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CD40L-CD40-Mediated Crosstalk between Eosinophils and Mast Cells Exacerbates Nasal Epithelial Barrier Dysfunction and Inflammatory Injury in Allergic Rhinitis

Jie Deng, Hongyi Liu, Yiran Zang, Zhihan Wei, Xue Yue, and Zhanguo Jin

Department of Otorhinolaryngology Head and Neck Surgery, Air Force Medical Center, PLA, Beijing, China

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ABSTRACT

The research intended to elucidate the synergistic effects of eosinophils (Eos) and mast cells (MCs) on human nasal epithelial cells (HNEpCs) in the context of allergic rhinitis (AR), focusing on inflammation, tight junction protein expression, and DNA damage.

Cell proliferation capacity was measured using the Cell Counting Kit-8 (CCK-8) method, apoptosis was examined via the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, and inflammatory cytokine levels were assayed via enzyme-linked immunosorbent assay. Western blotting evaluated the protein abundance of tight junction proteins (ZO-1, Occludin) and CD40L/CD40. Immunofluorescence was used to detect phosphorylated histone H2AX (γH2AX) (DNA damage), as well as subcellular ZO-1/Occludin distribution. Co-immunoprecipitation (Co-IP) was used to analyze the CD40L-CD40 interaction between Eos and MCs.

Eos and MCs significantly reduced HNEpC viability and enhanced apoptosis, with the most pronounced effects in the AR+Eos+MC group. Inflammatory cytokine levels were markedly elevated in the Eos+MC and AR+Eos+MC groups, with the highest concentrations observed in the AR+Eos+MC group. Western blot and immunofluorescence analyses showed decreased expression of ZO-1 and Occludin in treatment groups compared to Control, along with a shift in their localization from the cell membrane to the cytoplasm. γH2AX expression, indicating DNA damage, was significantly elevated, with the highest levels observed in the AR+Eos+MC group. Co-IP analysis confirmed enhanced CD40L-CD40 interaction involving Eos and MCs within the Eos+MC and AR+Eos+MC groups.

Eosinophils and MCs synergistically promote inflammation, disrupt the nasal epithelial barrier, and exacerbate DNA damage. The CD40L-CD40 pathway serves an essential function in their interaction, providing a potential therapeutic target for AR.

Keywords: Allergic rhinitis; Eosinophils; CD40 ligand; DNA damage; Inflammation; Mast cells

Corresponding Author: Zhanguo Jin, MD;
Department of Otorhinolaryngology Head and Neck Surgery, Air Force Medical Center, PLA, Beijing, China. Tel: (+86 137) 1761 5123, Fax: (+86 137) 1761 5123, Email: ccjzg@126.com

INTRODUCTION

Allergic rhinitis (AR) represents a globally common long-term immune-mediated condition, characterized by nasal congestion, rhinorrhea, sneezing, and nasal

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itching. It significantly affects patients' quality of life and increases asthma risk and other allergic diseases.[1] The pathogenesis of AR involves complex immune responses associated with aberrant immune cell activation and persistent secretion of inflammatory mediators.[2] Among these, eosinophils and mast cells serve as pivotal regulators of the inflammatory response in AR.[3] Eosinophils accumulate at inflammation sites and exacerbate inflammation via the production of cytokines like interleukin (IL)-4 and IL-5, together with tumor necrosis factor α (TNF-α), while also inducing epithelial cell damage.[4] Mast cells (MCs), on the other hand, release histamine, chemokines, and inflammatory mediators, increasing microvascular permeability and resulting in nasal mucosal congestion as well as edema.[5] The synergistic interaction between these 2 cell types may be fundamentally involved in the persistence of inflammation in AR. Nevertheless, the precise molecular basis underlying their effects on epithelial barrier integrity remains unclear.

The nasal epithelial barrier plays a critical role in maintaining local immune homeostasis and preventing the invasion of external allergens. Its integrity largely depends on the proper expression of tight junction proteins, such as zonula occludens (ZO)-1 and Occludin.[6,7] Disruption of epithelial tight junctions has been documented in AR patients, correlating with increased allergen permeability and exaggerated immune activation.[6,8] These cellular and molecular alterations, particularly epithelial barrier disruption and the release of proinflammatory cytokines, are closely linked to the hallmark symptoms of AR such as nasal obstruction, rhinorrhea, sneezing, and itching, and may also contribute to disease persistence and the heightened risk of asthma comorbidity. However, the cellular and molecular mechanisms leading to epithelial barrier breakdown, particularly the direct involvement of eosinophils and MCs, remain incompletely understood.

In addition to structural impairment, chronic allergic inflammation is known to induce oxidative stress and DNA damage, further compromising epithelial viability and promoting apoptosis.[9] Yet, whether eosinophils and MCs synergistically contribute to epithelial DNA damage in the AR context is largely unknown. Emerging studies highlight the CD40L-CD40 signaling pathway as critical for immune cell interactions, influencing cytokine release, survival, and activation of various effector cells.[10-12] While CD40L-CD40 expression has been identified in both eosinophils and MCs, their potential interaction in modulating epithelial barrier function in AR has not been elucidated.

