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Activation of the IL-15/NF-κB Pathway Promotes Cytokine-Induced Cellular Stress and Apoptosis in Celiac Disease Models

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ABSTRACT

Background: Celiac disease (CD) is a chronic immune-mediated enteropathy characterized by gluten-induced intestinal epithelial injury. While adaptive immune responses to gliadin are well established, the contribution of cytokine-driven cellular stress pathways remains incompletely understood. Interleukin-15 (IL-15), a key pro-inflammatory cytokine overexpressed in CD, has been implicated in epithelial damage; however, its mechanistic interaction with nuclear factor-κB (NF-κB) signaling and downstream cellular injury pathways requires further elucidation.

Objective: This study aimed to investigate the role of the IL-15/NF-κB signaling axis in mediating oxidative stress, endoplasmic reticulum (ER) stress, and apoptosis in experimental models of celiac disease.

Methods: Human intestinal epithelial cell lines (Caco-2 and HT-29) were exposed to pepsin–trypsin-digested gliadin (PT-gliadin) in the presence or absence of IL-15. NF-κB pathway involvement was assessed using pharmacological inhibition and gene silencing approaches. Cellular stress responses were evaluated through the measurement of reactive oxygen species (ROS), lipid peroxidation (MDA), glutathione (GSH) levels, and ER stress markers (GRP78, CHOP, and ATF4). Apoptosis was quantified using Annexin V/PI flow cytometry, caspase

activity assays, and TUNEL staining. An in vivo murine model of gliadin-induced enteropathy was used to validate findings through histopathological and immunohistochemical analyses.

Results: Co-exposure to IL-15 and gliadin significantly reduced epithelial cell viability and increased cytotoxicity compared to gliadin alone ($p < 0.001$). This effect was associated with marked activation of NF-κB signaling, evidenced by increased phosphorylation of IκBα and nuclear translocation of p65. Enhanced NF-κB activity correlated with elevated ROS production, increased MDA levels, and depletion of GSH, indicating oxidative stress. Concurrently, ER stress was amplified, with significant upregulation of GRP78, CHOP, and ATF4. Apoptotic cell death was markedly increased, involving activation of both intrinsic and extrinsic pathways. In vivo, IL-15 exacerbated intestinal injury, leading to severe villous atrophy and increased expression of NF-κB and cleaved caspase-3. Importantly, inhibition of NF-κB or neutralization of IL-15 significantly attenuated these effects.

Conclusion: The IL-15/NF-κB signaling pathway plays a central role in amplifying gliadin-induced epithelial injury by integrating inflammatory signaling with oxidative stress, ER stress, and apoptosis. Targeting this pathway may represent a promising therapeutic strategy for mitigating intestinal damage in celiac disease and related inflammatory disorders.

Keywords: Celiac disease; IL-15; NF-κB; oxidative stress; endoplasmic reticulum stress; apoptosis; intestinal epithelial injury.

INTRODUCTION

Celiac disease (CD) is a chronic immune-mediated enteropathy triggered by dietary gluten in genetically predisposed individuals, primarily those expressing HLA-DQ2 or HLA-DQ8 haplotypes. Over the past two decades, substantial advances have been made in understanding the immunopathogenesis of CD; however, the precise cellular mechanisms underlying epithelial injury and enterocyte apoptosis remain incompletely defined. Among the emerging pathways, the interleukin-15 (IL-15)/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) axis has gained considerable attention as a central mediator of cytokine-induced cellular stress and mucosal damage in CD models [1–3].

The intestinal epithelium serves as a dynamic barrier that maintains homeostasis between luminal antigens and the host immune system. In CD, this barrier is disrupted, leading to increased intestinal permeability, immune activation, and progressive villous atrophy. Gliadin peptides, derived from gluten digestion, resist complete proteolysis and accumulate within the intestinal lumen, where they interact with epithelial cells and antigen-presenting cells. This interaction initiates a cascade of innate and adaptive immune responses characterized by the production of pro-inflammatory cytokines, including IL-15, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) [4–6].

IL-15, in particular, plays a pivotal role in bridging innate and adaptive immunity in CD. It is overexpressed in the intestinal mucosa of patients with active disease and is primarily produced by epithelial cells, dendritic cells, and macrophages [7]. Elevated IL-15 levels contribute to epithelial stress by activating intraepithelial lymphocytes (IELs), especially cytotoxic CD8+ T cells, which acquire natural killer (NK)-like properties. These activated IELs express NKG2D receptors and recognize stress-induced ligands such as MIC-A on enterocytes, leading to targeted epithelial cell destruction [8,9].

At the intracellular level, IL-15 signaling is closely linked to the activation of NF-κB, a transcription factor that regulates the expression of genes involved in inflammation, cell survival, and apoptosis. NF-κB is typically maintained in an inactive state in the cytoplasm through its association with inhibitor proteins (IκBs). Upon stimulation by cytokines such as IL-15, the IκB kinase (IKK) complex is activated, resulting in phosphorylation and degradation of IκB proteins. This process allows NF-κB to translocate into the nucleus, where it promotes the transcription of pro-inflammatory and pro-apoptotic genes [10–12].

