

NF-κB p65 and NF-κB p50 of the Rel Family. A Comparison between Irritable Bowel Syndrome and Inflammatory Bowel Disease Patients

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ABSTRACT

Background/aim: This study aims to explore the role of NF-κB p65/p50 expression in patients with inflammatory bowel disease (IBD), those with irritable bowel syndrome (IBS) and normal controls.

Patients and methods: Twenty patients with IBS, 19 subjects with IBD and 18 normal controls, who underwent colonoscopy, provided us with biopsies from the terminal ileum, cecum and rectum. Patients with IBS were categorized according to bowel habits. Two polyclonal antibodies, alpha-p65 rabbit Ab and alpha-p50 rabbit Ab, were applied for immunohistochemical examination.

Results: It was found that in patients with IBD, the expression of intensity and distribution of NF-κB p65/p50 were lower than in IBS patients and normal controls ($p > 0.05$), but the NF-κB p65/p50 ratio was elevated ($p > 0.05$). The NF-κB p65/p50 ratio was lower than in IBD patients and rather similar to normal controls. The distribution of NF-κB p65 as well as the distribution and intensity of NF-κB p50 expression were higher in IBS patients with predominant diarrhea, and the difference was statistically

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significant in the cecum epithelium. The NF- κ B p65/p50 expression ratio was higher in IBS patients with predominant diarrhea, but statistical significance was documented only in cecum epithelium specimens.

Conclusion: The NF- κ B p65/p50 ratio offers more significant information than each subunit. The increased expression of NF- κ B p50 in IBS patients compared to IBD subjects or controls seems to be an early event in the process of inflammation. The similar pattern of NF- κ B p65/p50 expression in IBS patients with predominant diarrhea and IBD patients may suggest possible common pathogenetic pathways.

Keywords: irritable bowel syndrome, inflammatory bowel disease, NF- κ B p65 and NF- κ B p50.

ABBREVIATIONS

IBS: irritable bowel syndrome
IBD: inflammatory bowel disease
IL: interleukin
UC: ulcerative colitis

INTRODUCTION

The nuclear factor kappa B (NF- κ B) consists of dimeric combinations of the Rel family members, which act as transcription factors, regulating many genes involved in inflammation. The NF- κ B family comprises five members: c-Rel, RelA (p65), RelB, NF- κ B1 (p50/p105) and NF- κ B2 (p52/p100). The dimeric combination of p50 (NF- κ B1) and p65 (RelA) forms the predominant type of NF- κ B, that can bind with specific promoter sequences and stimulate the transcription of numerous pro-inflammatory genes (1, 2). The NF- κ B pathway may be triggered through the AKT pathway via the I κ B kinase beta (IKK β , canonical NF- κ B pathway) or by the interaction of TRAF with the activated NF- κ B-inducing kinase (NIK), resulting in the subsequent activation of NF- κ B by the I κ B kinase alpha (IKK α) (3, 4). Cytoplasmic inhibitory proteins, namely I κ B proteins, are responsible for binding with NF- κ B and retaining it in the cytoplasmic region in the absence of inflammation. After receiving various cellular signals, I κ B proteins are dissociated from the NF- κ B complex and are broken down by special enzymes. As a consequence, NF- κ B is free to move from the cytoplasm and enter the nucleus, where it binds to specific DNA promoter regions and initiates the transcription of genes related to inflammation. The activated p65 subunit of NF- κ B can move to the intranuclear space and promote the expression of genes that protect the cell from apoptosis, known as inhibitors of apoptosis (IAP). IAP proteins are crucial regulators of both NF- κ B pathways (3, 4). TNF- α ,

IL-1 and many more proinflammatory cytokines as well as chemokines and vascular endothelial adhesion molecules are under the regulation of NF- κ B (1, 2, 5). It is not surprising, therefore, that NF- κ B activation is positively correlated with the degree of inflammation in many sites of several pathological conditions (6).

