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ORIGINAL ARTICLE

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Nuclear factor κB is activated in small intestinal mucosa of celiac patients

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Abstract NF-κB regulates inflammatory and immune response by increasing the expression of specific genes. In celiac disease proinflammatory cytokines, adhesion molecules, and enzymes whose gene expression is known to be regulated by NF-κB are involved. This study investigated the activation of NF-κB in inflamed mucosa from patients with untreated celiac disease. Biopsy specimens from control, untreated, and treated patients were subjected to molecular biology analysis. NF-κB activation was evaluated by electrophoretic mobility shift assay. NF-κB related subunit protein level, and inducible nitric oxide synthase and cyclo-oxygenase 2 protein expression was analyzed by western blot. Both NF-κB/DNA binding activity and p50/p65 nuclear levels were higher in biopsy specimens from untreated patients than in those from treated patients and controls. The degradation of IκBβ in the cytosol and the reappearance in the nucleus indicated a persistent NF-κB activation in celiac disease. NF-κB activity was maintained in cultured biopsy specimens up to 6 h and decreased at 24 h, and then the addition of peptic-tryptic digest of gliadin caused the recovery of NF-κB activity at 6 h. NF-κB/DNA binding activity was correlated with inducible nitric oxide synthase and cyclo-oxygenase-2 protein expression. These results show for the first time that NF-κB is activated in the inflamed mucosa of celiac patients and suggest that it may represent a molecular target for the modulation of inflammatory response in celiac disease.

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Keywords Celiac disease · Cyclo-oxygenase-2 · Inducible nitric oxide synthase · Nuclear factor κB · Peptic-tryptic digest of gliadin

Abbreviations *CD:* Celiac disease · *COX:* Cyclo-oxygenase · *iNOS:* Inducible nitric oxide synthase · *IκB:* Inhibitory protein κB · *NF-κB:* Nuclear factor κB · *Pt-G:* Peptic-tryptic digest of gliadin

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Introduction

Celiac disease (CD) is a gluten-sensitive enteropathy in genetically predisposed individuals that generally leads to a wide spectrum of clinical symptoms. This pathology is characterized by the presence of antitissue transglutaminase antibodies in the serum and by damage at the level of the small intestine with villous atrophy, intraepithelial lymphocyte infiltration, chronic inflammation, and activation of lamina propria T cells. Nevertheless, patients go into remission when they are put on a gluten-

free diet [1]. There is increasing evidence to support a T-cell mediated immune response to gliadin as a key event in the pathogenic cascade of CD. Gluten induces the activation of lamina propria $CD4^+$ T cells, followed by secretion of high levels of interferon-γ [2]. Moreover, interferon-γ alone or in combination with tumor necrosis factor-α may activate macrophages to produce proinflammatory cytokines able to damage the mucosal matrix [3, 4]. It has been reported that nitric oxide and prostaglandins may play an important role in the mucosal lesion [5, 6]. High levels of nitric oxide products (nitrate/nitrite) in the urine of children with active CD have been found to be correlated with the expression of inducible nitric oxide synthase (iNOS) in the small intestine [7, 8]. Increased amounts of prostaglandin E_2 have been detected in homogenized small bowel biopsy specimens from patients with active CD [6]. Recently it has been reported that lamina propria cells from celiac patients produce high levels of cyclo-oxygenase (COX) 2 [9]. A common paradigm for the pathogenesis of CD is that several genes whose expression is induced in the inflamed mucosa, such as those encoding for iNOS and COX-2, contain κB sites for nuclear factor κB (NF-κB) [10, 11]. The most common form of NF-κB is a heterodimer composed of the p50 and p65 subunits. In quiescent cells NF-κB resides in the cytosol in latent form bound to inhibitory proteins, called IκBs. Several proteins have been identified including IκBα, IκBβ, IκBε, p100, p102, and Bcl-3. Stimulation of cells triggers a series of signaling events that ultimately lead to the phosphorylation, polyubiquitination, and proteosomal degradation IκB. Activated NF-κB is free to enter into the nucleus and stimulate transcription by binding to cognate κB sites in the promoter regions of various target genes. It has been suggested that differential patterns of degradation of the IκB isoforms represent an important mechanism in the regulation of NF-κB activation. Although IκBα and IκBβ likely interact with the same set of NF-κB/Rel family members, it appears that IκBβ activates persistently in a cell type and stimulus-specific manner, whereas the regulation of NF-κB by IκBα is rapid but transient [12]. However, the mechanism basis for the persistent activation of NF-κB has not yet been elucidated. Several NF-κB target genes coding for cytokines, adhesion molecules, and enzymes have been shown to be up-regulated in other gastrointestinal diseases [13]. The present study investigated whether NF-κB activation occurs in intestinal biopsy specimens from patients with active (untreated) or inactive (treated with gluten-free diet) CD. We provide evidence for the first time that NF-κB is constitutively activated in intestinal biopsy specimens from untreated patients.

