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Induction of Proinflamatuar Response by *Pseudomonas aeruginosa* Lipopolyssaccharide in Human Bronchial Epithelial Cells: Insights into Airway Inflammation

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Abstract

Introduction: *Pseudomonas aeruginosa* is a prominent opportunistic pathogen frequently implicated in persistent respiratory infections, especially among patients with impaired pulmonary function, including those suffering from cystic fibrosis, bronchiectasis, or ventilator-associated pneumonia. The airway epithelium serves as a primary site for host-pathogen interaction and actively contributes to innate immune responses via the production of proinflammatory cytokines. This study aimed to evaluate the dose-dependent and temporal effects of *P. aeruginosa* lipopolysaccharide (LPS) on inflammatory gene expression and cytokine secretion in BEAS-2B bronchial epithelial cells.

Materials and Methods: BEAS-2B cells were treated with increasing concentrations of *P. aeruginosa* LPS, and the optimal non-toxic dose was determined. Subsequently, cells were stimulated with 100 µg/mL LPS and harvested at 12, 24, 48, and 72 hours to assess the mRNA expression levels of IL-6, TNF-α, and IFN-γ using qRT-PCR. Cytokine secretion into the culture medium was quantified by ELISA.

Results: Stimulation with *P. aeruginosa* LPS significantly increased the expression and secretion of proinflammatory cytokines in a time-dependent manner. The highest levels of IL-6, TNF-α, and IFN-γ secretion were observed at 48 hours' post-stimulation ($p < 0.05$), indicating a delayed but robust inflammatory response.

Conclusion: These findings demonstrate that *P. aeruginosa* LPS elicits a marked proinflammatory response in human bronchial epithelial cells, highlighting the pivotal role of the epithelium in airway inflammation. The BEAS-2B cell line provides a valuable *in vitro* model for dissecting early immune responses to bacterial pathogens in the respiratory tract.

Key words: Pseudomonas aeruginosa; BEAS-2B; lipopolysaccharide; IL-6, TNF-α, IFN-γ; inflammation; epithelial cells.

Introduction

The airway epithelium functions as the frontline barrier against inhaled pathogens, allergens, and environmental pollutants. *Pseudomonas aeruginosa*, a Gram-negative opportunistic bacterium, frequently contributes to persistent infections of the respiratory tract, especially in individuals with weakened immune systems or chronic pulmonary disorders such as cystic fibrosis and COPD (1,2,3). A major factor driving its pathogenicity is lipopolysaccharide (LPS), a key component of the bacterial outer membrane that triggers strong immune responses via Toll-like receptor 4 (TLR4) signaling (4,5). Activation by LPS promotes the release of pro-inflammatory mediators, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and interferon-gamma (IFN-γ), thereby driving inflammation, oxidative stress and subsequent tissue injury (6,7). Although LPS-induced inflammation is a protective mechanism, excessive or prolonged activation may contribute to lung pathology, including airway remodeling and impaired epithelial barrier function (8,9). The human bronchial epithelial cell line BEAS-2B is a widely utilized and relevant in vitro model for studying host-pathogen interactions and epithelial immune responses to pathogen-associated molecular patterns (PAMPs) like LPS (10). While the immunomodulatory effects of *P. aeruginosa* LPS on macrophages are well-characterized, significant knowledge gaps persist regarding its temporal effects on human bronchial epithelium(11). Specifically, a comprehensive, systematic analysis of the time- dependent kinetics governing the expression (mRNA) and secretion (protein) of critical cytokines (TNF-α,

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Table 1: RNA primers used in gene expression analysis.

