or in combination, depending on the stage of the disease (1). Treatment with pharmacological and biological methods does not always avoid surgical intervention consisting in the strictureplasty of strictures in the small intestine or resection in jejuno-ileal CD, resection of narrowed segments of the small or large intestines, or fistulas, abscess drainage and many other methods (3, 4). Long-term observations indicate that 50 – 80% of patients with CD undergo at least one surgery during their lifetime (6, 12).

Understanding the multifactorial pathogenesis of CD is crucial for the diagnosis and monitoring of the disease, and, primarily, the treatment, because it can improve its effectiveness, reduce the side effects of therapy, and contribute to improving the quality of life (1, 6, 13). Hence the growing interest in CD pathomechanism at the molecular level, including understanding of the complex mechanisms modulating NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) activity, which is considered a critical factor in inflammatory processes and the immune response (14, 15).

EPIDEMIOLOGY

The prevalence of IBD, *i.e.* CD and ulcerative colitis (UC), is increasing (1, 6, 12). The Swedish IBD register (SWIBREG) contained over 46,000 patients in its database as of April 2019, and the number is systematically increasing (13). Based on the analysis of the directional coefficient of the incidence estimate, the number of IBD patients in Sweden alone will increase to 90,000 in 2030 and to 170,000 in 2050 (13). The Polish CD register covers over 6,000 patients, but the number of new patients, as in other European countries, is increasing (14, 15). Incidence of CD is highest in Western Europe and North America. Annual incidence in North America is estimated at 3.1 – 20.2 cases per 100,000 inhabitants, and at 5 cases per 100,000 inhabitants in the European Union (6, 13, 16). Over 2 million people are affected by IBD in North America, and up to 3.2 million in Europe. In Europe, incidence of IBD is higher in northern countries of the continent than in southern countries (16-18). Worldwide, however, the disease is much more common in Caucasians and in developed countries in Europe and North America, therefore northern latitude, not the season, is associated with increased IBD hospitalization rates (19).

Due to the appearance of the disease in late childhood and early adolescence, it seems important to ensure sufficient knowledge about the disease in adolescents and their legal guardians in order to achieve better self-control in the transition from pediatric healthcare to adult gastroenterological care (20).

In the 20thcentury, IBD occurred mainly in North America, Europe, and Oceania (16). The incidence of these diseases was the lowest in Africa and Asia. At the beginning of the 21stcentury, IBD became a global problem, with an increase in incidence in newly industrialized Asian countries, including Korea, but also in Africa and South America (mainly in Brazil) (17, 18, 21, 22).

The peak incidence of CD falls between 15 and 35 years of age, but it is possible that the first symptoms of the disease may occur at any age (17, 18, 23, 24). There is no difference in incidence between males and females. A steady increase in morbidity is attributed to the dominant role of the environment, which has an adverse effect on the intestinal mucosal barrier, and by modulating the composition of intestinal mucus and intestinal microbiota, is involved in the pathogenesis of IBD (17, 21, 25).

PATHOPHYSIOLOGY OF INFLAMMATORY BOWEL DISEASE

Etiopathogenesis of IBD is not fully understood and is the subject of many studies. Many different factors are taken into account, such as genetic, environmental, microbiological, and immunological (6, 25-29). According to current knowledge, CD is an incurable disease, therefore the goal is to reduce inflammation of the intestine, preserve its function, prevent complications, and enable patients to maintain a normal quality of life (1, 30-32).

IBD-related inflammation is characterized by an accumulation of B lymphocytes and plasma cells (cytotoxic CD19+ and IgA+ cells expressing granzyme B, GrB) in the mucosa, demonstrating the role of these cells in IBD-related epithelial damage (33).

Complications of CD in various organs, such as the osteoarticular system, skin, eyeballs, liver, and bile ducts, are a serious clinical problem (1). More than half of the patients suffer from complications in the form of arthritis, erythema nodosum, pyoderma gangrenosum, iritis, choroiditis, fatty liver disease, gallstones, primary sclerosing cholangitis (PSC), osteoporosis, as well as changes in the mouth, *e.g.* aphthous ulcers, relatively frequent perianal lesions in the form of fissures, fistulas, or abscesses (1, 6). Fistulas between small intestine, as well as entero-cutaneous, entero-bladder, and other loops, are extremely difficult to treat (1). Deep intestinal ulcers in the course of CD can lead to intestinal perforation and pose a direct threat to life, with high mortality rates of up to 20% (1). Patients with chronic inflammatory process in CD have an increased risk of cancer, in particular colorectal and lymphoid (1). Studies carried out in 1964 – 2014 on 10,000 patients with IBD showed that the introduction of highly effective biological therapy, despite the reduction of episodes of exacerbations of the disease, did not cause a decrease in the frequency of tumors (12, 34).

Inflammatory infiltration in CD is characterized by the presence of non-caseous granulomas with macrophages recruited from blood monocytes and CD4+, Th1, Th2, and Th17 lymphocytes, in the wall of the gastrointestinal tract (GIT) (1, 12). These processes are associated with the activation of NF-κB transcription factor (26, 35, 36).

Chronic recurrent immune disorders, which cause dysregulation of the expression of molecules involved in pro-inflammatory and anti-inflammatory processes, are taken into account in the pathogenesis of IBD (29, 36). Studies suggest a strong influence of hyperactivation of the immune system with an increased level of pro-inflammatory cytokines secreted by the activated T cells (27, 29).

Modulation of NF-κB activity leads to constant overexpression of pro-inflammatory cytokines and is associated with many autoimmune diseases, including CD (36-38). Increased NF-κB activation correlates with an increased number of pro-inflammatory IL-1, TNF-α, IL-6 cytokines, which closely correlates with the degree of inflammatory cachexia in IBD (34, 36). The genes involved in the etiopathogenesis of CD are listed in the *Table 1*.