Materials and methods

Patients

Biopsy specimens from the distal duodenum were obtained by upper gastrointestinal endoscopy from six children with CD on a normal gluten containing diet (untreated) and seven with CD following gluten-free diet from almost 3 years (treated). Histological examination was performed on one half of the specimen, while one half of the sample tissue was immediately frozen in liquid nitrogen and then tested. Diagnosis of CD was performed in all patients for anti-endomisial antibody positivity and typical mucosal lesions with crypt hyperplasia, villous atrophy, increased number of intraepithelial lymphocytes. Control pediatric patients (n=5) underwent upper gastrointestinal endoscopy for gastrointestinal symptoms but were anti-endomisial antibody negative, and their duodenal histology was normal. This study was approved by the local ethics committee (University Federico II, Naples, Italy).

Organ culture

The mucosal specimens from other untreated children (n=4) were cultured as previously described [14]. The specimens were incubated with medium alone at different time points (0, 2, 4, 6, 12, and 24 h). After 24 h incubation with medium alone the specimens were incubated for 2, 4, and 6 h with peptic-tryptic digest of gliadin (Pt-G; 1 mg/ml). Pt-G, purified prolamin fraction, was prepared as previously described [15]. The dishes were placed in a tight container with 95% O_2/5% CO_2 at 37°C, at 1 bar. Biopsies were snap-frozen and stored at −80°C until used.

Cytosolic and nuclear extracts

Cytosolic and nuclear extracts of biopsy specimens were prepared as previously described with some modification [16]. Briefly, each biopsy specimen was frozen in liquid nitrogen, immediately suspended in 150 µl ice-cold hypotonic lysis buffer (10 mM hydroxyethylpiperazine ethanesulfonic acid, 10 mM KCl, 0.5 mM phenylmethylsulfonyl fluoride, 1.5 µg/ml soybean trypsin inhibitor, 7 µg/ml pepstatin A, 5 µg/ml leupeptin, 0.1 mM benzamidine, 0.5 mM dithiothreitol) and homogenized using a glass homogenizer and a Teflon pestle. The homogenates were chilled on ice for 15 min and then vigorously shaken for another 15 min in the presence of 20 µl 10% Nonidet P-40. The nuclear fraction was precipitated by centrifugation at 1500 g for 5 min, and the supernatant containing the cytosolic fraction was removed and stored at −80°C. The nuclear pellet was resuspended in 100 µl high salt extraction buffer (20 mM hydroxyethylpiperazine ethanesulfonic acid pH 7.9, 10 mM NaCl, 0.2 mM EDTA, 25% v/v glycerol, 0.5 mM phenylmethylsulfonyl fluoride, 1.5 µg/ml soybean trypsin inhibitor, 7 µg/ml pepstatin A, 5 µg/ml leupeptin, 0.1 mM benzamidine, 0.5 mM dithiothreitol) and incubated with shaking at 4°C for 30 min. The nuclear extract was then centrifuged for 15 min at 13,000 *g* and supernatant was aliquoted and stored at −80°C. Protein concentration was determined by Bio-Rad (Milan, Italy) protein assay kit.