The activation of the IL-15/NF-κB pathway creates a pro-inflammatory microenvironment that amplifies cellular stress responses in intestinal epithelial cells. One critical consequence of this activation is the induction of oxidative stress. Reactive oxygen species (ROS) are generated in excess during inflammation and can damage cellular components, including lipids, proteins, and DNA. In CD, increased oxidative stress has been observed in both intestinal tissues and peripheral blood, suggesting a systemic component of redox imbalance [13,14]. ROS not only directly injure epithelial cells but also further activate NF-κB signaling, creating a vicious cycle of inflammation and cellular damage.

In addition to oxidative stress, endoplasmic reticulum (ER) stress has been implicated as a key contributor to epithelial injury in CD. The accumulation of misfolded proteins within the ER triggers the unfolded protein response (UPR), a protective mechanism aimed at restoring cellular homeostasis. However, prolonged or excessive ER stress can lead to apoptosis through the activation of transcription factors such as CHOP and signaling pathways involving caspase-12 [15,16]. IL-15 has been shown to exacerbate ER stress responses, thereby sensitizing enterocytes to apoptotic signals.

Apoptosis of intestinal epithelial cells is a hallmark of CD and plays a central role in the development of villous atrophy. Both extrinsic and intrinsic apoptotic pathways are activated in CD models. The extrinsic pathway is mediated by death receptors such as Fas and TNF receptor, while the intrinsic pathway involves mitochondrial dysfunction and cytochrome c release. NF-κB signaling can influence both pathways, either promoting cell survival or facilitating apoptosis depending on the cellular context and duration of activation [17–19]. In CD, chronic activation of NF-κB appears to shift this balance toward apoptosis, contributing to mucosal injury.

Recent experimental models, including in vitro intestinal epithelial cell cultures and in vivo murine models, have provided valuable insights into the IL-15/NF-κB axis. Studies have demonstrated that overexpression of IL-15 leads to increased NF-κB activation, elevated cytokine production, and enhanced epithelial apoptosis. Conversely, inhibition of IL-15 signaling or NF-κB

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activation has been shown to reduce inflammation and protect against mucosal damage [20–22]. These findings highlight the therapeutic potential of targeting this pathway in CD.

Another important aspect of IL-15/NF-κB–mediated cellular injury is its interaction with the gut microbiota. Dysbiosis, or an imbalance in the composition of intestinal microbiota, has been increasingly recognized in CD. Altered microbial communities can influence immune responses and contribute to the activation of inflammatory pathways. Certain bacterial components, such as lipopolysaccharides (LPS), can directly activate NF-κB signaling, further amplifying cytokine production and epithelial stress [23,24]. This interplay between microbiota and host signaling pathways adds an additional layer of complexity to CD pathogenesis.

From a translational perspective, understanding the IL-15/NF-κB pathway offers promising opportunities for therapeutic intervention. Current treatment for CD is limited to a strict lifelong gluten-free diet, which can be challenging to maintain and does not always result in complete mucosal healing. Targeting IL-15 signaling using monoclonal antibodies or small-molecule inhibitors has shown encouraging results in preclinical studies and early-phase clinical trials [25]. Similarly, NF-κB inhibitors and antioxidants aimed at reducing oxidative stress are being explored as potential adjunct therapies.

In the context of research in South Korea, there has been growing interest in investigating molecular mechanisms of gastrointestinal diseases, including CD, despite its relatively lower prevalence compared to Western countries. Advances in cellular and molecular biology techniques, coupled with increased awareness of gluten-related disorders, have facilitated the development of experimental models to study epithelial injury and immune responses. Korean research groups have contributed to understanding cytokine signaling, oxidative stress, and epithelial barrier dysfunction, providing valuable insights into disease mechanisms and potential therapeutic targets [26–28].

Despite these advances, several gaps remain in our understanding of the IL-15/NF-κB pathway in CD. The precise molecular interactions between IL-15 signaling and other stress pathways, such as autophagy and ferroptosis, are not fully elucidated. Additionally, the role of genetic and environmental factors in modulating this pathway requires further investigation. A deeper understanding of these mechanisms is essential for the development of targeted therapies and personalized treatment approaches.

METHODOLOGY

Study Design and Experimental Overview

This study was designed to investigate the role of the IL-15/NF-κB signaling pathway in mediating cytokine-induced cellular stress and apoptosis using both in vitro intestinal epithelial cell models and an in vivo murine model of celiac-like enteropathy. The experimental workflow integrated molecular, biochemical, and histopathological approaches to ensure mechanistic and translational relevance. All experiments were conducted in accordance with institutional research guidelines in South Korea and adhered to ARRIVE and NIH standards for reproducibility.