In this research, we employed a coculture system in vitro to investigate the interaction between Eos and MCs and their effects on nasal epithelial cells (HNEpCs). This research sought to fill a critical gap in defining the cooperative inflammatory mechanisms in AR and identify CD40L-CD40 as a potential immunotherapeutic target. In contrast to previous studies mainly emphasizing mediator release, this study highlights CD40L-CD40-mediated crosstalk between eosinophils and MCs as a novel driver of epithelial barrier dysfunction and DNA damage (Figure 1).

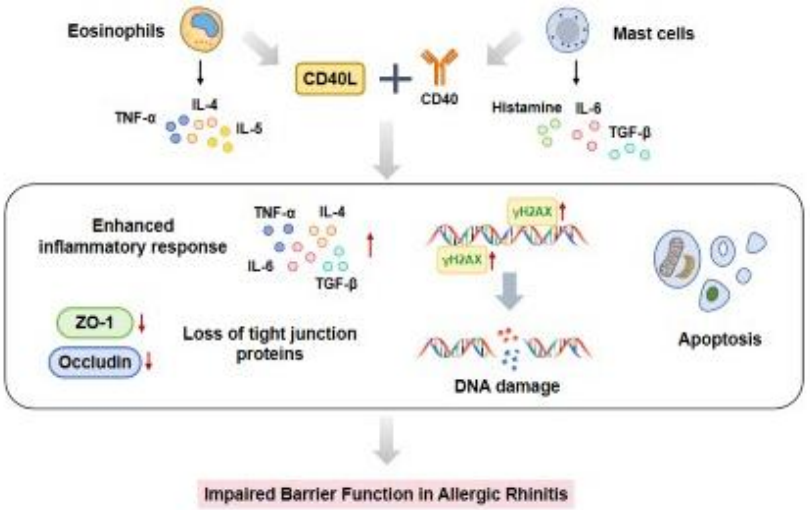


Figure 1. Schematic diagram of the proposed hypothesis: CD40L-CD40–mediated eosinophil–mast cell crosstalk in allergic rhinitis.

MATERIALS AND METHODS

Cell Culture and Experimental Grouping

To investigate the synergistic effects of eosinophils and MCs on nasal epithelial inflammation and barrier function, an in vitro co-culture model was established using three human-derived cell types. HNEpCs were purchased from PromoCell (#C-12620, Germany) and grown in PromoCell's airway epithelial culture medium under typical humidified conditions (37 °C, 5% CO_2). The HMC-1 human mast cell line was purchased from DSMZ (#ACC-283, Germany) and maintained in Iscove's Modified Dulbecco's Medium (IMDM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin-streptomycin. Cryopreserved human peripheral blood eosinophils were purchased from STEMCELL Technologies (#200-0385, Canada). Upon thawing, cells were immediately washed, counted, and reconstituted in X-VIVO 15 serum-free formulation (Lonza, Switzerland) for subsequent coculture. According to the manufacturer, these cells were isolated from healthy donors and exhibited >95% purity and high viability post-thaw. No additional enrichment procedures were performed.

Five experimental groups were established: (1) Control: HNEpCs cultured alone; (2) Eos: HNEpCs cocultured with eosinophils; (3) MC: HNEpCs cocultured with HMC-1 cells; (4) Eos+MC: HNEpCs cocultured with both eosinophils and HMC-1 cells; and (5) AR+Eos+MC group: HNEpCs pretreated with 10 ng/mL each, of recombinant human IL-4 and IL-13 (PeproTech, USA) for 24 hours, followed by coculture with eosinophils and HMC-1 cells to simulate the inflammatory microenvironment of AR.

CCK-8 Assay

HNEpCs were plated into 96-well plates (5×10^4 cells/well) and cultured in line with the experimental grouping. Following 24 hours of coculture, 10 μL CCK-8 solution (#C0041, Beyotime, China) was introduced into each well, then incubated at 37 °C with 5% CO_2 for 2 more hours. Optical density (OD) at 450 nm was determined via a microplate reader to assess metabolic activity. A blank containing only culture medium and CCK-8 reagent served as background absorbance correction. Each experimental group was repeated 3 times, and the mean OD values were utilized to compute relative viability.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) Assay

HNEpCs were distributed onto sterile coverslips placed inside 24-well plates and cultured according to the experimental grouping. After 24 hours, cells were fixed using 4% paraformaldehyde (10 minutes), rinsed thrice with phosphate-buffered saline (PBS), and subjected to 0.1% Triton X-100 permeabilization to allow TUNEL reagents to enter the nuclei. According to the manufacturer's instructions, terminal deoxynucleotidyl transferase (TdT) was mixed with fluorescein isothiocyanate (FITC)-labeled dUTP and applied to the samples, followed by incubation at 37 °C in the dark for 1 hour. After staining, the samples were washed 3 times with PBS and observed under a fluorescence microscope (FITC channel, excitation wavelength 488 nm). TUNEL-positive cells were quantified across at least 5 randomly picked visual fields, and their percentage compared to the entire cell population was calculated to determine the apoptosis rate. A negative control group (without TdT enzyme) was included to eliminate nonspecific staining. All experiments were repeated 3 times.