Studies in laboratory animals have shown that mice lacking p50 were immunodeficient, with increased susceptibility to infections. Targeted disruption (or knockout) of the p65 is incompatible with life as it is associated with developmental abnormalities (7).

p50 lacks a C-terminal transactivating domain and, therefore, p50 homodimers are not transcriptionally active. Instead, they are believed to act as transcriptional repressor molecules and suppress the expression of proinflammatory genes, since they can still bind with NF- κ B sites (8, 9). The intracellular concentrations of each member of the NF- κ B/Rel family set another parameter that may influence the progression, nature and degree of the innate immune response. It is suggested that, in terms of gene expression, the ratio of p50 to the other Rel family members in the nucleus may be very important. P50 homodimers have a dual role: they can serve as a brake by inhibiting the production of pro-inflammatory cytokines through repressive homodimers and can also function as an engine that promotes the production of anti-inflammatory cytokines through binding to the IL-10 promoter and initiating transcription. IL-10, initially known as "cytokine synthesis inhibiting factor", has the ability to hinder the transcription and translation of various inflammatory cytokines (10). It reduces antigen presentation and either inhibits or biases T-cell activation (8).

Most interestingly, NF- κ B dimers containing p65 appear to have profound pro-inflammatory activity, whereas the p50 homodimer (NF- κ B1) is

either inactive or may even be involved in blocking NF- κ B sites in some inflammation gene promoters against binding of p65 dimers (8). The fact that p50-p50 homodimers may persist in the course of the resolution of the inflammatory response is not something unexpected, considering the reported role of p50-p50 in the repression of pro-inflammatory gene transcription (11). Increased p65 and c-Rel concentrations may compete with p50 for binding and, as a consequence, inhibit the formation of p50 homodimers. Since IL-10 synthesis is promoted by the p50 homodimers concentrations, it is not surprising that increased levels of p65 and c-Rel negatively affect IL-10 production. Exploring the roles of different NF- κ B members in regulating pro- and anti-inflammatory cytokines as well as identifying other proteins that interact with p50 could help in controlling macrophage p50 levels to boost immunity or prevent autoimmune response. (12).

There is evidence that NF- κ B may be an important regulatory component in the pathogenesis of inflammation in IBD patients. More precisely, macrophages and epithelial cells from inflamed gut tissue in patients with IBD patients exhibited increased levels of NF- κ B p65 (13). In individuals with IBD, NF- κ B p65 can be simultaneously expressed and activated in epithelial cells as well as mucosal macrophages. Primary colonic lamina propria fibroblasts are also believed to contribute to inflammation in IBD via the NF- κ B pathway (9, 14). Interestingly, the amount of activated NF- κ B is significantly correlated with the severity of intestinal inflammation (14, 15).

During the last few years, another assumption regarding the pathophysiology of IBS has emerged. Inflammatory findings at the mucosa and a pro-inflammatory inclination of cytokines in patients with IBS suggest that the irritable bowel syndrome possibly constitutes a low-grade bowel inflammation (16-18). This hypothesis is rather interesting but needs further investigation. $\square$

PATIENTS AND METHODS

Fifty-seven patients (19 patients with IBD and 20 patients with IBS and 18 normal controls) participated in the present study. All participants, among whom there were no differences regarding their demographic characteristics, underwent a colonoscopy after a complete colon cleanse using a polyethylene glycol-electrolyte lavage solution.

Two pinch biopsies were obtained from the terminal ileum, cecum and rectum, totaling six biopsies per case. Biopsies from IBD patients were used as inflammatory disease controls. The normal control biopsies were taken from participants who underwent colonoscopy in the context of colon cancer screening and were found negative. To be part of the control group, each participant must have exhibited normal colonic mucosa both macroscopically and histologically, must have been free of persistent bowel symptoms and must not have been diagnosed with a chronic bowel disease, either organic or functional. The patients with IBS were further divided into three categories according to their bowel habits, including IBS-D patients (nine subjects with diarrhea as the predominant symptom), IBS-C patients (eight subjects with constipation as the main symptom) and IBS-M patients (three subjects whose symptoms may alternate), in order to detect any variations in NF- κ B p65 or NF- κ B p50 expression among these subgroups. Tissue specimens were formalin-fixed paraffin-embedded (4 mm slides) and obtained for immunohistochemical study. An automated immunostainer and the relative protocols provided by the company (Ventana Benchmark, Strasbourg, France) were used for immunohistochemistry. After deparaffinization in xylene and graded alcohols, endogenous peroxidase was blocked with 0.03% hydrogen peroxide for five minutes. Slides were then washed with Tris-buffered saline solution containing 0.1% Tween 20 at pH 7.6 and incubated with the primary antibody using an IgG1 class rabbit polyclonal antibody against the carboxy-terminus of the p65/RelA and p50 components of the NF- κ B complex (Thermo Fisher Scientific Inc) at a 1/100 dilution. Sections were then treated with 3,3'-Diaminobenzidine as chromogen for five minutes and counterstained with hematoxylin. The slides were rinsed with tap water, dried and covered with glass coverslips. To confirm the specificity of the primary antibody, immunoreactivity was blocked by reabsorption of the primary antibody with an excess amount of p65 and p50 antigen peptides. Certain specimens, which had low quality and were not suitable for immunohistochemistry, were not included in the analysis.