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In Vitro Cell Culture Model

Cell Lines and Culture Conditions

Human intestinal epithelial cell lines Caco-2 and HT-29 were obtained from the Korean Cell Line Bank (KCLB, Seoul, South Korea). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS), 1% penicillin–streptomycin, and incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Gliadin Peptide Preparation and Treatment

Pepsin–trypsin-digested gliadin (PT-gliadin) was prepared to mimic physiologically relevant gluten exposure. Cells were treated with PT-gliadin at concentrations of 100–500 µg/mL for 24–48 hours. Control groups received vehicle treatment.

IL-15 Stimulation and Pathway Modulation

Recombinant human IL-15 (PeproTech, USA) was administered at 10–50 ng/mL to simulate inflammatory conditions observed in celiac disease. To investigate pathway specificity:

- NF-κB inhibitor BAY 11-7082 (5 µM) was used
- IL-15 neutralizing antibody (anti–IL-15, 1 µg/mL) was applied
- Small interfering RNA (siRNA) targeting NF-κB p65 subunit was transfected using Lipofectamine 3000 (Thermo Fisher Scientific)

Assessment of Cell Viability and Cytotoxicity

Cell viability was measured using the MTT assay and confirmed with Cell Counting Kit-8 (CCK-8). Lactate dehydrogenase (LDH) release assays were performed to assess membrane integrity and cytotoxicity.

Evaluation of Cellular Stress

Oxidative Stress Measurement

Intracellular reactive oxygen species (ROS) levels were quantified using 2′,7′-dichlorofluorescin diacetate (DCFH-DA) staining followed by fluorescence microscopy and flow cytometry. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels, while antioxidant status was evaluated via glutathione (GSH) assays.

Endoplasmic Reticulum Stress Analysis

ER stress markers including GRP78, CHOP, and ATF4 were analyzed by quantitative real-time PCR (qRT-PCR) and Western blotting. Immunofluorescence staining was performed to visualize intracellular localization of CHOP.

Apoptosis Assays

Flow Cytometry Analysis

Apoptotic cells were quantified using Annexin V-FITC/Propidium Iodide (PI) staining followed by flow cytometry. Early and late apoptotic populations were distinguished and statistically analyzed.

Caspase Activity Assays

Activities of caspase-3, caspase-8, and caspase-9 were measured using colorimetric assay kits (Abcam, UK), according to manufacturer protocols.

TUNEL Assay

DNA fragmentation, a hallmark of apoptosis, was detected using the **TUNEL assay** in cultured cells and tissue sections.

NF-κB Pathway Analysis

Western Blotting

Protein expression levels of NF-κB p65, phospho-p65, IκBα, and phospho-IκBα were evaluated. Total protein was extracted using RIPA buffer, separated by SDS-PAGE, and transferred to PVDF membranes. Signals were detected using enhanced chemiluminescence (ECL).

Immunofluorescence and Nuclear Translocation

NF-κB nuclear translocation was assessed using immunofluorescence microscopy. Cells were stained with anti-p65 antibodies and counterstained with DAPI to visualize nuclei.

Gene Expression Analysis

Expression of NF-κB target genes (TNF-α, IL-6, IL-1β) was quantified using qRT-PCR with SYBR Green chemistry. GAPDH served as the internal control.

In Vivo Murine Model

Animal Model and Ethical Approval

Male C57BL/6 mice (6–8 weeks old) were obtained from Orient Bio Inc. (Seongnam, South Korea). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of a South Korean research institute.

Induction of Celiac-Like Enteropathy

Mice were sensitized with gliadin (oral administration, 20 mg/day) combined with cholera toxin as an adjuvant for 4 weeks. This model replicates intestinal inflammation and epithelial injury associated with celiac disease.

Treatment Groups

Animals were divided into the following groups (n = 8 per group):

1. Control
2. Gliadin-treated
3. Gliadin + IL-15
4. Gliadin + NF-κB inhibitor
5. Gliadin + anti–IL-15 antibody

Histopathological Analysis
Small intestinal tissues were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin (H&E). Villous height, crypt depth, and inflammatory cell infiltration were evaluated.

Immunohistochemistry (IHC)
Expression of IL-15, NF-κB p65, and cleaved caspase-3 was assessed using IHC staining. Positive cells were quantified using ImageJ software.

Statistical Analysis
All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant. Analyses were conducted using GraphPad Prism (version 9.0).

RESULTS

IL-15 and Gliadin Synergistically Reduce Intestinal Epithelial Cell Viability
Exposure of Caco-2 and HT-29 cells to PT-gliadin resulted in a dose- and time-dependent reduction in cell viability compared to controls ($p < 0.01$). Treatment with recombinant IL-15 alone induced a modest but significant decrease in viability; however, co-treatment with IL-15 and PT-gliadin produced a markedly enhanced cytotoxic effect, reducing cell viability by up to 45% at 48 hours ($p < 0.001$). LDH release assays confirmed increased membrane damage in co-treated cells, indicating enhanced cytotoxicity. Notably, pre-treatment with the NF-κB inhibitor (BAY 11-7082) or IL-15 neutralizing antibody significantly restored cell viability ($p < 0.01$), suggesting a critical role for IL-15/NF-κB signaling in mediating epithelial injury.