Electrophoretic mobility shift assay

Double-stranded oligonucleotides containing the NF-κB recognition sequence (5′-CAACGGCAGGGGAATCTCCCTCTCCTT-3′) were end-labeled with [^{32}P-γ]ATP. Nuclear extracts containing 5 µg protein were incubated for 15 min with radiolabeled oligonucleotides (2.5–5.0×10^4 cpm) in 20 µl reaction buffer containing 2 µg poly deoxyinosine- deoxycytidine, 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 10% (v/v) glycerol. The specificity of the DNA/protein binding was determined by competition reaction in which a 50-fold molar excess of unlabeled wild-type, mutant, or Sp-1 oligonucleotide was added to the binding reaction 15 min before addition of radiolabeled probe. In supershift assay antibodies reactive to p50 or p65 proteins were added to the reaction mixture 15 min before the addition of radiolabeled NF-κB probe. Nuclear protein–oligonucleotide complexes were resolved by electrophoresis on a 6% nondenaturing polyacrylamide gel in 1× Tris-borate-EDTA buffer at 150 V for 2 h at

4°C. The gel was dried and autoradiographed with intensifying screen at −80°C for 20 h. Subsequently the relative bands were quantified by densitometric scanning of the radiographic films with a GS-700 Imaging Densitometer (Bio-Rad) and a computer program (Molecular Analyst; IBM).

Western blot analysis

Immunoblotting analysis of anti-p50, anti-p65, anti-iκBα, anti-iκBβ, anti-iNOS, anti-COX-2, and anti-β-actin was performed on biopsy specimens. Cytosolic and nuclear fraction proteins were mixed with gel loading buffer (50 mM Tris, 10% sodium dodecyl sulfate, 10% glycerol, 10% 2-mercaptoethanol, 2 mg/ml bromophenol) at a ratio of 1:1, boiled for 3 min and centrifuged at 10,000 *g* for 5 min. Protein concentration was determined and equivalent amounts (50 µg) of each sample were electrophoresed in a 12% discontinuous polyacrylamide minigel. The proteins were transferred onto nitrocellulose membranes, according to the manufacturer's instructions (Bio-Rad). The membranes were saturated by incubation at 4°C overnight with 10% nonfat dry milk in phosphate-buffered solution and then incubated with (1: 1000) anti-p50, anti-p65, anti-iκBα, anti-iκBβ, anti-iNOS, and anti-COX-2 for 1 h at room temperature. The membranes were washed three times with 0.05% Triton 100x in phosphate-buffered solution and then incubated with anti-rabbit or anti-goat immunoglobulins coupled to peroxidase (1: 1000). The immunocomplexes were visualized by the enhanced chemiluminescence method (Amersham, Milan, Italy). The membranes were stripped and reprobed with β-actin antibody to verify equal loading of proteins. Subsequently the relative bands of p50 and p65 in nuclear fraction, and iNOS and COX-2 in cytosolic fraction were quantified by densitometric scanning of the radiographic films with a GS 700 Imaging Densitometer (Bio-Rad) and a computer program (Molecular Analyst, IBM).

Reagents

[^{32}P]γ-ATP was from Amersham (Milan, Italy). Poly-deoxyinosine-deoxycytidine and T4 polynucleotide kinase were from Boehringer-Mannheim (Milan, Italy). Anti-p50, anti-p65, anti-iNOS, anti-COX-2, anti-iκBα, anti-iκBβ, and β-actin antibodies were from Santa Cruz (Milan, Italy). Oligonucleotide synthesis was performed to our specifications by Tib Molbiol (Boehringer-Mannheim, Genoa, Italy). Nonfat dry milk was from Bio-Rad. DL-Dithiothreitol, pepstatin A, leupeptin, benzamidine, phenylmethylsulfonyl fluoride were from Applichem (Darmstadt, Germany). Pt-G from pure bread wheat (*Triticum aestivum*, var. San Pastore) was kindly supplied by the Istituto Sperimentale per la Cerealicoltura (Rome, Italy). All other reagents were from Sigma (Milan, Italy).