MicroRNA is a group of non-coding molecules, about 20 – 25 nucleotides in length (55). It joins complementary to mRNA3' untranslated region (3'UTR) causing degradation or inhibition of translation. Many studies point to impaired microRNA expression in the intestinal mucosa in patients with IBD (40, 56, 57). This, in turn, can lead to abnormalities in the immune response, dysfunction of the intestinal mucosa, and, consequently, inflammatory bowel disease (57, 58). The change in miRNA expression in patients with CD is the subject of many studies that show clear decrease (miR-3194, miR-196A, miR-

Table 1. Genes involved in the etiopathogenesis of Crohn's disease (CD).

Genes and their importance in CD	Higher prevalence	Lower prevalence	Ref.
Differentiation of CD and UC	HLAA * 03 DRB1 * 13 DRB1 * 01: 03	DRB1 * 15	(39)
Genetic markers of inflammatory bowel disease	IBD3 (6p21.1-23) IBD1 (16p13.1-16q12.2) CARD15	–	(39-41)
CD phenotypes	NOD2 / CARD15 TLR4	–	(42)
Endothelial dysfunction of importance in CD	CDH1	Encodes epithelial E-cadherin and beta-catenin that play an important role in maintaining the integrity of the intestinal barrier	(43)
	LAMB1	Encodes the laminin-beta-1 subunit	(44)
	EMC1	ER membrane protein complex subunit 1	
	HNF4A	Hepatocyte nuclear factor 4 alpha associated with glucose and lipid metabolism, and with enteritis and abnormal mucosal permeability	(45)
	DAP	Cell death-related protein; a positive regulator of Rip2 and promoter of the induction of inflammatory cytokines through the Nod1/2-Rip2 pathway	(46)
	PRDM1	Zinc-finger protein 1 of PR domain	(47)
	NKX2-3	Homeobox Nkx-2,3 protein; plays a differential role in the pathogenesis of UC and CD through a regulation of EGR1	(48)
Regulation of the inflammatory response and adhesion of leukocytes to the vascular endothelial cells	COP9	–	(49)
Marker of damage to the intestinal mucosa in CD	T-UCR uc.261	–	(50)
Ectodermal dysplasia associated with X-linked immunodeficiency	IKBKG	Abnormalities of NF-κB inhibitors in the gamma subunit	(51)
Change in the recognition of microbial components through excessive activation of NF-κB in monocytes	NOD2	Intracellular receptor for components of pathogenic microorganisms	(52)
Autophagy	ATG16L1 and ATG16L2	Cellular ontogenesis in CD	(53)
ER-stress-related autophagy	ATG16L1T300A	Age-dependent transmural ileitis driven by the endoplasmic reticulum (ER) stress sensor	(54)

192, miR-378B, miR-422A, miR-611, miR-3184, miR-4284, miR-29a, miR-204, miR-30c, miR-708) or increase (miR-19A, miR-1273D, miR-886-5P, miR-3138, miR-612, miR-551B, miR-185, miR-505, miR-206) in expression (55, 57-62).

Studies on Hsa-miR-375 have shown that low expression of this microRNA leads to induction of NF-κB activation, Toll-like-receptor 4 (TLR4) disorders, increase of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-8, while reducing the level of anti-inflammatory IL-10 and as a result of IBD progression (55).

Many studies are devoted to infectious agents, viruses, bacteria, and fungi that are found in the intestines and determine their functioning. There are about 160 species of bacteria that colonize the human digestive tract (27, 28). A clear decrease in the number of *Bacteroides* producing polysaccharide A was observed in IBD patients, which may affect IL-10 secretion and inhibition of inflammation (25, 27, 28).

In addition, numerous studies indicate that T lymphocytes with low affinity for their own antigens (which may be the cause of the autoimmune response), may have been previously activated by molecular mimicry *via* microbial antigens (63). Interactions between microorganisms and the host are mediated by pattern recognition receptors (PRRs), *e.g*. TLRs and NLRs (nucleotide-binding oligomerization domain-likereceptors). In addition, LPS (lipopolysaccharide, *i.e*. a component of the cell membrane of Gram-negative bacteria) binds to TLR4, which leads to strong activation of NF-κB and an increase in pro-inflammatory cytokine production (64).

The intestinal microflora contributes to the initiation of the inflammatory response in CD (65), which leads to the abnormal activation of the NF-κB pathway and excessive production of pro-inflammatory cytokines that initiate chronic enteritis (66).

The NF-κB pathway seems to be pivotal in the progression of inflammatory lesions, and thus is a target for commonly used therapies of IBD that block the activity of this pathway (67). Targeting this transcription factor seems to be crucial in CD due to its direct relationship with disease activity, as presented in the work of Han *et al*., where NF-κB activation corresponds to a higher incidence of ileal involvement compared to patients with low NF-κB activity, which may correspond to specific clinical phenotypes of the disease (68). The observed changes are intertwined with oxidative stress, which intensifies inflammation in a mechanism dependent on the rapamycin (mTOR)/NLRP3 pathway (69). Hypoxia significantly reduces the expression of pro-inflammatory cytokines and increases the concentration of autophagy proteins in colon tissues of patients with CD, which inhibits NF-κB signaling as a result of hypoxia-induced autophagy.

Infections with such pathogens as *Norovirus*, *Campylobacter*, *Salmonellae*, *Shigella*, *Entamoeba histolytica*, *Cytomegalovirus*, and *Yersinia* are considered significant in CD. In contrast, exacerbations of the disease are associated with *Clostridium difficile*, *Shigella*, *Salmonella*, *Campylobacter*, *E. coli* and *Listeria* infections (28, 70).