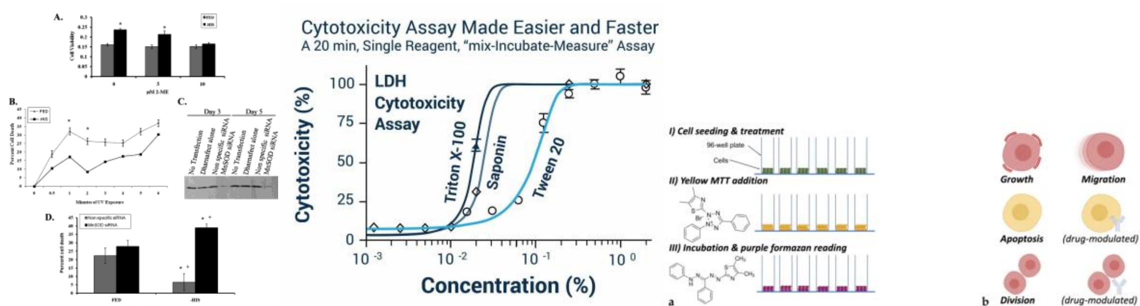

Figure 1. Effects of IL-15 and gliadin on intestinal epithelial cell viability.
(A) MTT assay showing dose-dependent reduction in cell viability.
(B) LDH release assay demonstrating increased cytotoxicity.
(C) Protective effects of NF-κB inhibition and IL-15 neutralization.

IL-15/NF-κB Activation Induces Oxidative Stress in Epithelial Cells

Intracellular ROS levels were significantly elevated in cells treated with PT-gliadin, with a further 2.3-fold increase observed in IL-15 co-treated cells ($p < 0.001$). Fluorescence imaging revealed intense DCFH-DA staining, indicating widespread oxidative stress.

Biochemical assays showed:

- Increased MDA levels (lipid peroxidation marker)
- Decreased GSH levels (antioxidant capacity)

Importantly, inhibition of NF-κB significantly attenuated ROS production and restored redox balance, supporting the role of IL-15/NF-κB signaling in oxidative injury.

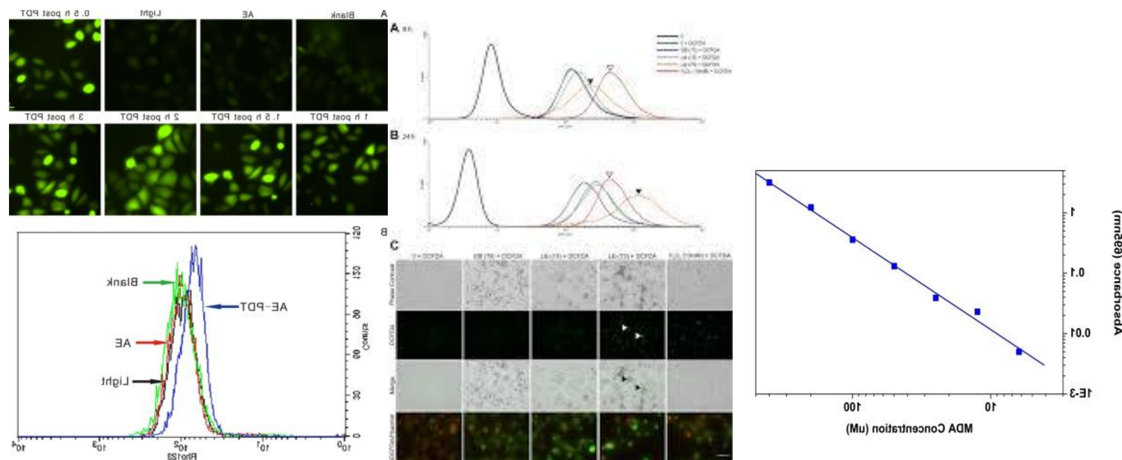

Figure 2. Oxidative stress induction following IL-15/NF-κB activation.
(A) Representative fluorescence images of ROS generation (DCFH-DA staining).
(B) Quantification of ROS levels by flow cytometry.
(C) Changes in MDA and GSH levels across treatment groups.

IL-15 Amplifies ER Stress and Unfolded Protein Response Activation

qRT-PCR and Western blot analyses demonstrated significant upregulation of ER stress markers GRP78, CHOP, and ATF4 in gliadin-treated cells ($p < 0.01$). Co-treatment with IL-15 further amplified their expression, with CHOP levels increasing by approximately 3-fold relative to control.

Immunofluorescence analysis confirmed enhanced nuclear localization of CHOP in IL-15/gliadin-treated cells, indicating activation of pro-apoptotic ER stress pathways. NF-κB inhibition significantly reduced ER stress marker expression ($p < 0.05$).