Genes	Forward primers	Reverse primers
IL-6	5'-CTCCACAAGCGCCTTCGGT-3'	5'-GAATCTTCTCCTGGGGGTACTGG-3'
TNF-α	5'-GCCCATGTTGTAGCAAACCCTC-3'	5'-GGTTATCTCTCAGCTCCACGCC-3'
GAPDH	5'-GTCTCCTCTGACTTCAACAGCG-3'	5'-ACCACCCTGTTGCTGTAGCCAA-3'
IL-1β	5'-CGAATCTCCGACCACCACTA-3'	5'-AGCCTCGTTATCCCATGTGT-3'
IFN-γ	5'-GCTGTTACTGCCAGGACCC-3'	5'-TTTTCTGTCACTCTCCTCTTTCC-3'

IFN-γ, IL-1β, IL-6) in response to *P. aeruginosa* LPS in human bronchial epithelial cells (BEAS-2B), alongside concomitant assessment of cell viability/proliferation, is lacking(12,13). Furthermore, the correlation dynamics between mRNA expression and protein secretion for these cytokines over an extended time course remain underexplored in this context. Understanding these precise temporal profiles is crucial for elucidating the complex regulation of inflammation, identifying potential therapeutic windows, and developing strategies to mitigate LPS-induced lung injury (14,15). We hypothesized that *P. aeruginosa* LPS induces distinct, time-dependent profiles of pro-inflammatory cytokine gene expression and protein secretion in BEAS-2B cells, and that these inflammatory dynamics correlate with modulations in epithelial cell viability and/or proliferation. Understanding the kinetics of LPS-induced cytokine production in airway epithelial cells may provide insights into the pathogenesis of bacterial lung infections and support the development of targeted anti-inflammatory therapies.

Material and Methods

Reagents: All cell culture reagents were purchased from Capricorn Scientific (Germany). Pseudomonas aeruginosa lipopolysaccharide (LPS) (catalog number L9143) was obtained from Sigma-Aldrich (USA) in a lyophilized form and stored at 2–8 °C until use. The Cell Proliferation Kit II (XTT) was also procured from Sigma-Aldrich. Quantitative reverse transcription PCR (qRT-PCR) reagents were used following manufacturer instructions. The SYBR-green 2X qPCR mastermix was supplied by ATLAS Biotechnology (Turkey). All materials and equipment used in the study were either purchased sterile or sterilized by autoclaving prior to application.

Cell culture: Human bronchial epithelial cells (BEAS-2B, ATCC no:CRL-3588 ™) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with L-glutamine, 10% fetal bovine serum, and antibiotics (100 µg/mL penicillin and 100 µg/mL streptomycin). Cultures were incubated at 37 °C in a humidified environment with 5% CO_2. Experimental procedures were initiated when cells reached 70–80% confluency (16,17).

Cell proliferation: Cell viability and proliferation were evaluated using an 2,3 - bis- (2-methoxy-4-nitro - 5 - sulfophenyl) - 2H-tetrazolium-5 carboxanilide (XTT) assay based on the sodium salt of benzene sulfonic acid hydrate. BEAS-2B cells were seeded into 96-well plates at a density of 1×10^4 cells per well and allowed to adhere for 24 hours. After incubation, cells were exposed to P. aeruginosa LPS at concentrations of 0.1, 1, 5, 10, 50, and 100 ng/mL for 12, 24, 48, and 72 hours in the presence of assay reagents. Subsequently, 50 µL of XTT solution (1 mg/mL) was added to each well, and plates were incubated for 4 hours. Absorbance was then measured at 450 nm using a Biotek Synergy H1 microplate reader (18,19).

Gene expression analysis: To examine the immunological effects of *P. aeruginosa* LPS, BEAS-2B cells were exposed to 100 ng/mL LPS for 12, 24, 48, and 72 hours. After treatment, total RNA was isolated using TRIzol reagent (ABP Biosciences, China). mRNA levels of TNF-α, IFN-γ, IL-1β, and IL-6 were quantified via qRT-PCR using 100 ng of RNA on the Lightcycler® 96 system (Roche Diagnostics, USA) and A.B.T.™ 2X SYBR-green mastermix. Primers specific for GAPDH, IL-1β, IL-6, TNF-α, and IFN-γ were utilized (17). The PCR protocol consisted of an initial incubation at 55 °C for 5 minutes, followed by 95 °C for 5 minutes, then 40 cycles of denaturation at 95 °C for 15 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 1 minute. Melting curve analysis confirmed product specificity. Gene expression levels were normalized against GAPDH cycle thresholds (18). Primer sequences are listed in Table 1.