One of the theories regarding the external environment assumes that hygiene associated with developed countries causes reduced contact with pathogens in childhood, which significantly reduces immune tolerance and increases the likelihood of IBD. Smoking is considered a factor that doubles the incidence and risk of relapse in patients with CD, so patients are advised to quit smoking (1, 28). Living in the city, appendectomy or tonsillectomy in the past, and the use of oral hormonal contraceptives are considered to significantly increase the risk of CD (1, 28). On the other hand, physical activity, high levels of vitamin D, and *Helicobacter pylori* infection are considered protective and reduce the risk of CD. A slight protective effect is also attributed to having pets and high levels of vitamins A, K, and E (71). The mechanism of the protective effect of vitamin D is based on its anti-inflammatory effect on the *in vivo* production of TH1 and TH17 cytokines in patients with CD (10). In addition, interestingly, patients with active CD and current infection have lower serum T-25(OH)D levels than those without infection, which explains the negative correlation of vitamin D with CDAI, serum IL-6 levels, and inflammatory markers in CD patients, which are lower in active CD than in people without infection (72). The phenomenon of delayed neutrophil recruitment to the inflammation site is also observed in patients with CD. This condition negatively affects the immune system response to chronic and destructive inflammation in the intestine. Studies have shown that neutrophils of patients respond normally *in vitro*, and this recruitment delay was considered a phenomenon secondary to CD (*Fig. 1*) (70, 73).

Apart from invasive methods, markers of inflammation found in the stool (calprotectin and lactoferrin produced by neutrophils) are useful in the diagnosis of gastrointestinal disorders of CD and in the assessment of its progression in hospital conditions. Their clinical usefulness is related to treatment monitoring, in particular in the context of relapses or in the assessment of mucosal healing in IBD (74, 75).

In addition, other new markers appear that compete with fecal calprotectin with respect to endoscopic methods representing the gold standard for IBD activity assessment, such as metalloprotease-9 (MMP-9) and lipakalin-2 (76).

DIAGNOSTICS

The diagnosis of CD is based on several elements: clinical symptoms, blood analytical tests, imaging tests, such as ultrasound, CT, or MRI, endoscopic methods, and histological examination of intestinal iopsies (1). Endoscopic investigations are of key use to differentiate CD from UC and other disease entities that may resemble IBD, including mesenteric ischemia, segmental colitis associated with diverticulosis, cancer, radiation enteritis, and drug-induced colitis (6, 77, 78). Ileocolonoscopy with biopsy is considered the gold standard in the diagnosis of most IBD patients, but it is also costly and burdensome. Many studies are thus on the way to work with prediction models based on symptoms and biomarkers for identifying endoscopic activity in CD as an alternative for invasive methods (1, 78-80).

The clinical course of IBD is featured by remission and relapse, is heterogeneous and unpredictable. Monitoring of IBD activity can avoid the poor prognosis. One of the clinically useful analytical tests is assessment of fecal calprotectin. In

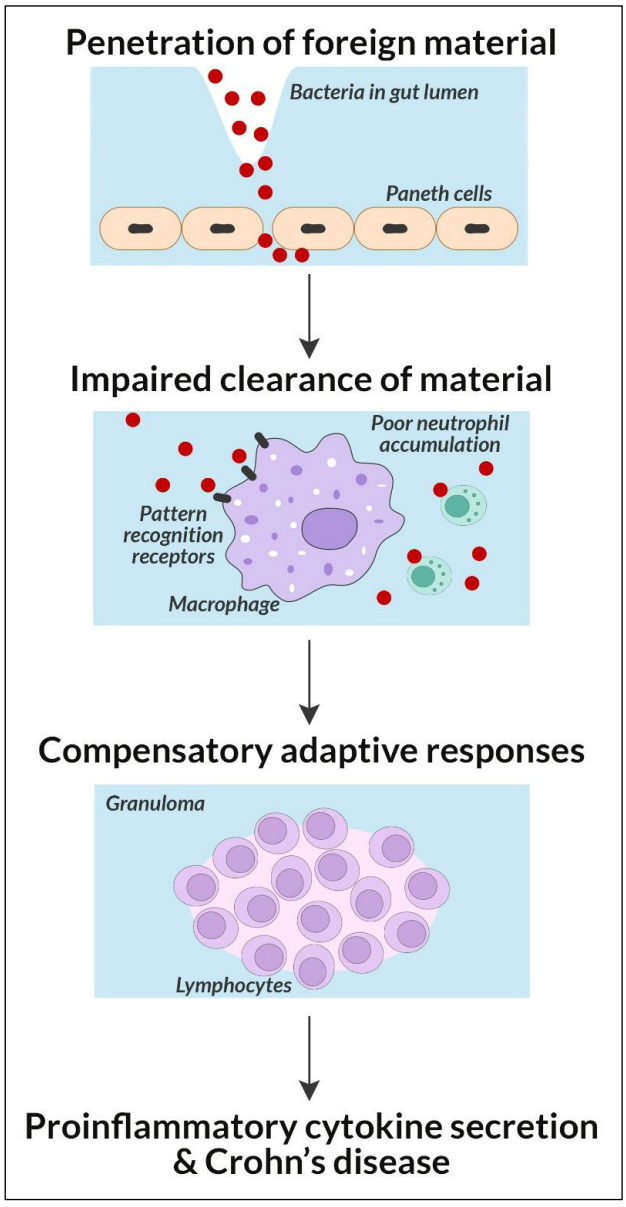

Fig. 1. Inflammatory response dysfunction in Crohn's disease.

inflammatory infiltration, neutrophils migrate to the intestinal lumen and release calprotectin, which is excreted in the feces (58). It is a non-invasive test in assessing colitis activity, it can also be used to assess the response to treatment, and thus limit the use of invasive endoscopic methods (58, 75, 78, 80). Recent meta-analyses revealed that fecal calprotectin could predict clinical relapse for UC patients in remission (81). Fecal calprotectin levels measured by ELISA remains the gold standard but the recently developed rapid semi quantitative bedside tests highly correlate with ELISA results (75). Fecal lactoferrin assesment is the other useful screening marker with high sensitivity and modest specificity for assessing IBD activity (82).

Lectins such as galectins were recently studied as potential biomarkers linked with problems in diagnosis of active IBD (83). Despite not being a specific marker for CD, serum galectin 3-binding proteins might be used as an adjuvant biomarker for disease activity (83).

Despite the progress in the diagnosis of diseases and the differentiation of CD and UC by endoscopy with histological examination of intestinal biopsies, assessment of the history of the disease, serological markers, and radiological tests, 4 – 6% of patients cannot make a definitive diagnosis (78, 79). In such cases, the disease is referred to as inflammatory bowel disease - unclassified (79).