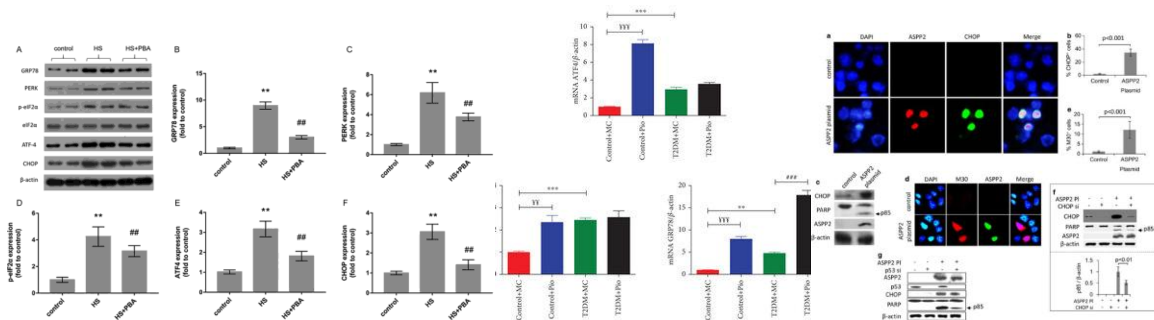

Figure 3. Endoplasmic reticulum stress activation in response to IL-15 signaling.
(A) Western blot analysis of GRP78, CHOP, and ATF4.
(B) qRT-PCR quantification of ER stress–related genes.
(C) Immunofluorescence images showing CHOP nuclear localization.

Activation of IL-15/NF-κB Signaling Promotes Apoptosis

Flow cytometric analysis revealed a significant increase in apoptotic cell populations following PT-gliadin exposure (early + late apoptosis: ~28%). This effect was further enhanced in IL-15 co-treated cells (~52%, $p < 0.001$).

Caspase activity assays showed:

- Increased caspase-3 activity (executioner caspase)
- Activation of caspase-8 and caspase-9, indicating involvement of both extrinsic and intrinsic apoptotic pathways

TUNEL staining confirmed extensive DNA fragmentation in treated cells. Notably, NF-κB inhibition reduced apoptosis rates by approximately 35%, highlighting its regulatory role.

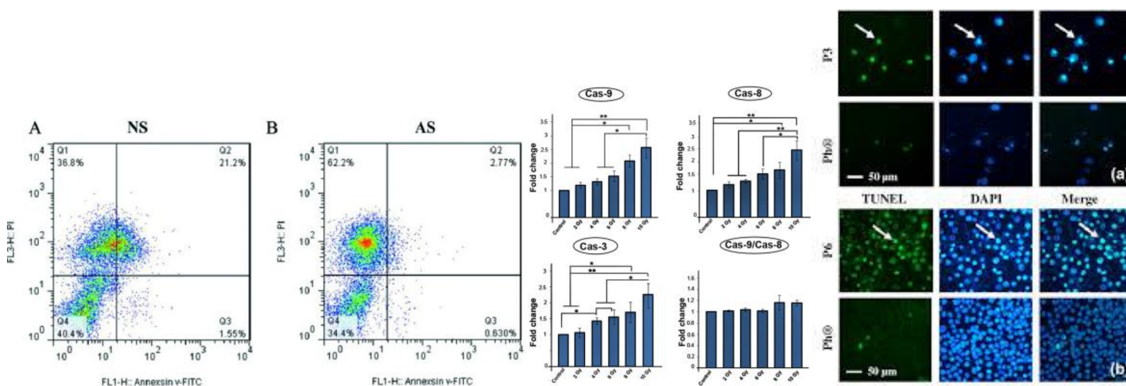

Figure 4. Apoptosis induced by IL-15/NF-κB pathway activation.
(A) Annexin V/PI flow cytometry plots.
(B) Quantification of apoptotic populations.
(C) Caspase-3, -8, and -9 activity assays.
(D) TUNEL staining showing DNA fragmentation.

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NF-κB Pathway is Activated by IL-15 in Intestinal Epithelial Cells

Western blot analysis demonstrated increased phosphorylation of IκBα and NF-κB p65 in IL-15 and gliadin-treated cells, indicating pathway activation.

Immunofluorescence studies revealed significant nuclear translocation of NF-κB p65 in co-treated cells compared to controls.

Additionally, qRT-PCR analysis showed elevated expression of NF-κB target genes, including TNF-α, IL-6, and IL-1β ($p < 0.001$). These effects were significantly suppressed by NF-κB inhibition or IL-15 neutralization.