Inflammatory Cytokine Detection

HNEpCs were cultured for 24 hours according to the experimental grouping, and the culture supernatant was collected. The samples underwent centrifugation (3000 rpm, 10 minutes) to eliminate cell debris, and the resulting supernatant was used for enzyme-linked immunosorbent assay (ELISA). The cytokines measured included IL-4 (Cat# DY204), IL-6 (Cat# DY206), TNF-α (Cat# DY210), along with transforming growth factor-β TGF-β (Cat# DY240), each assessed using the corresponding ELISA kit (R&D Systems, USA). All detection steps were carried out in accordance with the manufacturer's protocols, including the preparation of standard curves, sample and standard additions, 37 °C incubation, washing, and enzyme-labeled antibody incubation. After the colorimetric reaction, the OD_{450} was acquired with a plate reader, and the inflammatory cytokine concentrations (pg/mL) were calculated based on the standard curve.

Western Blot Analysis

Protein extraction was carried out after cell collection using radioimmunoprecipitation assay (RIPA) buffer system (#P0013B, Beyotime, China).

After centrifugation (12 000 rpm, 10 minutes), the upper phase was transferred, and protein levels were assessed via a bicinchoninic acid (BCA)-based protein assay (#ST2222, Guangzhou YuJia, China). Protein concentrations were adjusted to ensure equal protein loading across samples. 30 μg aliquots were electrophoresed via sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (#FFP39, Beyotime, China), then blocked using 5% non-fat milk for 1 hour, followed by overnight incubation at 4 °C with the following primary antibodies: ZO-1 (ab307799, Abcam, UK), Occludin (ab216327, Abcam, UK), CD40L (ab303610, Abcam, UK), and CD40 (ab224639, Abcam, UK). β-actin (ab8227, Abcam, UK) was used as a loading control. The next day, the membranes underwent triple PBS-T washing and were exposed to horseradish peroxidase (HRP)-labeled secondary antibodies (1:5000, Abcam, USA) for 1 hour. Signal detection was performed via enhanced chemiluminescence (ECL)-based chemiluminescence, and grayscale intensity was analyzed using ImageJ software to determine the target protein expression levels. Target protein band intensities were normalized to β-actin, and relative expression levels were calculated as fold changes compared with the control group.

Immunofluorescence Analysis

After cell culture, cells underwent fixation using 4% paraformaldehyde (10 minutes), rinsed 3 times in PBS (5 minutes each), and processed with 0.1% Triton X-100 (Beyotime, P0096, China) for 10 minutes. To reduce nonspecific binding, cells were blocked with 5% bovine serum albumin (BSA) for 1 hour at ambient temperature and subsequently subjected to overnight incubation at 4°C with antibodies, including ZO-1, Occludin, and phosphorylated histone H2AX (γH2AX) (Abcam, UK; ab307799, ab216327, ab81299). On the following day, cells underwent 3 PBS washes, were exposed to Alexa Fluor 488– (green) or 594– (red) conjugated secondary antibodies at ambient temperature for a 1-hour incubation in the dark. Subsequently, Nuclei were labeled with 4′,6-diamidino-2-phenylindole (DAPI) (1 μg/mL) for 5 minutes, rinsed 3 times in PBS, and sealed with an antifade mounting medium. A Leica confocal microscope (Germany) was employed to examine fluorescence intensity and intracellular localization of target proteins within cells. γH2AX staining was used to assess DNA damage. Fluorescence intensity was quantified using ImageJ software under identical exposure settings, with background subtraction and a consistent threshold applied across all groups. At least 5 random fields were analyzed per group.

Co-Immunoprecipitation (Co-IP) Assay

Cells from the Eos+MC and AR+Eos+MC groups were harvested, and protein extraction was performed with RIPA buffer containing protease and phosphatase inhibitors (Roche, Switzerland) to prevent protein degradation. After centrifugation (12 000 rpm, 10 minutes, 4°C), protein content was subjected to BCA-based quantification. For immunoprecipitation, samples were incubated with CD40L antibody (ab303610, Abcam, UK), CD40 antibody (ab224639, Abcam, UK), or with normal rabbit immunuglobulin (Ig) G (ab172730, Abcam, UK) as a negative control. Incubation was carried out at 4 °C for 4 hours with gentle rotation. Then, Protein A/G magnetic beads (Thermo Fisher, USA) were added and incubated for an additional 2 hours to allow antibody-antigen complexes to bind to the beads. The beads were rinsed 3 times to clear nonspecific proteins. Precipitated complexes were boiled (95 °C, 5 minutes) and examined via immunoblotting to assess the expression levels of CD40L and CD40 in immune complexes, thereby evaluating the interaction between Eos and MCs. IgG was used as a negative control. In addition, 10% of the total cell lysates (input) were loaded in parallel as controls to verify equal protein loading and confirm the specificity of interactions.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8.0.2. The Shapiro–Wilk test was first applied to assess data normality, followed by one-way analysis of variance (ANOVA) with Tukey's post hoc test for multiple group comparisons. Data are presented as mean ± standard deviation (SD), and statistical significance was set at $p<0.05$. All experiments were conducted with at least 3 independent biological replicates.