Determination of ASCA (anti-*Saccharomyces-cervisiae* antibodies) and p-ANCA (perinuclear anti-neutrophil cytoplasmic antibodies) is helpful in differentiating CD and UC, although not recommended by current guidelines (77, 84, 85). The configuration of ASCA+, p-ANCA$^-$ is characteristic for CD (sensitivity and specificity equal to 55.0% and 86.4% for ASCA+; 47.0% and 94.8% for ASCA+/pANCA$^-$, respectively) while ASCA$^-$, p-ANCA+ for UC (sensitivity and specificity equal to 61.6% and 84.8% for pANCA+; 53.0% and 91.6% for pANCA+/ASCA$^-$, respectively) (84, 85).

INFLAMMATORY MECHANISMS IN CROHN'S DISEASE

Activation of NF-κB is one of the key mechanisms involved in inflammation in the intestinal mucosa (36-38).

The NF-κB transcription factor family contains five subunits, p65 or RelA, p50, c-Rel, p52, and RelB, which may exist as homo- or hetero-dimers (86). Dimers are maintained inactive in the cytoplasm by binding through κB inhibitory proteins (IκB). Upon activation, IκB degrades and active NF-dimB dimers move to the nucleus, where they regulate transcription (86). The course of this activation is shown in *Fig. 2*, (87-90).

NF-κB activation involves two major signaling pathways, *i.e.* canonical and non-canonical (alternative), both of which are important for regulating immune and inflammatory responses despite differences in the signaling mechanisms (38, 91).

The canonical NF-κB pathway responds to many factors, including ligands of various cytokines, pattern recognition receptors (PRRs), TNF-α, as well as T-cell (TCR) and B-cell (BCR) receptors (91, 92). The basic mechanism of canonical activation of NF-κB is the induction of IκBα degradation caused by its phosphorylation by the IκB kinase complex (IKK) (93).

IKK consists of two catalytic subunits (IKKα and IKKβ), and the NF-κB essential modulator (NEMO) regulatory subunit, or IKKγ (94). IKK can be activated by various stimuli, including cytokines, growth factors, mitogens, microbial components, and stress factors. After activation, IKK phosphorylates IκBα at the two N-terminal serines, thereby triggering ubiquitin-dependent

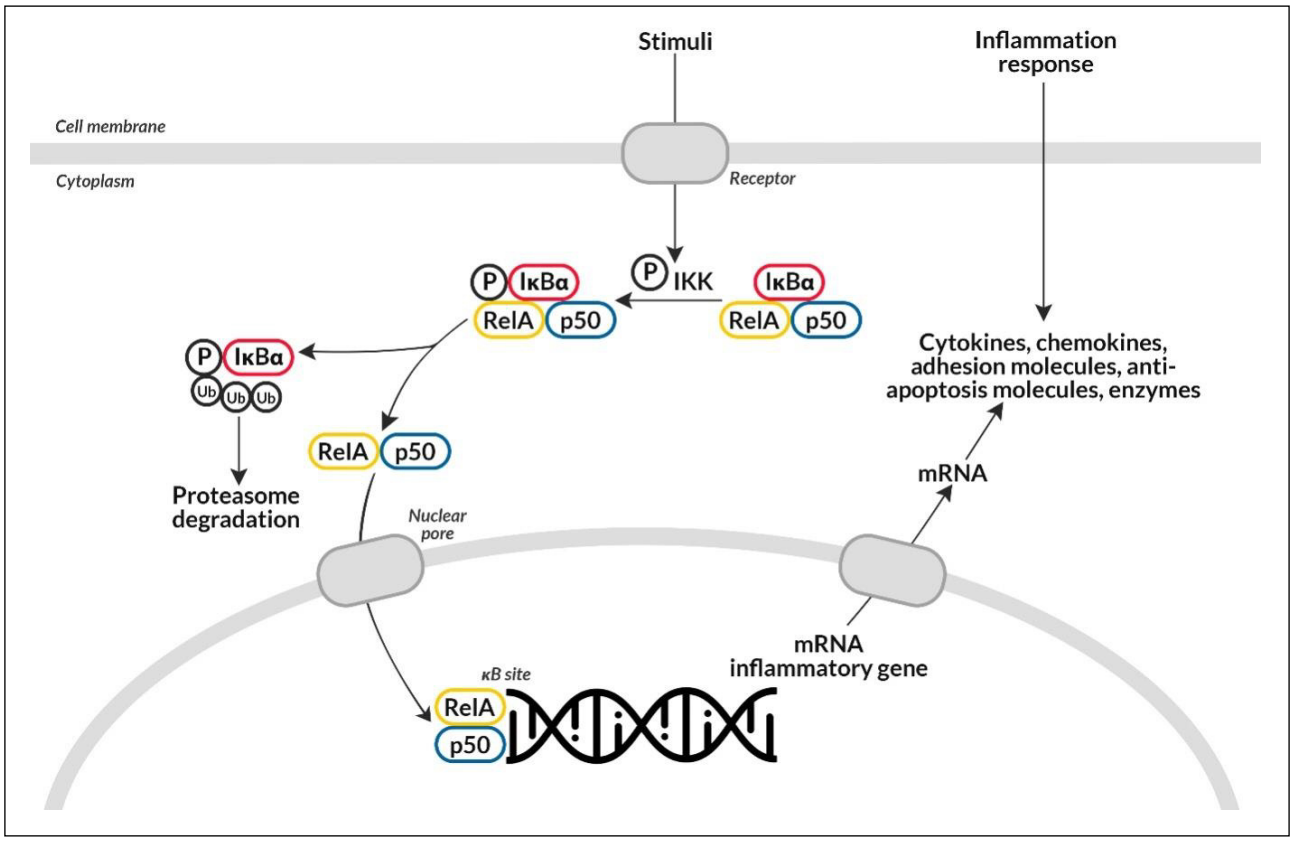

Fig. 2. Nuclear factor-κB activation (see text for details of NFκB pathway).

degradation of IκBα in the proteasome, causing rapid and transient nuclear translocation of canonical NF-κB members, mainly p50/RelA and p50/c-Rel dimers (95).