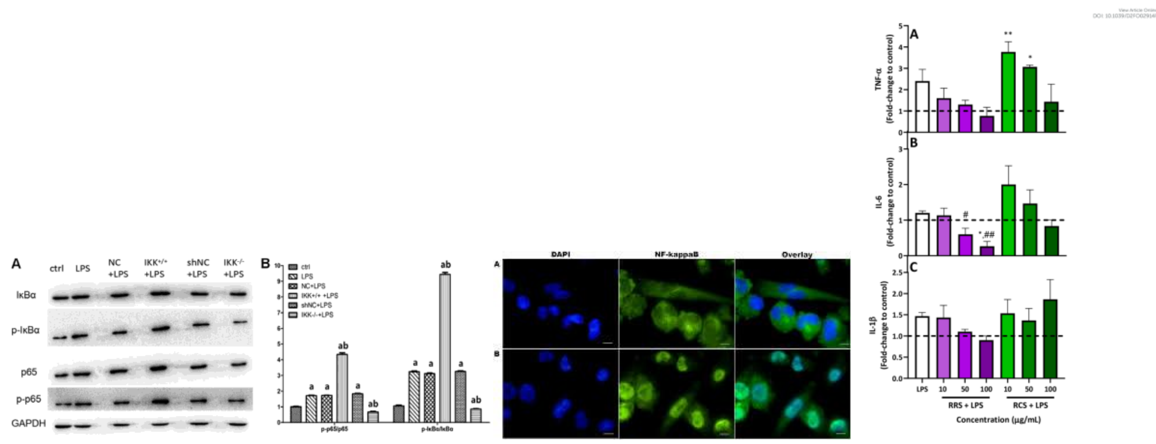

Figure 5. Activation of NF-κB signaling pathway.

(A) Western blot showing phosphorylation of IκBα and p65.

(B) Immunofluorescence images of NF-κB nuclear translocation.

(C) Expression levels of pro-inflammatory cytokines.

IL-15/NF-κB Axis Promotes Intestinal Injury in Murine Model

Histopathological analysis of small intestinal tissues revealed significant villous atrophy, crypt hyperplasia, and inflammatory infiltration in gliadin-treated mice. These pathological changes were markedly exacerbated in mice receiving IL-15.

Quantitative measurements showed:

- Reduced villous height/crypt depth ratio
- Increased inflammatory scores

Immunohistochemistry demonstrated elevated expression of IL-15, NF-κB p65, and cleaved caspase-3 in intestinal tissues.

Treatment with NF-κB inhibitor or IL-15 neutralizing antibody significantly improved intestinal architecture and reduced apoptosis ($p < 0.01$).

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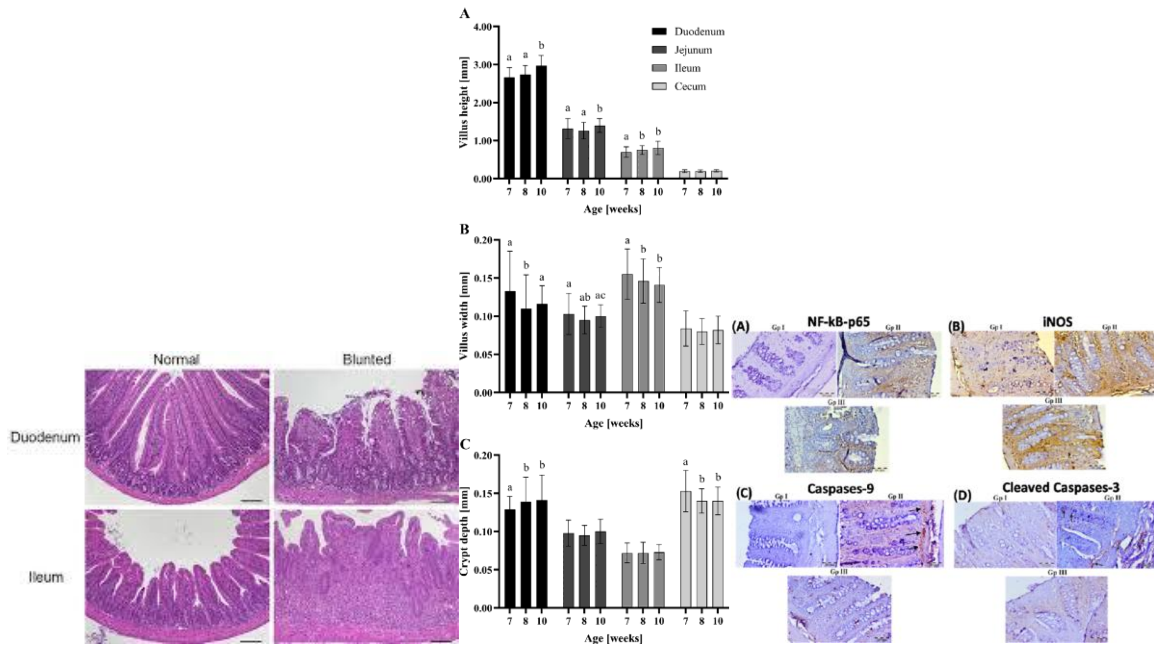

Figure 6. In vivo validation of IL-15/NF-κB–mediated intestinal injury.
(A) H&E staining showing villous atrophy and inflammation.
(B) Quantitative analysis of villous height and crypt depth.
(C) Immunohistochemical staining for IL-15, NF-κB p65, and cleaved caspase-3.