NF- κ Bp65/p50 immunohistochemistry was scored independently by two experienced pathologists who had been blinded about the patients. A semiquantitative score that considered

both NF-κB p65/p50 staining intensity and extension was used to evaluate the expression on epithelium and crypts. Distribution of NF-κB p65&p50 immunostaining was graded as focal (<10%), regional (11–50%) or diffuse (>50%). The intensity of NF-κB p65&p50 staining was evaluated as weak (+0), moderate (+1), intense (+2), or severe (+3). NF-κB p65&p50 were considered positive when the sample presented severe or intense diffuse, moderate diffuse and severe or intense regional staining. Immunohistochemically positive-stained cells were counted in six consecutive non-overlapping microscopic fields (three sections × two biopsy samples) with areas of 0.024 mm² at ×400 magnification. The degree of inflammation was evaluated using the same light microscope at a magnification of ×20. All the tissue slides were evaluated by the same experienced pathologist.

IBM SPSS software was used for the statistical analysis, and *p* < 0.05 indicated statistical significance.

The Ethics Committee and the Scientific Research Council approved the study (decision of approval No. 515α/11-03). □

RESULTS

Terminal ileum

The expression of intensity of NF-κB p65 among IBD, IBS patients and controls appeared higher in IBS patients, particularly at the crypts, but the differences were not statistically significant (Figure 1). Results were similar regarding the di-

stribution of NF-κB p65 in the epithelium and crypts in the same groups, except for a nonsignificant decrease of NF-κB p65 distribution at the epithelium in IBS patients (Table 1).

The expression of the intensity of NF-κB p50 among IBD, IBS patients and controls seemed to be higher in IBS patients and lower in IBD ones. The difference between IBS and IBD patients was not statically significant at the epithelium (*p*=0.24) or crypts (*p*=0.07). The expression of NF-κB p50 distribution in IBS patients and controls was not statistically significant, but it was higher in IBS patients when compared to IBD patients. There was a higher NF-κB p50 expression in the IBS group compared to IBD particularly at the superficial epithelium [*p*=0.09 (superficial epithelium), *p*=0.02 (epithelium of crypts)]. The expression of

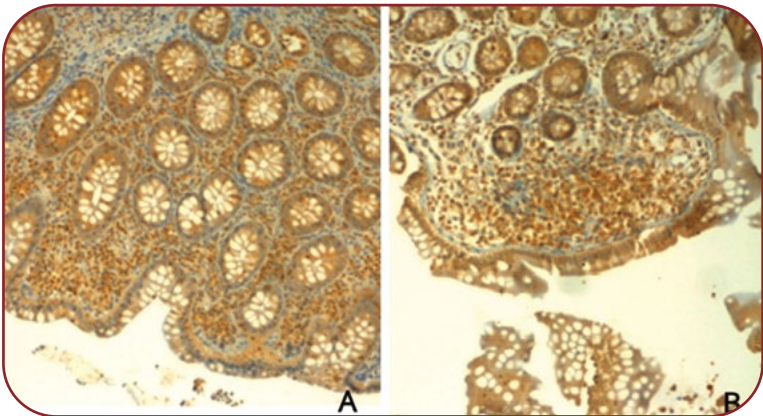


FIGURE 1. NF-κB p65 expression in the terminal ileum of a patient with IBD (A) and a control (B). Magnification at 10x

TABLE 1. Comparison of NF-κB p65, NF-κB p50, NF-κB p65/p50 ratio in the terminal ileum, cecum and rectum of IBD patients, IBS patients and controls. Significance results are identified using superscript letters; different superscript letters express a statistically significant difference between the compared groups; the same superscript letter depicts that the difference between the compared groups is not statistically significant.