The canonical NF-κB pathway responds quickly to activation, which results in production of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), leading to cell apoptosis. TNF-α receptor signaling plays an important role in the canonical NF-κB pathway in cell death by Jun-N-terminal kinases (JNK), p38, and caspase 8 (26, 87, 91).

The non-canonical pathway is responsible for the activation of the p100/Rel-B complex (91). This pathway uses the IKK complex, which includes two IKKα subunits, but no NEMO subunit. In the non-canonical pathway, it comes to the ligand-induced activation of NF-κB inducing kinase (NIK), which phosphorylates and activates the IKKα complex. In turn, it phosphorylates p100, leading to the processing and release of active p52/Rel-B heterodimers (88, 91).

In most cells, the NF-κB complex remains in the cytoplasm in a transcription-wise inactivated form until it is stimulated. Proteins from the IκBs group (IκBα, IκBβ, and IκBε) bind NF-κB and occur in this form in the cytoplasm (88). Stimulation causes phosphorylation of proteins from the IκB group, which leads to their ubiquitination in proteasomes. Then NF-κB can enter the cell nucleus and becomes a transcription factor (88).

NF-κB activates the expression of numerous pro-inflammatory genes encoding cytokines, chemokines, and adhesion molecules that play an important role in inflammation and the immune response (90). Activation of NF-κB, as well as a number of other reactions listed below, has an inflammatory route and is associated with the presence of pro-inflammatory cytokines (87, 93, 94, 96). The scheme of response to NF-κB activation is shown in *Fig. 3*.

NF-κB and oxidative stress

Increased cellular oxidative stress activates NF-κB, which increases the production of pro-inflammatory cytokines, acute phase proteins, and adhesion proteins. Peroxide anion ($O_2^{\bullet-}$), in addition to stimulating NF-κB, reacts with nitric oxide (NO), which, despite increased production by NO synthase (iNOS), is thus depleted (35, 89). The product is peroxynitrite, arising at the expense of cellular NO availability and being important for vasodilation, under conditions of limited oxygenation in the intestines. Peroxynitrite itself is toxic to endothelial cells, it causes protein oxidation, lipid peroxidation, and amino acid nitration (35, 89). In addition, the phenomenon of inactivation of superoxide dismutase by peroxide anion and peroxynitrite has been demonstrated. Such a multidirectional attack on a cell results in serious damage and functional impairment including cell death (96, 97).

HYPOXIA OF THE INTESTINAL MUCOSA IN CROHN'S DISEASE

Hypoxia is a phenomenon when the demand for oxygen exceeds the actual supply, which also happens in the case of inflammation in the intestines in the course of IBD (98). The body then activates adaptive mechanisms, as HIF (hypoxia inducible factor) and NF-κB activation (98, 99). The canonical NF-κB pathway is activated by phosphorylation of IκBα, which causes an increase in pro-inflammatory cytokine production. It seems important that NF-κB and HIF may act independently, but may also influence each other (99). Activated NF-κB is involved in the regulation of HIF1α mRNA by binding to the p50 and p65 κB subunit to the HIF1α promoter. In NF-κB overexpression

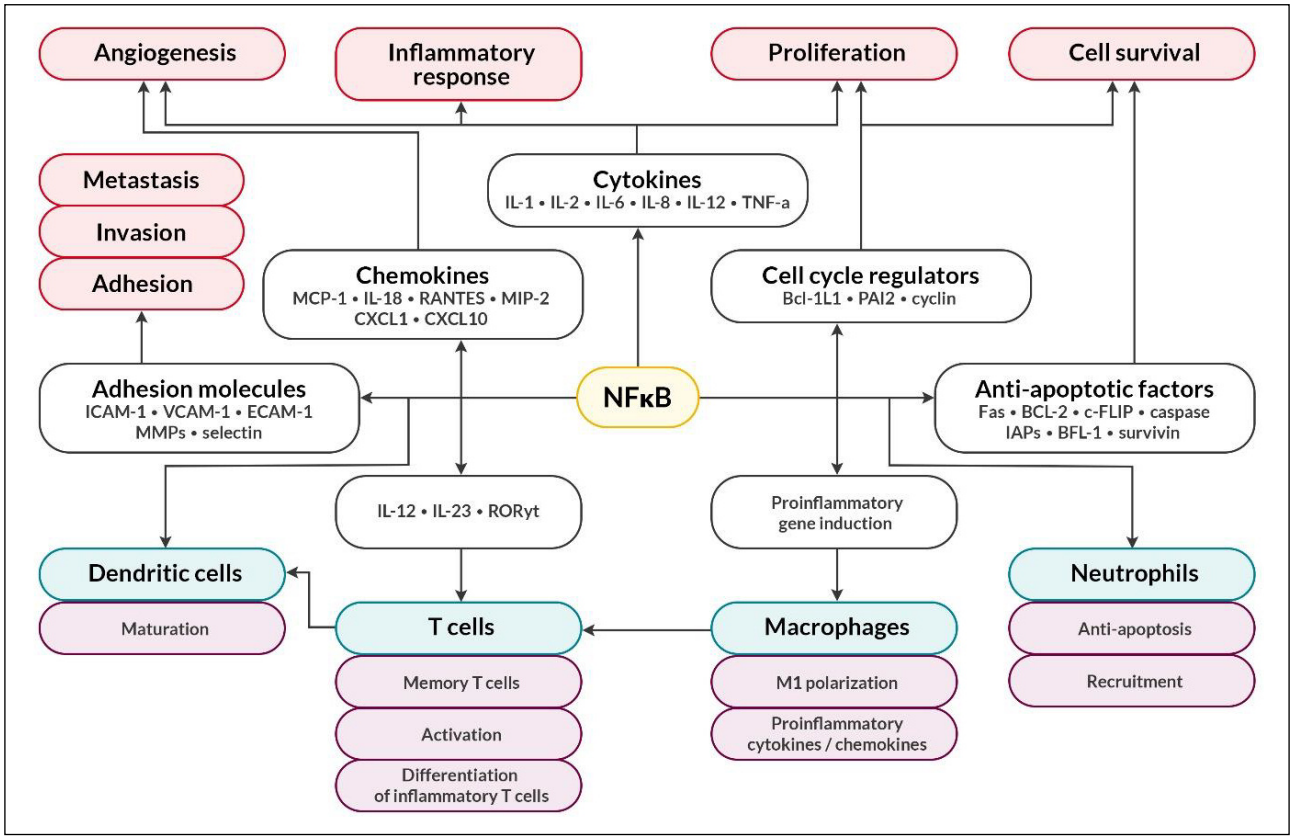

Fig. 3. Local callular response to nuclear factor-κB.