Statistics

Results are expressed as the means ±SEM of *n* experiments. Statistical significance was calculated by one-way analysis of variance and Bonferroni-corrected *P* value for multiple comparison test. The level of statistically significant difference was defined as 0.05. Linear associations between variables were assessed by the use of standard-least-square linear regression. The correlation coefficient (*r*) is presented as measure of linear association for regression relationship.

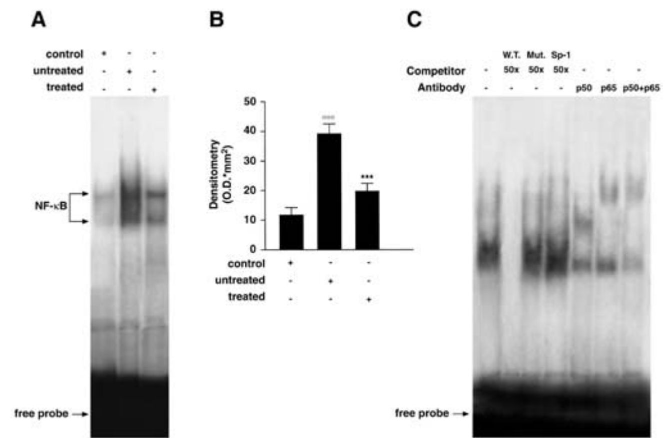

Fig. 1 NF-κB activation and characterization of NF-κB complex. (**A**, **B**) Representative electrophoretic mobility shift assay (**A**) and densitometric analysis (**B**) show NF-κB/DNA binding activity in nuclear extracts from biopsy specimens of control, untreated, and treated patients. Nuclear extracts from biopsy specimens were prepared as described in the text and incubated with ^{32}P-labeled NF-κB probe. (**A**) Data are from a single experiment. (**B**) Mean ±SEM of 14 experiments. °°°*P*<0.0001 vs. control; ****P*<0.0001 vs. untreated. (**C**) Characterization of NF-κB complex. In competition reaction nuclear extracts from biopsy specimens of untreated patients were incubated with radiolabeled NF-κB probe in absence or presence of identical but unlabeled oligonucleotides (W.T., 50×), mutated nonfunctional κB probe (Mut., 50×) or unlabeled oligonucleotide containing the consensus sequence for Sp-1 (Sp-1, 50×). In supershift experiments nuclear extracts were incubated with antibodies against p50, p65, or p50 + p65 15 min before incubation with radiolabeled NF-κB probe. Data are from a single experiment and representative of six experiments

Results

NF-κB activity is increased in intestinal mucosa of CD patients

To detect NF-κB/DNA binding activity nuclear extracts from biopsy specimens of untreated patients, treated patients, and normal controls were analyzed by electrophoretic mobility shift assay. As shown in Fig. 1 panels A and B, a low basal level of NF-κB/DNA binding activity was detected in nuclear extracts from biopsy specimens of controls. The NF-κB/DNA binding activity markedly increased in nuclear extracts obtained from biopsy specimens of untreated patients, while it significantly decreased in nuclear extracts from biopsy specimens of patients treated. The composition of the NF-κB complex was determined by competition and supershift experiments in nuclear extracts from untreated patients. The specificity of NF-κB/DNA binding complex was demonstrated by the complete displacement of NF-κB/DNA binding in the presence of a 50-fold molar excess of unlabeled NF-κB probe (W.T., 50×) in the competition reaction. In contrast, a 50-fold molar excess of unlabeled mutated NF-κB probe (Mut., 50×), or Sp-1 oligonucleotide (Sp-1, 50×) had no effect on this DNA-binding activity. The subunit composition of the NF-κB complexes