RESULTS

Synergistic Effects of Eosinophils and MCs on HNEpC Survival and Apoptosis

To evaluate the impact of eosinophils and MCs on

The viability of HNEpCs, we performed CCK-8 assays to assess cell viability and TUNEL assays to determine apoptosis levels. As shown in Figure 2A, both the Eosinophils and MC groups exhibited a marked decrease in cell viability relative to the control group (p<0.01). Co-culture with both Eosinophils and mast cells (Eos+MC) further exacerbated the reduction in viability, while the AR+Eos+MC group (IL-4/IL-13 pretreated HNEpCs co-cultured with eosinophils and mast cells) led to the most pronounced decrease (p<0.01). Apoptotic cell death was assessed via TUNEL staining (Figure 2B). Minimal TUNEL-positive nuclei were observed in the control group, whereas the eosinophils and mast cell groups demonstrated moderate increases in apoptosis (p<0.01). Notably, the Eos+MC group showed a further increase, and the highest apoptosis rate was recorded in the AR+Eos+MC group (p<0.01).

These data indicated that eosinophils and MCs acted synergistically to promote epithelial cell apoptosis, especially under Th2-inflammatory conditions, potentially contributing to epithelial barrier disruption in AR.

Eosinophils and MCs Cooperatively Amplify Proinflammatory Cytokine Secretion in HNEpCs

To investigate the proinflammatory effects of eosinophils and MCs on HNEpCs, we measured IL-4, IL-6, TNF-α, and TGF-β cytokine concentrations in cell culture supernatants using ELISA. As shown in Figure 3A–D, HNEpCs cocultured with either eosinophils or MCs showed significantly elevated cytokine levels relative to the control group (p<0.01). Notably, coculture with both cell types further elevated cytokine levels compared to the single-cell-type groups (p<0.01). This synergistic enhancement was particularly pronounced in the AR+Eos+MC group, which exhibited the highest concentrations of IL-4, IL-6, TNF-α, and TGF-β across all conditions (p<0.01).

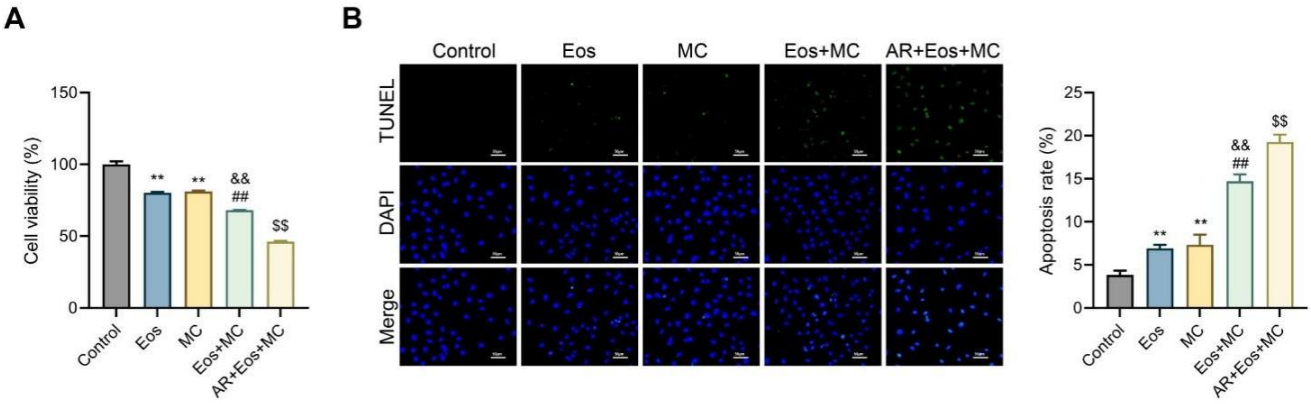
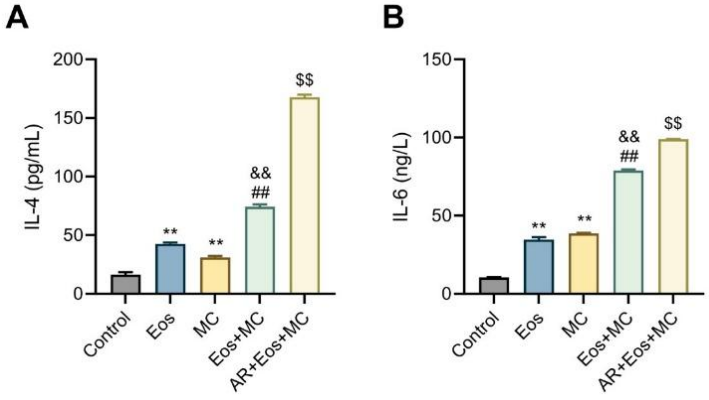