states, there is also overexpression of HIF1α-related proteins, even in normal oxygenation states. The relationship is also observed in case of HIF1α, which may activate NF-κB in neutrophils and keratinocytes (98, 99).

MODULATORS OF NUCLEAR FACTOR-KB ACTIVATION

Activators

Many factors are involved in NF-κB activation, such as viruses, bacteria, parasites, fungi, their metabolites, endotoxins, LPS, pro-inflammatory cytokines (especially IL-1 and TNF-α), mitogens, oxidative stress, environmental factors, heavy metals, UV light, X and γ radiation, carcinogens, hydrogen peroxide, mechanical stress, hypoxia, adhesive molecules, cell cycle regulators, and angiogenic factors (99). It is thought that almost 400 different genes are involved in the activation process (99).

Among the techniques for quantifying NF-κB activation, assays that measure NF-κB activity are noteworthy, including those analyzing p65 cell localization, EMSA NF-κB DNA binding, *in situ* RNA hybridization, and NF-κB target gene expression. Localization of NF-κB activation and inhibition, p65 localization, or transcriptional activity can be measured by immunostaining or *in situ* RNA hybridization for NF-κB regulated genes along with methods such as γH2AX immunostaining or *in situ* RNA for aging markers. such as p16INK4a and p21 (100).

Studies on NF-κB activity measurement have been conducted using luciferase in mononuclear peripheral blood cells in patients with IBD. A statistically significant increase in NF-κB activity was shown when stimulating cells with LPS (101). In patients with CD, a higher percentage of macrophages responded to LPS compared to the control group. In addition, patients with CD showed significantly higher NF-κB activity in the smoking group compared to the group of non-smokers. Smoking can be considered an epigenetic modulator, affecting NF-κB activity, which may explain the impact of environmental factors on the occurrence of the disease (99, 101).

Other studies, in turn, showed a relationship between TNF-α, vitamin C, and activation of the NF-κB pathway in IBD patients. TNF-α inhibits absorption of ascorbic acid in the intestine and transcription of the *SLC23A1* (sodium-dependent vitamin C transporter-1) gene *via* the NF-κB pathway (102). Low levels of ascorbic acid occur in patients with IBD; this condition may be associated with oxidative stress, chronic inflammation, and elevated levels of pro-inflammatory cytokines, including TNF-α (102).

RIPK1 (receptor interacting serin/threonine kinase 1) is another key element in the regulation of NF-κB activity (103). It is a cytosolic protein kinase that is expressed in various cell types and mediates signal transduction of necrosis, apoptosis, and inflammation. Signaling *via* RIPK1 is activated in response to TNF-α. It is regulated by phosphorylation and ubiquitination. When RIPK1 undergoes ubiquitination, it leads to NF-κB activation and pro-inflammatory signaling (103).

Inhibitors

NF-κB activation is tightly regulated by negative feedback, which responds to the presence of active NF-κB in the cell nucleus. Negative feedback includes NF-κB, which induces IκBα, A20 protein, and cezanne protein. IκBα has a nuclear sequence that allows the removal of NF-κB from the nucleus, and thus termination of transcriptional activation (94, 95). This enables feedback, which is the mechanism of NF-κB oscillation between the nucleus and cytoplasm. CYLD (cylindromatosis gene) deubiquitinating enzymes, A20 protein, and cezanne protein are critical NF-κB negative regulators. They are induced by pro-inflammatory signals and can block IKK activation by disconnecting the polyubiquitin chain. This suppresses NF-κB oscillation between the nucleus and cytoplasm due to its stabilization in the cytoplasm by IκBα (104, 107).

ABIN-1, ABIN-2, and PXR molecules may play a catalytic role in the modulation of inflammation in the course of IBD (108). This is connected to the fact that severe colitis causes decreased ABIN-2 signaling more often than blocked TPL-2 signaling, which can be used to assess the potential effect of TPL-2 inhibitors on the disease progression (109).

Studies on TFA (total flavone of *Abelmoschus manihot* L. Medic), which was administered to mice with intestinal inflammation induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS), showed suppressive action against NF-κB and MAPK (mitogen-activated protein kinase) (110). A decrease in pro-inflammatory cytokines, also in inflammatory bowel tissues, was observed in TFA-administered mice (111). TFA has been found to contain, among others: quercetin, isoquercetin, quercetin-3-O-robinobioside, quercetin-3'-O-glucoside, gossypetin, gossypetin-3-O-glucoside, hyperoside, and myricetin. This indicates that TFA may suppress the inflammatory response in mice with TNBS-induced colitis by inhibiting the NF-κB and MAPK signaling pathways (111). The results may improve understanding of TFA function and provide a new potential treatment for CD (111). Resveratrol has a similar effect of suppression on NF-κB and MAPK (107). After LPS stimulation, it was found that activated macrophages treated with RM (product of resveratrol radiolysis) cause a decrease in pro-inflammatory cytokine (TNF-α, IL-6, IL-1β, and IL-12p70) and NO production, and a decrease in activation of the IRAK-1 (interleukin-1 receptor-associated kinase 1), MAPK and NF-κB signaling pathways (107).