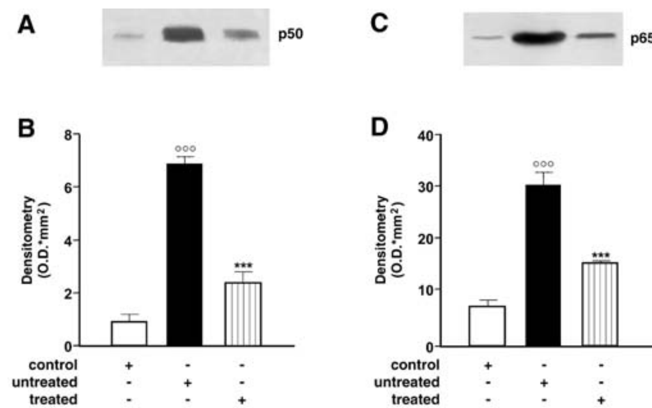

Fig. 2 Nuclear level of p50 and p65 subunits. Representative western blots of p50 (**A**) and p65 (**C**) and densitometric analysis (**B**, **D**) show the nuclear level in biopsy specimens from control, untreated, and treated patients. (**A**, **C**) Data are from a single experiment. (**B**, **D**) Mean ±SEM of five experiments. °°°$P<0.0001$ vs. control, ***$P<0.0001$ vs. untreated

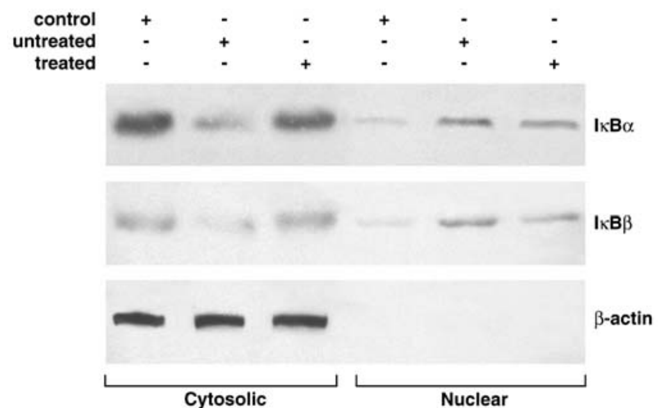

Fig. 3 Cytosolic and nuclear level of IκB subunits. Representative western blots show the cytosolic and nuclear level of IκBα and IκBβ in biopsy specimens from control, untreated, and treated patients. β-Actin expression is shown as a control. Data are from a single experiment and representative of five experiments

was determined by incubating nuclear extracts with specific antibodies against p50 or p65 subunits and observing the effects on the electrophoretic mobility of NF-κB DNA complexes. Addition of anti-p65 to the binding reaction caused the appearance of low mobility complex whereas addition of anti-p50 caused the appearance of the faster migrating complex. Concomitant addition of anti-p50 and anti-p65 to the binding reaction resulted in a marked reduction in the levels of NF-κB complexes, suggesting that NF-κB consists primarily of p50 and p65 dimers (Fig. 1C). The NF-κB activation was confirmed by immunofluorescence analysis performed on these specimens and a larger number of patients and controls. A higher expression of nuclear p65 was detectable in both crypt epithelial cells and in lamina propria mononuclear cells from untreated patients than in treated and controls (data not shown).

Nuclear level of p50 and p65 subunits

The level of p50 and p65 in nuclear extracts from biopsy specimens was examined by western blot analysis. Biopsy specimens from controls expressed a basal level of p50 and p65, whereas from untreated patients the levels of p50 and p65 were higher than in treated patients (Fig. 2).

Cytoplasmic and nuclear level of IκB proteins

Since NF-κB activation is controlled by inhibitory IκB proteins, we examined the presence of IκBα and IκBβ proteins in cytosolic and nuclear extracts from biopsy specimens of untreated, treated patients, and controls in an attempt to underlying mechanisms to sustained activation of NF-κB. In biopsy specimens from untreated patients IκBα and even more IκBβ disappeared from the cytosolic fraction whereas high levels of IκBα and lower levels of IκBβ were detectable in specimens from treated patients and controls. Significant amounts of IκBα and IκBβ were observed in the nuclear extracts from biopsy specimens of untreated patients, while lower amounts of nuclear IκBα and IκBβ were observed in specimens from treated patients. Basal levels of IκBα and IκBβ were present in the nuclear extracts from specimens of controls (Fig. 3).

iNOS and COX-2 protein expression

iNOS and COX-2 protein level in cytosolic extracts from biopsy specimens were determined by western blot analysis. As shown in Fig. 4, a significantly higher level of either iNOS and COX-2 protein expression was detected in biopsy specimens from untreated patients than in those from controls. In biopsy specimens from treated patients the level of either iNOS or COX-2 protein expression was significantly lower than in those from untreated patients.