Figure 2. Synergistic effects of eosinophils and mast cells (MCs) on human nasal epithelial cells (HNEpCs) viability and apoptosis. A. Cell Counting Kit-8 (CCK-8)-8 evaluation of the HNEpC viability. B. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining and quantification of HNEpC cells (scale bar = 20 μm). Values indicate mean ± SD (n = 3). $^{**}p$<0.01 vs control; $^{\#\#}p$<0.01 vs Eos; $^{\&\&}p$<0.01 vs MC; $^{\$\$}p$<0.01 vs Eos+MC.



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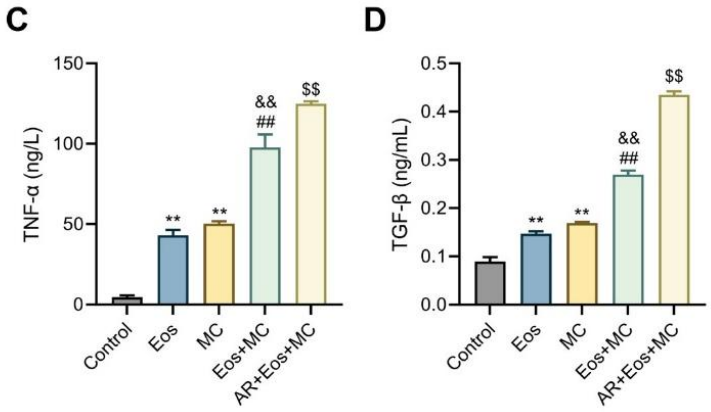

Figure 3. Eosinophils (Eos) and mast cells (MCs) cooperatively amplify proinflammatory cytokine secretion in human nasal epithelial cells (HNEpCs). A–D. Enzyme-linked immunosorbent assay (ELISA) analysis of interleukin-4 (IL-4) (A), IL-6 (B), tumor necrosis factor-α (TNF-α) (C), and transforming growth factor-β (TGF-β) (D) levels in the culture supernatants of HNEpCs after coculture with eosinophils, mast cells, both (Eos+MC), or under AR+Eos+MC conditions. Data are presented as mean ± SD (n = 3). **p<0.01 vs control; ##p<0.01 vs Eos; &&p<0.01 vs MC; $$$p$<0.01 vs Eos+MC.

These results indicated that eosinophils and MCs synergistically enhanced inflammatory cytokine production in HNEpCs, especially under Th2-polarized conditions that mimic AR.

Eosinophils and MCs Induce DNA Damage, Disrupt Epithelial Barrier Integrity, and Enhance CD40/CD40L-mediated Immune Signaling in HNEpCs

To investigate the structural and molecular mechanisms by which eosinophils and MCs impair epithelial barrier function, we assessed DNA damage, tight junction integrity, and CD40/CD40L signaling in HNEpCs. As shown in Figure 4A, γH2AX-positive nuclear foci were significantly increased in the eosinophil and mast cell groups and further amplified in the Eos+MC and AR+Eos+MC conditions, indicating enhanced double-strand DNA breaks (p<0.01).

Tight junction disruption was assessed by evaluating ZO-1 and Occludin distribution and expression. Immunofluorescence staining (Figure 4B–C) showed markedly decreased fluorescence intensity of both tight junction proteins following eosinophil or mast cell stimulation, along with cytoplasmic redistribution indicative of junctional disassembly. Western blot analysis (Figure 5A) confirmed these observations at the protein level, with significantly reduced ZO-1 and Occludin protein levels across the coculture and AR+Eos+MC group (p<0.01).

We further investigated CD40/CD40L signaling pathway participation in mediating Eos–MC interactions. As shown in Figure 5B, Western blot revealed that CD40 and CD40L expression levels were progressively downregulated in response to inflammatory stimulation. However, Co-IP analysis (Figure 5C) demonstrated enhanced physical interaction between CD40 and CD40L in the Eos+MC and AR+Eos+MC groups, despite the reduced total protein levels. This suggested that under AR-like conditions, increased receptor-ligand engagement might facilitate intercellular signaling between eosinophils and mast cells.

Collectively, these findings indicated that eosinophils and MCs synergistically induced epithelial DNA damage, disrupted tight junction integrity, and activated CD40/CD40L-mediated immune interactions, which together contributed to the pathogenesis of AR.