Protein Quantification: Supernatants collected at each time point were analyzed for concentrations

of IFN-γ, TNF-α, and IL-6 using ELISA kits (ELK Biotechnology, USA). Absorbance measurements were performed at 450 nm, and cytokine levels were determined via standard curves (20).

Ethical Approval: This study does not require ethics committee approval because it involves publicly available data and does not involve human participants.

Statistical Analysis: Each experiment was carried out in triplicate to ensure reproducibility. Data were processed using GraphPad Prism version 9.0.1 (USA), and group comparisons were conducted via two-tailed t-tests. A p-value of less than 0.05 was regarded as statistically significant, with significance indicated as *$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

Results

Impact of *P. aeruginosa* LPS on BEAS-2B Cell Viability: The viability of BEAS-2B cells treated with varying concentrations of *P. aeruginosa* LPS was compared to untreated control cells. At the 12 h time point, treatment with 100 µg/mL LPS led to a slight decrease in cell viability relative to other concentrations. No significant alterations in viability were observed at 24 h. However, an increase in cell viability was detected at 48 h and persisted through 72 h in the 100 µg/mL treatment group (Figure 1).

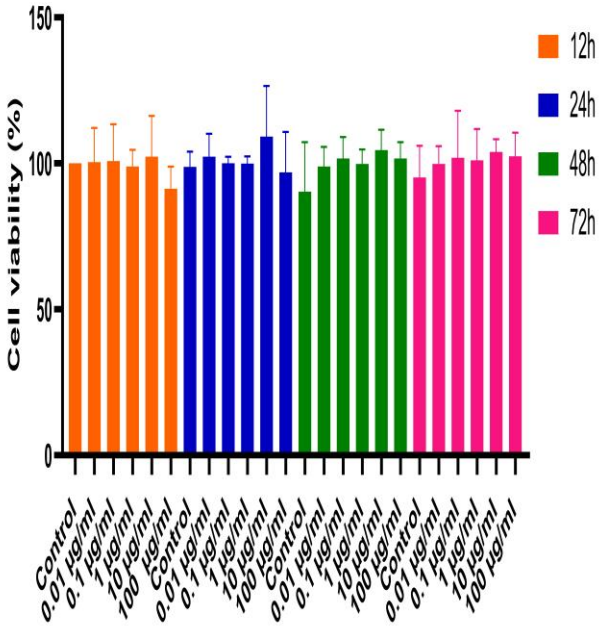

Figure 1: Changes in BEAS-2B cell viability following treatment with different concentrations of *P. aeruginosa* LPS.

Effect of LPS on pro-inflammatory gene expression: LPS stimulation induced time-dependent changes in pro-inflammatory gene expression. IFN-γ expression was significantly upregulated at 12 h post-treatment, followed by a notable decrease at 24 h compared to the control. TNF-α levels were elevated at 12 h and remained upregulated throughout the time course. IL-1β mRNA levels were consistently increased at all time points, with the most pronounced expression detected at 48 h. IL-6 expression was significantly downregulated within the first 24 h, then sharply increased at 48 h, and decreased again by 72 h (Figure 2a).

Effect of LPS on cytokine secretion: ELISA analysis of cell culture supernatants revealed that IFN-γ levels increased notably at 24 h. TNF-α concentrations were initially low at 12 h but showed a significant rise by 24 h. In contrast, IL-6 secretion was significantly reduced at 24 h when compared to the control group (Figure 2b).