Kinetic analysis of NF-κB activation in cultured biopsy specimens from untreated patients

To determine whether NF-κB activity is also sustained ex vivo we used an in vitro model of mucosal biopsies. Biopsy specimens from untreated patients were cultured with medium alone for 0, 2, 4, 6, 12, and 24 h before assessment of NF-κB/DNA binding activity. The results in Fig. 5A show that in nuclear extracts from specimens cultured for 0, 2, 4, and 6 h NF-κB activity was maintained at high levels, while that in those cultured for 12 and 24 h NF-κB activity was decreased. When NF-κB activity was sustained at high levels, both iNOS and COX-2 protein expression was also maintained at high

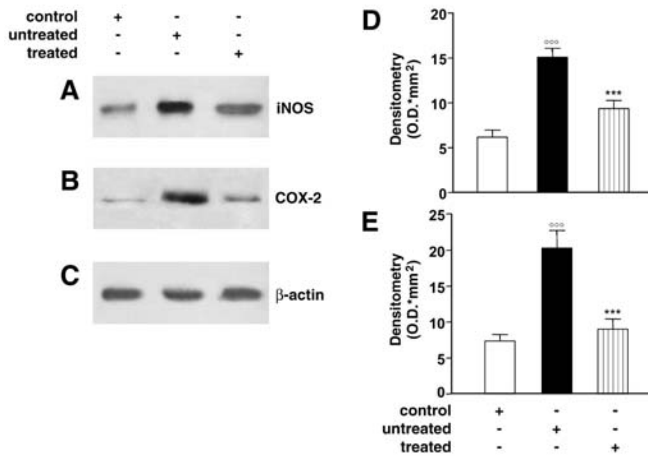

Fig. 4 Expression of iNOS and COX-2. Representative western blots of iNOS (**A**) and COX-2 (**B**) and densitometric analysis (**D**, **E**) show the protein expression in cytosolic extracts from biopsy specimens of control, untreated, and treated patients. (**C**) β-Actin expression is shown as a control. (**A–C**) Data are from a single experiment. (**D**, **E**) Mean ±SEM of five experiments. °°°$P<0.0001$ vs. control, ***$P<0.0001$ vs. untreated