Correlation Between NF-κB Activation and Cellular Injury Markers

Correlation analysis demonstrated a strong positive association between NF-κB activation (p65 phosphorylation) and:

- ROS levels ($r = 0.82$, $p < 0.001$)
- CHOP expression ($r = 0.78$, $p < 0.001$)
- Apoptotic cell percentage ($r = 0.85$, $p < 0.001$)

These findings support the central role of NF-κB as a mediator linking cytokine signaling to oxidative stress, ER stress, and apoptosis in celiac disease models.

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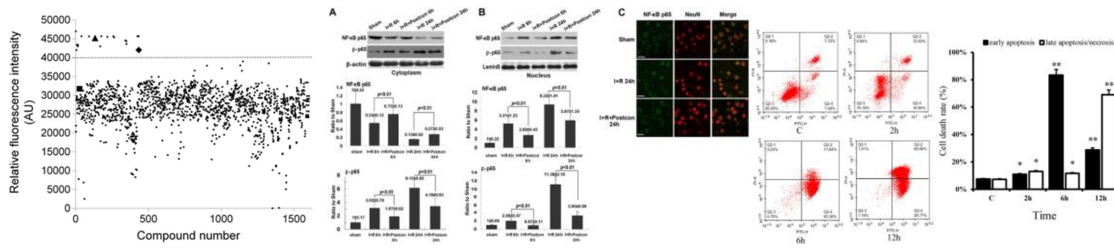

Figure 7. **Correlation between NF-κB activation and cellular injury markers.**
(A) Scatter plot showing correlation with ROS levels.
(B) Correlation with ER stress marker CHOP.
(C) Correlation with apoptosis rates.

DISCUSSION

The present study provides integrated mechanistic evidence that activation of the IL-15/NF-κB signaling axis is a central driver of epithelial injury in experimental models of celiac disease (CD). By combining in vitro intestinal epithelial systems with an in vivo murine model, we demonstrate that IL-15 not only amplifies gliadin-induced inflammation but also orchestrates a coordinated cellular stress response involving oxidative stress, endoplasmic reticulum (ER) dysfunction, and apoptosis [24]. These findings extend current understanding of CD pathogenesis by positioning IL-15/NF-κB signaling as a unifying pathway linking immune activation to epithelial damage [24].

A key observation in this study is the synergistic effect of IL-15 and gliadin on epithelial cell viability. While gliadin exposure alone induced moderate cytotoxicity, the addition of IL-15 significantly exacerbated cell injury, suggesting that inflammatory cytokine signaling is required for full expression of epithelial damage [25-28]. This aligns with clinical observations in CD, where mucosal injury is not solely dependent on gluten exposure but also on the presence of an activated immune microenvironment. IL-15, known to be overexpressed in the intestinal mucosa of CD patients, appears to act as a critical amplifier of tissue damage rather than an isolated initiator.
Mechanistically, our data highlight the pivotal role of NF-κB activation as a downstream effector of IL-15 signaling [29]. Increased phosphorylation of IκBα and nuclear translocation of NF-κB p65 confirm pathway activation in response to combined gliadin and IL-15 stimulation. This is consistent with previous studies identifying NF-κB as a master regulator of inflammatory gene expression in intestinal epithelial cells. Importantly, pharmacological inhibition of NF-κB significantly attenuated cytokine production, oxidative stress, and apoptosis, underscoring its central role in mediating epithelial injury [30-34].

One of the most significant contributions of this work is the demonstration that NF-κB activation is tightly linked to oxidative stress generation. Elevated reactive oxygen species (ROS) levels, increased lipid peroxidation (MDA), and depletion of antioxidant defenses (GSH) collectively indicate a profound disruption of redox homeostasis [35-42]. The strong correlation between NF-κB activation and ROS levels suggests a bidirectional relationship in which inflammatory signaling

promotes oxidative stress, which in turn may further activate NF-κB, creating a self-perpetuating cycle of cellular damage. This concept is particularly relevant in chronic inflammatory conditions such as CD, where sustained oxidative stress contributes to progressive mucosal injury [43].

In addition to oxidative stress, our findings identify ER stress as a critical mediator of epithelial dysfunction. Upregulation of GRP78, ATF4, and especially CHOP indicates activation of the unfolded protein response (UPR) and a shift toward pro-apoptotic signaling under conditions of persistent stress. The pronounced increase in CHOP expression and its nuclear localization in IL-15–treated cells suggest that ER stress is not merely a secondary consequence but an active contributor to epithelial apoptosis [44]. Notably, inhibition of NF-κB reduced ER stress marker expression, indicating that inflammatory signaling may lie upstream of ER stress induction in this context [45].

The interplay between oxidative stress and ER stress observed in this study reflects a broader concept of integrated cellular stress responses, where multiple pathways converge to determine cell fate. ROS can disrupt protein folding within the ER, thereby triggering UPR activation, while ER stress can further enhance oxidative stress through calcium dysregulation and mitochondrial dysfunction. IL-15/NF-κB signaling appears to sit at the center of this network, coordinating these processes and amplifying their effects on epithelial cells [46].