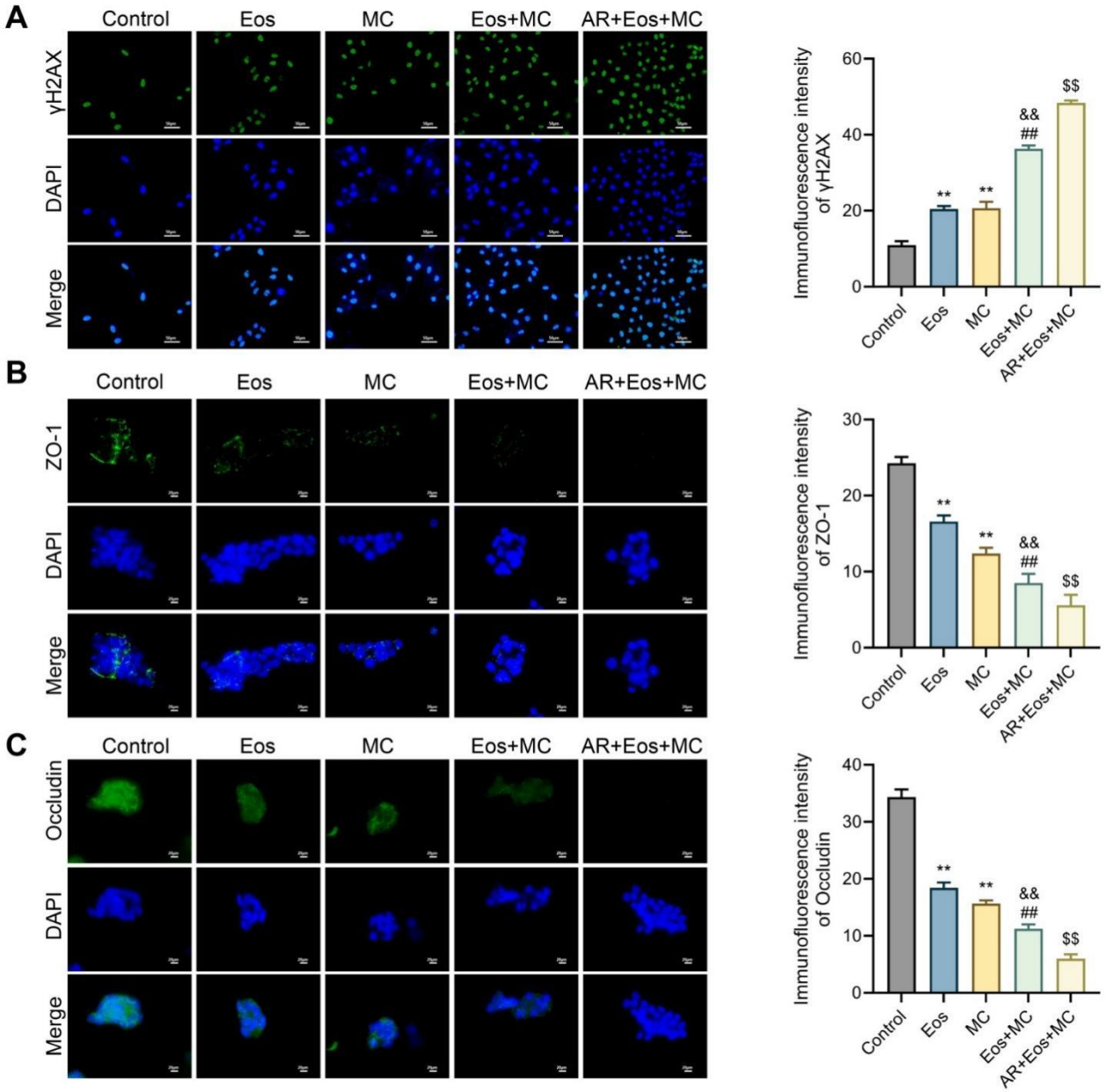

Figure 4. Eosinophils (Eos) and mast cells (MCs) induce DNA damage and disrupt epithelial barrier proteins in human nasal epithelial cells (HNEpCs). A. Immunofluorescence staining and quantification of phosphorylated histone H2AX (γH2AX) in HNEpCs indicating increased DNA damage. B–C. Immunofluorescence staining and quantification of ZO-1 (B) and Occludin (C), showing reduced protein levels along with altered subcellular localization. Data are presented as mean ± SD (n = 3). $^{**}p<0.01$ vs control; $^{\#\#}p<0.01$ vs Eos; $^{\&\&}p<0.01$ vs MC; $^{\$\$}p<0.01$ vs Eos+MC.