		IBD		IBS		Normal colonic mucosa	
		Epithelium	Crypts	Epithelium	Crypts	Epithelium	Crypts
		Intensity	Intensity	Intensity	Intensity	Intensity	Intensity
Terminal ileum	NF-κB p65	(15*) 1,33 ^a	1,67 ^a	(14*) 1,38 ^a	1,88 ^a	(14*) 1,15 ^a	1,77 ^a
	NF-κB p50	(19*) 1,26 ^a	1,42 ^a	(18*) 1,61 ^a	1,83	(16*) 1,38 ^a	1,56 ^a
	NF-κB p65/p50	1,05 ^a	1,17 ^a	0,88 ^a	1,02 ^a	0,74 ^a	1,13 ^a
Cecum	NF-κB p65	(15*) 1,5 ^a	2,07 ^a	(14*) 1,93 ^a	2,36 ^a	(16*) 1,71 ^a	2 ^a
	NF-κB p50	(15*) 1,2 ^a	1,2 ^a	(20*) 2,15 ^b	1,9 ^b	(18*) 1,89 ^b	1,72 ^b
	NF-κB p65/p50	1,33 ^a	1,66 ^a	1,08 ^a	1,25 ^a	0,9 ^a	1,17 ^a
Rectum	NF-κB p65	(16*) 1,33 ^a	2,0 ^a	(12*) 1,75 ^b	2,58 ^b	(16*) 1,86 ^b	2,2 ^b
	NF-κB p50	(18*) 1,11 ^a	1,1 ^a	(20*) 1,91 ^b	1,91 ^b	(18*) 1,61 ^c	1,67 ^c
	NF-κB p65/p50	1,2 ^a	1,8 ^a	0,9 ^a	1,37 ^a	1,16 ^a	1,32 ^a

*Number of samples examined in the study

the extension of the NF- κ B p65 and NF- κ B p50 was diffuse in all three groups.

In addition, the NF- κ B p65/p50 ratio expression was higher in IBD patients, especially at the superficial epithelium, but the difference was not statistically significant. The expression of the NF- κ B p65/p50 ratio was almost identical in IBS patients and controls at the superficial epithelium, but at crypts it was higher in controls, almost approaching that of IBD patients.

Cecum

The expression of the NF- κ B p65 intensity was higher in IBS patients compared to IBD ones or controls in both the superficial epithelium and epithelium of crypts (statistically not significant), similarly to what was found at the ileum. The expression of the distribution of NF- κ B p65 among the same groups in the superficial epithelium and crypts' epithelium was similar (Table 1).

The comparison of the expression of the NF- κ B p50 intensity among IBD, IBS patients and controls showed a statistically significant increase in IBS patients (IBS vs IBD, $p=0.001$). Also, the expression of NF- κ B p50 was significantly higher in controls compared to IBD patients (controls vs IBD, $p=0.01$). The distribution of NF- κ B p50 was similar in IBS patients and controls and a little lower in IBD patients ($p>0.05$). The expression of the extension of NF- κ B p65 and NF- κ B p50 was diffuse in all three groups.

The expression of the NF- κ B p65/p50 ratio was higher in IBD patients at both superficial and cryptical epithelium and lower in controls, without any statistically significant variation.

Rectum

The comparison of the NF- κ B p65 expression of intensity among IBD, IBS patients and controls showed an increase in IBS patients especially in the epithelium of the crypts, and a decrease in IBD patients [IBS vs IBD ($p=0.001$), IBD vs controls ($p=0.01$)]. It is noteworthy that the expression of the intensity of NF- κ B p65 in the epithelium of controls was higher among the aforementioned groups (Figure 2). In addition, when comparing IBD patients with IBS patients and controls, the expression of the extension of NF- κ B p65 was found to be lower in the superficial epithelium and the epithelium of the crypts of the former group [IBD vs IBS ($p=0.001$), IBD vs controls ($p=0.02$)] (Table 1).