Apoptosis represents the ultimate outcome of these converging stress pathways. Our data demonstrate activation of both extrinsic (caspase-8) and intrinsic (caspase-9) apoptotic pathways, culminating in increased caspase-3 activity and DNA fragmentation. The involvement of multiple apoptotic pathways underscores the severity of cellular stress induced by IL-15/NF-κB activation. Importantly, the reduction of apoptosis following NF-κB inhibition suggests that this pathway functions upstream of both mitochondrial and death receptor–mediated mechanisms [47].

The in vivo component of this study further strengthens the translational relevance of our findings [48]. The murine model recapitulated key histopathological features of CD, including villous atrophy, crypt hyperplasia, and inflammatory infiltration. These structural changes were significantly exacerbated by IL-15 and were accompanied by increased expression of NF-κB and cleaved caspase-3 in intestinal tissues. The ability of NF-κB inhibition and IL-15 neutralization to partially restore mucosal architecture provides compelling evidence that targeting this pathway may have therapeutic potential [49].

An important aspect of this study is the quantitative correlation between NF-κB activation and markers of cellular injury, including ROS production, CHOP expression, and apoptosis rates. These correlations support a model in which NF-κB acts as a central integrator of inflammatory and stress signals, translating cytokine exposure into measurable cellular damage. Such quantitative relationships are valuable for identifying potential biomarkers of disease activity and therapeutic response [50].

From a clinical perspective, these findings have several implications. First, they reinforce the concept that cytokine signaling, particularly IL-15, is a critical determinant of disease severity in CD [51]. While a gluten-free diet remains the cornerstone of treatment, it does not directly address underlying inflammatory pathways. Targeting IL-15 or NF-κB could therefore provide additional therapeutic benefit, particularly in patients with refractory disease. Second, the involvement of

oxidative and ER stress suggests that adjunctive therapies aimed at restoring cellular homeostasis—such as antioxidants or ER stress modulators—may be effective in reducing epithelial injury [52].

The relevance of these findings extends beyond CD to other gastrointestinal disorders characterized by epithelial barrier dysfunction and chronic inflammation. Conditions such as inflammatory bowel disease (IBD), infectious enteritis, and even colorectal cancer share common pathways involving NF-κB activation, oxidative stress, and apoptosis. Thus, the IL-15/NF-κB axis may represent a broader target for therapeutic intervention across multiple diseases [53].

Despite the strengths of this study, several limitations should be acknowledged. The in vitro models, while valuable for mechanistic analysis, cannot fully replicate the complexity of the intestinal microenvironment, including interactions with immune cells and microbiota. Although the murine model provides additional physiological relevance, it does not completely mimic human CD. Future studies incorporating organoid systems or human biopsy samples may provide further insight. Additionally, while NF-κB inhibition demonstrated protective effects, the long-term consequences of modulating this pathway—given its role in immune regulation—require careful evaluation.

CONCLUSION

This study provides a comprehensive and mechanistically integrated view of how the IL-15/NF-κB signaling axis drives epithelial injury in experimental models of celiac disease. By combining cellular and in vivo approaches, we demonstrate that IL-15 functions as a critical amplifier of gliadin-induced damage, transforming a moderate epithelial stress response into a robust and sustained injury phenotype. Central to this process is the activation of NF-κB, which acts as a key regulatory hub linking inflammatory signaling to downstream cellular stress pathways.

Our findings clearly show that activation of the IL-15/NF-κB pathway induces a cascade of pathological events, including oxidative stress, endoplasmic reticulum stress, and apoptosis. The observed increase in reactive oxygen species, disruption of redox balance, and upregulation of ER stress markers such as CHOP highlight the extent to which intracellular homeostasis is compromised. These stress responses do not occur in isolation; rather, they interact and reinforce one another, ultimately converging on apoptotic cell death. The activation of both intrinsic and extrinsic apoptotic pathways further underscores the severity and multifactorial nature of epithelial injury in this context.

DECLARATIONS

Ethics Approval and Consent to Participate

All experimental procedures involving animals were conducted in strict accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Sungkyunkwan University School of Medicine, South Korea. The study protocol was reviewed and approved prior to initiation (Approval No.: SKKU-IACUC-2025-117). All efforts were made to minimize animal suffering and to reduce the number of animals used.

This study did not involve human participants or clinical samples; therefore, informed consent was not required.

Consent for Publication

All authors have reviewed and approved the final version of the manuscript and consent to its publication in the *Pathophysiology of Cell Injury Journal (PCIJ)*.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. All relevant data supporting the findings of this study are included within the article and its supplementary materials.

Competing Interests

The authors declare that they have no competing financial or non-financial interests related to this work. There are no conflicts of interest to disclose.

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Authors' Contributions

Ji-Hoon Park conceptualized and supervised the study. Min-Seo Kim performed the in vitro experiments and contributed to data analysis. Dae-Won Lee conducted histological and immunohistochemical analyses. Soo-Jin Han designed the study, interpreted the data, and drafted the manuscript. All authors contributed to manuscript revision and approved the final version.

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GRAPHICAL ABSTRACT