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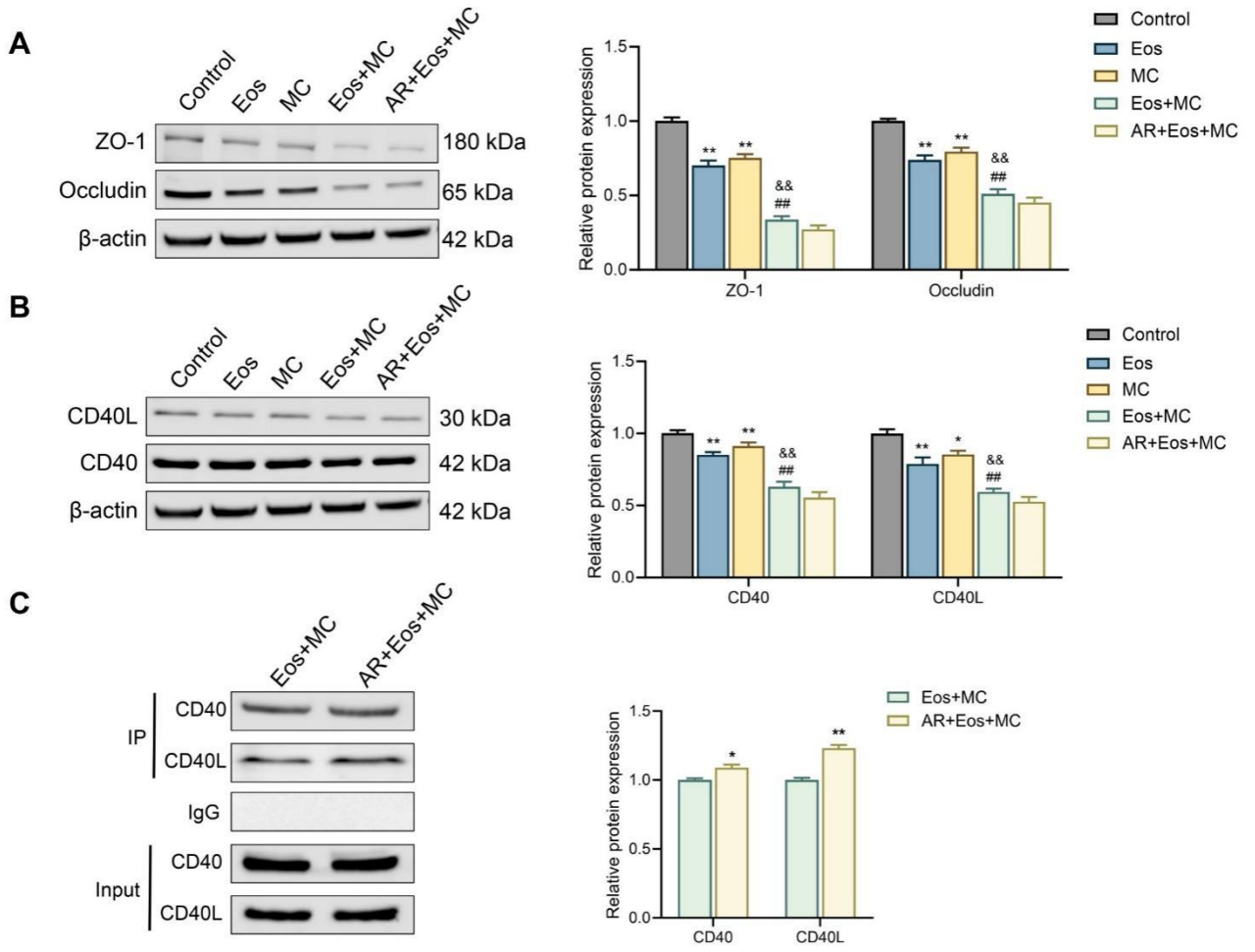

Figure 5. Eosinophils (Eos) and MCs impair tight junction protein expression and promote CD40/CD40L interaction in human nasal epithelial cells (HNEpCs). A. Immunoblotting results for ZO-1 and Occludin in HNEpCs after coculture. B. Western blot analysis of CD40 and CD40L in HNEpCs after coculture. C. Co-IP assay depicting binding of CD40 with CD40L among Eos+MC versus AR+Eos+MC ($^{*}p<0.05$, $^{**}p<0.01$ vs Eos+MC). Values are expressed as mean ± SD (n = 3). $^{*}p<0.05$, $^{**}p<0.01$ vs control; $^{\#\#}p<0.01$ vs Eos; $^{\&\&}p<0.01$ vs MC.

DISCUSSION

This study demonstrated that the interaction between eosinophils and MCs markedly compromised the viability of HNEpCs and promoted apoptosis, with the most pronounced effects observed under AR-like Th2-inflammatory conditions. Concurrently, coculture of eosinophils and MCs synergistically increased the secretion of IL-4, IL-6, TNF-α, and TGF-β, thereby amplifying the proinflammatory milieu and exacerbating epithelial injury.

In addition, eosinophils and MCs induced significant DNA damage in HNEpCs, as reflected by γH2AX upregulation, and disrupted epithelial barrier integrity through downregulation and cytoplasmic redistribution of ZO-1 and Occludin. Notably, despite a modest decrease in total CD40 and CD40L protein expression, Co-IP analysis revealed enhanced receptor–ligand engagement, suggesting clustering or altered membrane localization that facilitates intercellular signaling. Collectively, these findings indicate that Eos–MC crosstalk drives epithelial barrier dysfunction and inflammatory amplification via the CD40L-CD40 axis in AR.