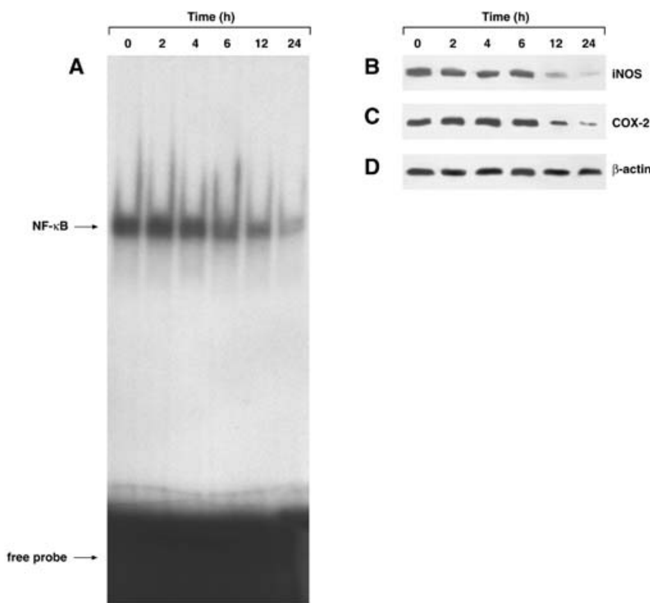

Fig. 5 Kinetic analysis of NF-κB activation in cultured biopsy specimens from untreated patients. (**A**) Representative electrophoretic mobility shift assay shows the NF-κB/DNA binding activity in nuclear extracts from biopsy specimens cultured with medium alone for 0, 2, 4, 6, 12, and 24 h. (**B**, **C**) Representative western blots of iNOS (**B**) and COX-2 (**C**) show the protein expression in cytosolic extracts from biopsy specimens cultured with medium alone for 0, 2, 4, 6, 12, and 24 h. (**D**) β-Actin expression is shown as a control. Correlation coefficients between the intensity of NF-κB/DNA binding activity and both iNOS and COX-2 protein expression bands, determined by densitometric analysis, were 0.99 ($P<0.0001$) and 0.98 ($P<0.0001$), respectively. (**A**) Data are from a single experiment and representative of seven experiments. (**B–D**) Data are from a single experiment and representative of four experiments

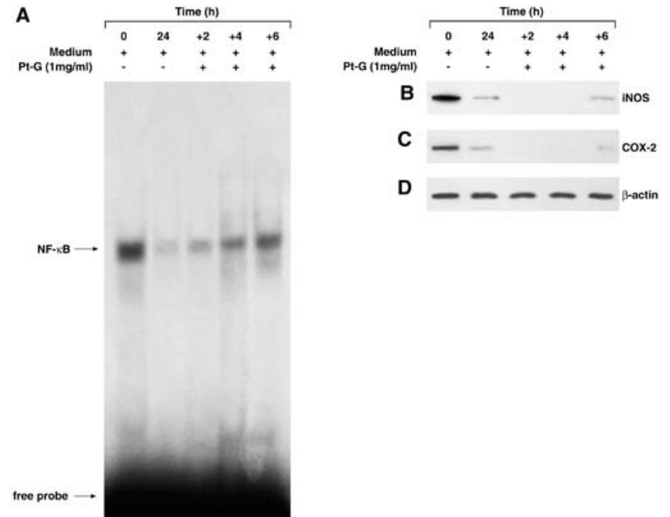

Fig. 6 Kinetic analysis of NF-κB activation in cultured biopsy specimens from untreated patients in the presence or absence of Pt-G. (**A**) Representative electrophoretic mobility shift assay shows the NF-κB/DNA binding activity in nuclear extracts from biopsy specimens cultured with medium alone for 0 and 24 h and then incubated with Pt-G (1 mg/ml) for 2, 4, and 6 h. (**B**, **C**) Representative western blots of iNOS (**B**) and COX-2 (**C**) show the protein expression in cytosolic extracts from biopsy specimens cultured with medium alone for 0 and 24 h and then incubated with Pt-G (1 mg/ml) for 2, 4, and 6 h. (**D**) β-Actin expression is shown as a control. (**A**) Data are from a single experiment and representative of three experiments. (**B–D**) Data are from a single experiment and representative of two experiments

levels. Conversely, reduced NF-κB activity was accompanied by a decrease in both iNOS and COX-2 protein expression (Fig. 5B, C, respectively). NF-κB/DNA binding activity and either iNOS and COX-2 protein expression were correlated ($r=0.99$, $P<0.0001$ and $r=0.98$, $P<0.0001$, respectively). In addition, to evaluate whether NF-κB activity decreased at 24 h recovered, the specimens were incubated with Pt-G (1 mg/ml) for 2, 4, and 6 h. As shown in Fig. 6A, NF-κB activity increased 6 h after addition of Pt-G whereas the levels of either iNOS and COX-2 protein expression were at the limit of detection (Fig. 6B, C, respectively). These results show that NF-κB activity is sustained in intestinal mucosa of patients with untreated CD even 6 h after removal from the causative environment, decreases at 12 and 24 h, and increases 6 h after the addition of Pt-G.