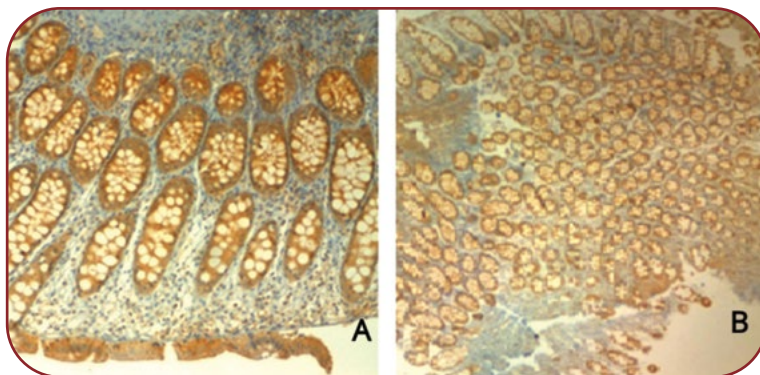


FIGURE 2. NF- κ B p65 expression in the rectum of a patient with IBS (A) and a control (B). The expression of the intensity of NF- κ B p65 in the epithelium of controls was generally higher compared to IBS patients. Original magnifications at 10x and 5x, respectively

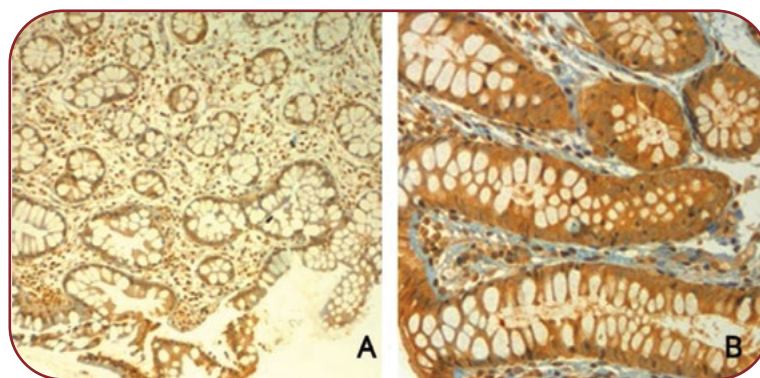


FIGURE 3. NF- κ B p50 expression in rectum in an IBD patient (A) and an IBS patient (B). Expression of NF- κ B p50 intensity is significantly increased in the superficial and cryptical epithelium of the patient with IBS. Original magnifications at 10x and 20x, respectively

Regarding the expression of NF- κ B p50 intensity among IBS, IBD patients and controls it was significantly increased in the superficial and cryptical epithelium of IBS patients [IBS vs IBD ($p=0.001$), IBS vs controls ($p=0.02$)] and statistically significantly lower in IBD patients [IBD vs controls ($p\sim 0.01$)] (Figure 3). Distribution of NF- κ B p50 among IBS, IBD patients and controls was higher in IBS patients, while there was a significant decrease in IBD patients [IBD vs IBS ($p=0.001$), IBD vs controls ($p=0.015$)]. The extension of NF- κ B p65 and NF- κ B p50 was diffuse in all three groups.

In addition, the ratio of NF- κ B p65/p50 was higher (non-statistically significant) in IBD patients, especially at the epithelium of the crypts. Comparing IBS patients and controls, the results were similar at the epithelium of the crypts while at the

superficial epithelium, the NF-κB p65/p50 ratio was not significantly higher in controls.

IBS subgroups

No statistically significant difference in the expression of the intensity of NF-κB p65 was noted among the IBS subgroups in the different anatomical sites of the colon. NF-κB p65 was elevated in IBS-D patients at the epithelium of ileum ($p=0.2$) compared to IBS-M/C patients and it was lower in crypts of ileum in IBS-C compared to equals IBS-M/IBS-D ($p=0.08$). At the cecum, the results were similar at epithelium (IBS-C, IBS-D patients), where at crypts NF-κB p65 was increased in IBS-D patients compared to IBS-C ones ($p=0.5$). At the rectum, the results were conflicting. At the epithelium of the rectum, the intensity of NF-κB p65 was lower in IBS-C comparing equal IBS-M/IBS-D, while at the crypts NF-κB p65 was higher in IBS-C patients compared to equal IBS-M/IBS-D ($p>0.05$). In addition, there are not any statistically significant differences in the expression of the extension of NF-κB p65 in all subgroups throughout the intestine, except for a decrease in the superficial epithelium of the ileum

in IBS-M patients, but this difference was insignificant (Table 2).