The present findings further elucidate the mechanisms by which eosinophils and MCs impair the nasal epithelial barrier through CD40L-CD40 signaling. Previous work by Galdiero et al[13] showed that

eosinophils can promote mast cell activation via IL-5 and enhance the release of histamine and chemokines, thereby intensifying local inflammation. Consistent with this, our results support the view that Eos–MC interactions amplify inflammatory cascades in AR. The observed downregulation of ZO-1 and Occludin is in line with earlier studies reporting tight junction disruption in AR patients.[7] Recent clinical and experimental studies have similarly demonstrated downregulation of tight junction proteins and impaired epithelial barrier function in AR patients, further supporting the relevance of our findings.[14,15] Importantly, cytokines, such as IL-6 and TNF-α, are known to induce reactive oxygen species production and activate DNA damage responses via the JNK/p38 MAPK pathway,[7] providing a plausible explanation for the γH2AX upregulation observed in our study. Furthermore, the CD40L-CD40 axis has been shown to amplify NF-κB signaling and promote immune activation,[16,17] suggesting that enhanced receptor–ligand engagement between eosinophils and MCs may exacerbate epithelial injury through this pathway.

Despite providing direct evidence for the synergistic role of eosinophils and MCs in AR inflammation, this study has certain limitations. First, it relied on an in vitro coculture model, which lacks in vivo validation. Although IL-4 and IL-13 pretreatment was applied to mimic a Th2-skewed milieu, additional confirmation using other Th2 cytokines (e.g., IL-5, IL-13) or epithelial alarmins (TSLP, IL-33) was not performed, potentially limiting the robustness of the AR-like model. Moreover, a group with IL-4/IL-13 pretreatment alone, without eosinophils or mast cells, was not included, restricting the ability to fully separate cytokine effects from immune cell interactions. In addition, only 4 cytokines (IL-4, IL-6, TNF-α, TGF-β) were measured, and omission of IL-5 and IL-13 reduces the comprehensiveness of cytokine profiling. Second, this study primarily focused on the CD40L-CD40 pathway, and direct evidence for its regulation of inflammation via NF-κB or MAPK signaling cascades was not assessed. Future studies should employ gene knockouts or specific pathway inhibitors to clarify these mechanisms. Third, other epithelial barrier–related proteins, such as Claudins and E-cadherin, were not examined, which may have provided a broader perspective on barrier dysfunction. Finally, DNA damage was evaluated solely by γH2AX staining. Although γH2AX is a widely recognized biomarker of DNA double-strand breaks, complementary approaches, such as comet analysis, 53BP1 staining, or inclusion of positive controls were not performed, which may limit the strength of our conclusions.

In terms of therapeutic implications, biologics, such as dupilumab, which blocks IL-4/IL-13 signaling, have demonstrated efficacy in allergic airway diseases,[18,19] yet not all patients achieve sufficient symptom control and epithelial barrier dysfunction may persist.[20] Targeting the CD40L-CD40 axis could therefore complement existing biologics by further attenuating inflammation and preserving barrier integrity. Monoclonal antibodies targeting CD40 or CD40L have shown promise in autoimmune diseases.[21] A recent phase 1 trial of the anti-CD40 antibody KPL-404 demonstrated favorable safety and pharmacodynamic activity,[22] supporting the translational potential of this pathway for AR. Building on these findings, future studies should validate CD40L-CD40–mediated mechanisms in animal models and clinical cohorts, and explore therapeutic strategies beyond current biologics. In particular, combined blockade of IL-4/IL-13 and CD40L-CD40 signaling, together with interventions aimed at mitigating oxidative stress and DNA damage, may provide more effective protection of epithelial barrier integrity and represent promising directions for clinical translation.

In conclusion, the interaction between eosinophils and MCs exacerbates HNEpC damage, leading to reduced cell viability, increased apoptosis, elevated inflammatory cytokine levels, decreased expression of tight junction proteins, and enhanced DNA damage. Further investigation revealed that the CD40L-CD40 signaling pathway plays a critical role in Eos-MC interactions, potentially amplifying inflammation and aggravating epithelial barrier dysfunction. This investigation contributes to clarifying the pathogenesis of AR and suggests that the CD40L-CD40 signaling pathway may serve as a novel therapeutic target. Future clinical studies should explore targeted interventions against this pathway to improve inflammation control and epithelial barrier protection in AR patients.

STATEMENT OF ETHICS

The immortalized cell line used in this study was obtained from PromoCell and DSMZ. Ethical approval for the use of these cells is not required in accordance with local or national guidelines.

FUNDING

This study did not receive any funding in any form.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

Data sharing does not apply to this article, as all data are already included in the manuscript. Further enquiries can be directed to the corresponding author.

AI ASSISTANCE DISCLOSURE

The authors used GPT-4 (OpenAI) to assist with language editing and polishing of the manuscript. All scientific content, interpretations, and conclusions were developed and verified by the authors.

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