Discussion

NF-κB is a transcriptional regulator that mediates key immune and inflammatory response [12]. In this report we present evidence for the first time that NF-κB is constitutively active in intestinal mucosa of patients with untreated CD. We found that NF-κB/DNA binding activity is significantly greater in biopsy specimens from untreated patients than in those from treated patients, indicating that NF-κB activation occurs in this mucosal compart-

ment and declines on removal of gluten from diet. Levels of p50 and p65 subunits were higher in nuclear extracts from biopsy specimens of untreated patients than in those from treated patients. IκBα and IκBβ were degraded in the cytosol and present in the nucleus, suggesting that IκBβ plays a role in maintaining NF-κB/DNA binding activity in inflamed mucosa of patients with untreated CD. It has been reported that agents promote persistent NF-κB activity induce IκBα and IκBβ degradation [17, 18]. IκBβ is implicated in regulating the persistent NF-κB activation in inflammatory chronic diseases [19, 20]. It has been shown that following degradation of the initial pool of IκBβ, newly unphosphorylated synthesized IκBβ, can act as a chaperone of NF-κB blocking the inhibitory effect of IκBα in the nucleus and therefore maintain NF-κB activity even after IκBα resynthesis [21]. Furthermore, other studies have demonstrated that the dynamic state of degradation and resynthesis of IκBβ may result in the continuous production of hypophosphorylated IκBβ form that is unable to mask the nuclear localization signal of RelA, permitting NF-κB/IκBβ complexes to enter into the nucleus and bind DNA [12, 22].

In this study we demonstrate that IκBβ is present in the nuclear fraction. At present we were unable to determinate whether IκBβ is as part of NF-κB/DNA complex and in hypo- or unphosphorylated state. Nevertheless, we have found that NF-κB activation persists for up 6 h in cultured biopsy specimens from untreated patients and is lower at 12 and 24 h. In addition, NF-κB activation was correlated with either iNOS and COX-2 protein expression. Previous studies have shown that iNOS is expressed more in enterocytes and COX-2 in the cells of lamina propria [7, 9]. These enzymes catalyzing the synthesis of nitric oxide and proinflammatory prostaglandins, respectively, have been shown to be involved in disease induction and maintenance [5, 6, 7, 8]. Our finding that both iNOS and COX-2 expression is increased in biopsy specimens from untreated patients is in agreement with previous observations, although in other studies iNOS and COX-2 appear to play a protective role in intestinal injury [9, 23, 24, 25, 26]. Interestingly, our findings show that removal of the inflamed mucosa from the causative environment reduces the expression of both iNOS and COX-2, two molecular events downstream of NF-κB activation, and suggest that NF-κB activation is diminished in patients with a strict gluten-free diet. Moreover, we observed that NF-κB activity is decreased at 24 h and increased in cultured biopsy specimens 6 h after the addition of Pt-G.

Taken together our results show that NF-κB is indeed activated in intestinal mucosa of untreated CD patients and suggest a role for IκBβ in regulating the persistent activation of NF-κB in this disease. Therefore NF-κB might play a pivotal role in the perpetuation of inflammatory process in CD and even at early stage. NF-κB appears to be an important mediator of antigen-induced T cell activation and promotes Th1 subset development through the induction of NF-κB-dependent cytokines such as interferon-γ [27]. Secreted products of activated T cells are capable of maintaining the activation of nonimmune cells within the lesion, thereby perpetuating the chronic inflammatory process [28]. Gluten induces activation of mucosal Th1 T cells in patients with susceptibility of CD, thereby leading to local secretion of high levels of interferon-γ, which alone or together with other mediators activates macrophages and directly or indirectly damages enterocytes or alter their maturation [29]. Activated macrophages secrete cytokines, adhesion molecules, and enzymes whose gene expression is known to be transcriptionally regulated by NF-κB [28]. These mediators may contribute to a perpetuation of the inflammatory reaction [1, 5]. Thus our findings may be of clinical relevance because the sustained activation of NF-κB in intestinal mucosa of CD patients leads to prolonged induction of inflammatory gene expression and thereby perpetuates the chronic inflammatory process. In conclusion, the presence of activated NF-κB in human mucosal lesion in CD may yield new insights into the understanding of the pathogenesis of this disorder.

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