In other studies, intestinal inflammation was induced in rats, and then thymol (2-isopropyl-5-methylphenol) was administered, which is found in many plants, *e.g.* thyme, and has antioxidant, anti-inflammatory, analgesic, antibacterial, and antifungal activity (110). Such observations show that this type of therapy significantly reduces mucosal damage. The results indicate that thymol exerts anti-inflammatory effects in colitis by inhibiting the NF-κB signaling pathway and reducing expression of TNF-α and MPO (110, 111).

PHARMACOTHERAPY

Introduction

CD treatment is multifactorial, it requires monitoring of the patient's clinical condition and modification of therapy depending on its effects. The treatment strategy focuses on two stages: the induction of remission leading to resolution of clinical symptoms, improvement of the patient's quality of life, and healing of changes in the intestinal mucosa. The second stage is the long-term maintenance of deep remission, defined as clinical, biochemical, and endoscopic remission, which aims to prevent recurrence of the disease and avoid CD complications (1, 6, 12). Pharmacotherapy is not always effective; it is necessary to undergo surgery with resection of the intestine in nearly half of the patients, and to leave the stoma in some cases (1, 78, 112). In many countries, a pharmacological strategy called step-up is recommended, which involves the inclusion of increasingly stronger drugs, depending on the progressive increase in inflammation (1, 6, 78). The second method of therapy is a top-down strategy. Aggressive escalation of treatment consists in extinguishing the inflammation and

achieving deep remission at the beginning of the disease by starting treatment with the most potent preparations, which are currently biological drugs. This therapy may reduce the incidence of intestinal and parenteral complications (1, 32). After achieving remission, the challenge is to continue treatment and make the decision of its de-escalation (113, 114).

Biological therapy that has been used for several years is effective in inducing remission and controlling the disease, but it is associated with adverse effects and significant costs. Studies conducted in a group of children with CD aged 6 – 17 years verified that the combination of anti-TNF-α with immunomodulators was similarly effective as anti-TNF-α monotherapy (115). Disease index, serum biomarkers, fecal calprotectin, minimal serum levels of adalimumab and anti-adalimumab antibodies were evaluated in the examined patients and no significant higher efficacy of the combination therapy against anti-TNF-α monotherapy was observed (115). The main groups of drugs used to treat CD include mesalazine (5-aminosalicylic acid; 5-ASA) and its derivatives (*e.g.* sulphasalazine), glucocorticoids, immunosuppressants, biological drugs, and, in some cases, antibiotics (*Table 2*). Patients require supplementation of deficiencies and treatment of disease complications (1, 6, 8, 12, 112).

An interesting effect of alternative therapy with polyphenols such as oligonol comparing with an effect of sulphasalazine was investigated in experimental colitis, and oligonol prevented the relapse in this model, however, it requires further explanations (116).

Aminosalicylates

Aminosalicylates have been used in IBD for over half a century and still remain the most important group, especially in UC (1, 6, 12, 32). They show anti-inflammatory and immunomodulatory effects. Representatives of this group are mesalazine (5-ASA), sulfasalazine, and less commonly used balsalazide or olsalazine. Mesalazine inhibits cyclooxygenase, the formation of prostaglandins and leukotrienes, pro-inflammatory cytokines that chemotactically affect neutrophils (119). Activation of NF-κB has been implicated in the pathogenesis of IBD, and various drugs, such as 5-ASA, sulfasalazine as well as glucocorticoids, interfere with NF-κB signaling (120, 121).

The efficacy of aminosalicylates in therapy inducing CD remission has recently been questioned, hence they are not recommended by some scientific societies. Guidelines in some countries, including Poland, allow administration of mesalazine or sulfasalazine preparations in patients with lesions located in the large intestine. Both of them do not prevent recurrence of the disease, while mesalazine is beneficial in preventing the development of colorectal cancer in patients with IBD. For the latter reason, it is recommended for chronic administration, especially in patients with UC (1, 6, 32).

Glucocorticoids

Glucocorticoids are the basic drugs in the therapy of the active IBD phase used for induction of remission (1, 6, 12). Preparations such as prednisone, prednisolone, hydrocortisone, methylprednisolone, or budesonide are administered (31, 32). They are not recommended for maintenance therapy due to their side effects, including an increased risk of developing osteoporosis (1, 6, 12). Immunosuppressive or biological drugs are introduced in patients with steroid-dependent disease or steroid resistance.

Glucocorticoids inhibit the early and late inflammatory response. The anti-inflammatory mechanisms include the inhibition of arachidonic acid metabolism, a decrease in radical formation by oxygen radical scavenging, an inhibition of peripheral and intestinal lymphocytes activation (121). They can interact directly with proteins that are part of transcription complexes, without interacting with DNA regulatory elements (this phenomenon is called transrepression). Glucocorticoids can interact with NF-κB, resulting in decreased production of pro-inflammatory cytokines, adhesion molecules, and nitric oxide synthesis. This is because IKBα is induced, which in turn reduces the transcription of pro-inflammatory cytokines (32).

Immunomodulating treatment

The purpose of using immunomodulatory drugs is to achieve remission and maintain it for a long time, as well as to allow withdrawal of glucocorticoids. Immunosuppressive drugs are recommended in patients with steroid resistance, requiring repeated glucocorticoids therapy due to CD exacerbation, in patients with relapsed disease after attempting to reduce the dose of glucocorticoids, with CD complicated by fistulas, and after surgery with a high risk of relapse (1, 31, 114). Purine analogs, such as azathioprine and mercaptopurine, are used in CD while methotrexate is used in patients with intolerance or side effects. These drugs require close monitoring of the patient because of numerous adverse effects (1, 6, 31, 32, 114).

The experimental study indicate that the action of immunomodulatory drugs are partially related to NF-κB. Quaglio *et al*. demonstrated that azathioprine, sulphasalazine and prednisolone modulate heparanase, NF-κB and Hsp70 gene expression, cytokine production and oxidative stress and participate in the inflammatory response (122). Chang *et al*. have showed that mercaptopurine through inhibiting IκB bioexpression and subsequently blocks bio-activation of NF-κB decreases NF-κB-related pro-inflammatory cytokines exerts anti-inflammatory properties in vascular model (123).