On the other hand, the intensity of NF-κB p50 expression at the ileum was higher in IBS-D patients rather than in IBS-C or IBS-M patients (not statistically significant), while the distribution of NF-κB p50 expression was slightly higher in IBS-D and IBS-C patients compared to IBS-M patients. The intensity of expression of NF-κB p50 at the cecum was higher in IBS-D patients, especially at the superficial epithelium [(IBS-D vs IBS-C ($p=0.04$)) and lower in IBS-C patients. The differences in the distribution of NF-κB p50 expression at the same area were not statistically significant. Furthermore, the intensity of NF-κB p50 at the rectum did not show a particular pattern among IBS subgroups, while the expression of extension NF-κB p50 distribution was slightly lower in IBS-D patients.

Regarding the NF-κB p65/p50 ratio, it showed overall higher in IBS-D patients compared to IBS-C ones, except for the cecum epithelium [IBS-D vs IBS-C ($p\sim 0.005$)]. The NF-κB p65/p50 ratio in IBS-M patients could not be estimated

	IBS subtype						
		Terminal ileum		Cecum		Rectum	
		Epithelium	Crypts	Epithelium	Crypts	Epithelium	Crypts
		Intensity	Intensity	Intensity	Intensity	Intensity	Intensity
NF-κB p65	C	(3*) 1,5 ^a	2 ^a	(7*) 2 ^a	2,16 ^a	(6*) 1,67 ^a	2,5 ^a
	M	(3*) 1,15 ^a	3 ^a	-	-	(1*) 2 ^a	2,0 ^a
	D	(8*) 2,5 ^a	3 ^a	(7*) 2 ^a	2,33 ^a	(5*) 2 ^a	2,33 ^a
NF-κB p50	C	(8*) 1,63 ^a	1,84 ^a	(8*) 1,87 ^a	1,63 ^a	(8*) 2 ^a	1,84 ^a
	M	(1*) 1 ^a	1 ^a	(3*) 2 ^a	2 ^a	(3*) 1,67 ^a	2 ^a
	D	(9*) 2 ^a	2 ^a	(9*) 2,75 ^b	2 ^a	(9*) 2 ^a	2 ^a
NF-κB p65/p50	C	0,9 ^a	1,1	1,07 ^a	1,26	0,88 ^a	1,24
	M	1,5 ^a	3	-	-	1,2 ^a	1
	D	1,25 ^a	1,5	0,72 ^b	1,65	1 ^a	1,65

*Number of samples examined in the study

TABLE 2. Comparison of NF-κB p65, NF-κB p50, and NF-κB p65/p50 in the ileum, cecum and rectum of IBS subgroups. Significance results are identified using superscript letters; different superscript letters express a statistically significant difference between the compared groups; the same superscript letter depicts that the difference between the compared groups is not statistically significant.

	Crohn's disease		Ulcerative colitis	
	Epithelium	Crypts	Epithelium	Crypts
	Intensity	Intensity	Intensity	Intensity
NF-κB p65	(3*) 1,3 ^a	2,3 ^a	(9*) 1,4 ^a	2,0 ^a
NF-κB p50	(3*) 1,3 ^a	1,3 ^a	(12*) 1,2 ^a	1,2 ^a
NF-κB p65/p50	1,0 ^a	1,78 ^a	1,17 ^a	1,67 ^a

*Number of samples examined in the study

TABLE 3. Comparison of NF-κB p65, NF-κB p50, NF-κB p65/p50 ratio in the cecum of patients with ulcerative colitis and Crohn's disease. Significance results are identified using superscript letters; different superscript letters express a statistically significant difference between the compared groups; the same superscript letter depicts that the difference between the compared groups is not statistically significant.

with accuracy due to the reduced size of the sample.

Crohn's disease vs ulcerative colitis

Finally, comparing NF- κ B p65 & p50 intensity and distribution in the cecum epithelium and crypts in patients with Crohn's disease and ulcerative colitis (UC), they seemed to be rather similar and so were NF- κ B p65/p50 ratios (Table 3). $\square$

DISCUSSION

NF- κ B plays a pivotal role in chronic inflammation and related diseases, as it upregulates the gene expression of many enzymes and adhesion molecules.

In IBS it was supposed that NF- κ B p65 was especially implicated in inflammatory procedures. It appears that NF- κ B p65 (RelA), which has been suggested to have a greater pro-inflammatory role in the induction of inflammatory gene expression than p50, is an important factor in the regulation of intestinal inflammation in IBD (19). It has been recently reported that MiR-15, which has higher levels in UC patients compared to IBS and normal controls, mimics an induced nuclear translocation of NF- κ B p65, leading to overexpression of proinflammatory cytokines in the colonic epithelial cells (20).

Besides, the Toll-like-receptor 9 (TLR-9) agonist cobitolimod (DIMS0150) targeting NF- κ B-p65 is a promising therapeutic option for UC (21), while other reports present evidence for a butyrate-associated inhibition of activation of NF- κ B (p65) in lamina propria macrophages of patients with distal UC (22). Probiotics have been reported to have the same effect (23).

It has been reported that MiR-15, whose levels are higher in UC patients compared to IBS and normal controls, mimics induced nuclear translocation of NF- κ B p65, leading to overexpression of proinflammatory cytokines in colonic epithelial cells (20).

In our study, it was shown that in IBD patients, the intensity and distribution of NF- κ B p65 was lower (at least in the ileum, cecum, and rectum), compared to patients with IBS or normal controls. On the other hand, it is intriguing that NF- κ B p50 was lower in inflammatory bowel diseases. Subsequent estimation of the ratio NF- κ B p65/p50 in IBD patients indicated that it was higher in those patients, compared to IBS patients or controls.

The ratio of NF- κ B p65/p50 rather than solely NF- κ B p65 or NF- κ B p50 may be more important in the induction of inflammation.

Concerning IBS, there is an open question regarding its classification, namely converging towards IBD implying some degree of inflammation in such patients or resembling more to normal controls. The intensity of both NF- κ B p65 and NF- κ B p50 was elevated in IBS patients compared to IBD patients and normal controls. On the other hand, the estimation of NF- κ B p65/50 ratio showed no definite variations between IBS patients and controls, whereas it was elevated in IBD patients. Determining NF- κ B p65 distribution in the aforementioned groups it was similar in IBS patients and controls as well as in IBD patients, while the distribution of NF- κ B p50 was elevated in IBS patients compared to IBD patients or controls. The elevated distribution of NF- κ B p50 may be associated with moderating the inflammatory process leading to minimal inflammation in IBS. Such an assumption is preliminary and many parameters should be estimated to support it.

A relatively recent study reported that NF- κ B p65 is elevated in IBS patients with diarrhea as a predominant symptom compared to IBS patients with alternating symptoms and controls (24). In our study, categorizing IBS patients into subgroups based on symptomatology showed that the intensity of NF- κ B p65 varied significantly in the intestine without a specific pattern. Distribution of NF- κ B p65 was higher at the ileum in IBS-D patients, while it was rather similar at the cecum and rectum in IBS-D&C patients. Distribution of NF- κ B p65 in IBS-M patients seemed to be lower than the other subgroups. On the other hand, the intensity of NF- κ B p50 was higher in IBS-D patients almost universally in the intestine, while the same expression pattern was found for NF- κ B p50 distribution at the cecum and rectum. Data concerning the intensity and distribution of NF- κ B p50 in IBS-C and IBS-M patients were conflicting. At last, the NF- κ B p65/NF- κ B p50 ratio was almost universally higher in IBS-D patients compared to IBS-C patients, except for the epithelium of the cecum. Taking into consideration both factors (NF- κ B p65 and p50), it seems that IBS-D patients could be closer to the model of inflammation in comparison to IBS-C or IBS-M patients.

To sum up, the role of the NF- κ B family in the inflammation in IBD and IBS is complex. NF- κ B p50 factor might play a significant initial

role in controlling or confining inflammation in certain conditions. The increased expression of NF- κ B p50 in IBS patients compared to the IBD patients or the control group implies that this step is possibly an early event in the process of inflammation and the development of the disease. It remains questionable whether the insignificant expression of the ratio of NF- κ B p65/p50 in IBS-D and IBD patients could suggest some common pathogenetic steps for these two entities. Estimation of the later findings requires further workup, possibly with the examination of interleukins such as TNF- α , IL-6 which correlates with neoplasia

and autoimmune disorders (25, 26), adhesion molecules, enzymes or other factors in an attempt to clarify possible pathophysiological mechanisms.

Limitations of the study

The rather small number of histological specimens in some categories is a limitation that may not permit generalization of the conclusions as far as statistics is concerned. $\square$

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