Table 2. Available agents and therapies for Crohn's disease (12, 32, 117, 118).

Cytokines A+	Tumor necrosis factor inhibitors	Infliximab, adalimumab, certolizumab
	IL-23/Th17	Ustekinumab, risankizumab, brazikumab
Integrins	Anti-integrin α4β7	Vedolizumab
JAK inhibitors		Filgotinib, upadacitinib, tofacitinib
Molecules	Antisense molecules	Mongersen
Anti-trafficking therapies	Anti-cell adhesion	Natalizumab, vedolizumab
Others	Mucosal vascular addressin cell adhesion molecule 1 (IgG2 anti-MaDCAM-1)	PF-00547659
	Anti-inflammatory properties	Laquinimod

Biological therapy

Biological therapy using monoclonal antibodies against TNF-α and directed to other biologically active molecules involved in the inflammatory process in CD is widely used because it induces and maintains deep remission of the disease (1, 6, 12, 32). Biological treatment involves the use of monoclonal antibodies, which aim to neutralize the action of pro-inflammatory cytokines, inhibit the function of adhesive proteins, lymphocytes, and certain transcription factors (113, 117).

Drugs from this group are used both in the induction of remission and its maintenance in patients with severe or moderately severe CD, when there is no improvement after other drugs, in patients with fistulas, and in patients intolerant to glucocorticoids or immunomodulatory drugs. Monoclonal antibodies that attach to the inflammation-promoting TNF-α protein, such as infliximab and adalimumab, are primarily used in induction and sustained remission therapy. Another medicines are: vedolizumab, a monoclonal antibody blocking α4β7 integrin and modulating inflammatory processes located in the intestine; ustekinumab, acting on IL-12 and IL-23 cytokines; certolizumab, a TNF-α inhibitor; and natalizumab, anti-alpha4 integrin IgG4 antibody (1, 6, 12, 32, 113, 117).

The action of several biologics are partially related to NF-κB. Guidi *et al*. have demonstrated that an increase of IκB inhibitors levels could be one of the mechanisms by which infliximab decreases NF-κB activity and exerts its anti-inflammatory effects (124).

Probiotics and prebiotics

According to some studies, oral probiotics have beneficial effects in patients with UC, but not in CD. Intestinal microbiome disorders have an important role in the development of inflammation (27-29). Probiotics containing two main types of bacteria are particularly used: *Lactobacillus* (*e.g. L. reuteri*, *L. plantarum*, *L. acidophilus*, *L. casei*) and *Bifidobacterium* (*e.g. B. longum*) (125). These bacteria exhibit an escalating effect on the release of anti-inflammatory agents (IL-22, IL-10) and reducing the production of pro-inflammatory TNF-α, IL-6, IL-1β, IFN-γ, and IL-12. In addition, they modulate the activity of immune cells (Treg lymphocytes, dendritic cells, macrophages) (27, 125). Studies on some strains have been shown to directly modulate NF-κB, thereby reducing the number of pro-inflammatory cytokines and the severity of inflammation (126). Bacteria directly influencing NF-κB include: *Lactobacillus longurum*, *L. johnsonii*, *L. sakei* K040706, *L. johnsonii* CJLJ103, *Bifidobacterium B. adolescentis* IM38, and *B. longum* CH57 (27, 125).

The action of probiotics can have both positive and negative effects. Thus, Derwa *et al*. confirmed that they reduce the symptoms of CD disease or lead to remission (126), however their importance in this disease has not been confirmed in the 2019 meta-analysis (127).

Prebiotics (*e.g*. lactulose, inulin) and functional fibers that can selectively stimulate the growth of beneficial intestinal microbiota and are fermented to short-chain fatty acids. However, there is no evidence of their efficacy in the treatment of CD (127-129). Probiotics, mainly containing three species of live *Bifidobacterium* (BTV) strains, can have a beneficial effect by reducing the side effects of ASA drugs, thus having a good clinical effect on UC (130).

Mesenchymal bone marrow stem cell therapy (MSC)

Research on the use of MSCs is conducted in a variety of disease states, including IBD. Rats with induced inflammatory bowel disease were subjected to this therapy, followed by the assessment of nuclear NF-κB p65 expression, serum TNF-α and IL-10 concentrations, and myeloperoxidase (MPO) activity. Rats showed an increased recruitment of MSCs to the intestinal mucosa, and expression of the abovementioned factors decreased (128, 129). Further studies will clarify whether MSC therapy, which has proven at the molecular level, will have positive effects in patients with IBD (128, 129).

PPARγ ligands

Peroxisome proliferator-activated receptors (PPARs) are transcription factors that belong to the family of nuclear hormone receptors. To date, three PPAR isotypes have been identified: α, β/δ, and γ, each being a product of a separate gene. The PPARγ isotype can also affect the proliferation, survival, and differentiation of both normal and cancer cells. In addition, they exhibit a different expression profile in tissues and activation by type-specific ligands, and are involved in different, although often complementary cellular processes. PPARγ ligands have been described as growth inhibitors, promoting differentiation and inducing tumor cell apoptosis. Currently, research is performed on a potential therapeutic target using PPARγ ligands to reduce inflammation in patients with CD (131-133).

Summary

Activation of the NF-κB transcription factor, which increases the production of pro-inflammatory cytokines, acute phase proteins, and adhesion proteins, is one of the key mechanisms involved in inflammation in the intestinal mucosa in CD. NF-κB activation involves many factors, including infectious, endotoxins, pro-inflammatory cytokines, oxidative stress, UV light, hypoxia, angiogenic factors, *etc*. NF-κB stimulates the expression of numerous pro-inflammatory genes encoding cytokines, chemokines, and adhesive molecules that play an important role in inflammation and immune responses. Disorders of regulation of NF-κB activation lead to overexpression of pro-inflammatory cytokines like IL-1, IL-6, TNF-α, and are associated with the progression of inflammation in IBD. The contribution of NF-κB stimulation to CD under the influence of therapy or new potential drugs used in this disease requires further research.

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