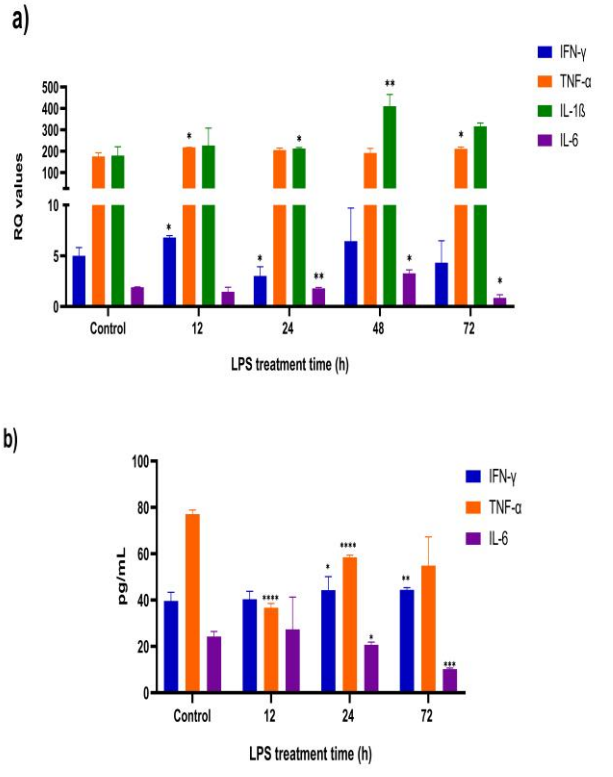

Figure 2: **a)** Temporal changes in IFN-γ, TNF-α, IL-1β, and IL-6 mRNA expression in BEAS-2B cells following 100 ng/mL LPS exposure.
b) Time-dependent effects of *P. aeruginosa* LPS (100 ng/mL) on IFN-γ, TNF-α, and IL-6 cytokine secretion in BEAS-2B bronchial epithelial cells, measured at 12, 24, 48, and 72 hours by ELISA.

Discussion

In this study, we investigated the proinflammatory responses of BEAS-2B human bronchial epithelial cells following stimulation with *Pseudomonas aeruginosa* lipopolysaccharide (LPS). Our data showed that treatment with 100 μg/mL LPS for up to 72 hours did not induce significant cytotoxicity, suggesting that the selected concentration was appropriate for modeling inflammation without compromising cell viability.
Importantly, LPS stimulation induced a time-dependent increase in the expression and secretion of key proinflammatory cytokines, including IFN-γ, TNF-α, and IL-6. These findings are consistent with previous studies reporting LPS-mediated activation of bronchial epithelial cells and subsequent cytokine release (21). TNF-α and IL-6 are central mediators in acute inflammation, promoting leukocyte recruitment, endothelial activation, and tissue remodeling. Moreover, the elevation of IFN-γ in our model suggests a potential link to Th1-type immune responses and macrophage activation, which are crucial in the host defense against bacterial pathogens. Our gene expression analysis revealed early and transient induction of IFN-γ and TNF-α, consistent with LPS-TLR4 signaling, which activates NF-ϰB and MAPK pathways leading to cytokine production (22) . The rapid increase in IFN-γ expression at 12 h followed by a decrease at 24 h may reflect a tightly regulated epithelial response aimed at preventing excessive inflammation. In contrast, TNF-α and IL-1β remained elevated over the entire 72-hour period, suggesting a more sustained inflammatory phenotype. Interestingly, IL-6 expression followed a biphasic pattern: it was suppressed at early time points (12–24 h) but markedly increased at 48 h. This delayed response may be associated with autocrine feedback loops or secondary transcriptional regulation, as seen in previous studies using bronchial epithelial cells (23,24). At the protein level, ELISA findings corroborated gene expression trends for TNF-α and IFN-γ, while IL-6 protein secretion was notably reduced at 24 h, supporting the transcriptional suppression observed in qRT-PCR results. These findings align with prior research indicating that airway epithelial cells are not passive barriers but active participants in immune signaling, capable of amplifying innate responses during bacterial invasion(25,26). The prolonged expression of IL-1β and TNF-α observed here may contribute to neutrophil recruitment and epithelial barrier disruption, hallmark features of chronic airway inflammation. From a clinical perspective, understanding epithelial cytokine dynamics in response to *P. aeruginosa* LPS is particularly relevant in the context of respiratory diseases like cystic fibrosis, where chronic colonization by *P. aeruginosa* drives persistent inflammation and progressive lung damage (27). Our results emphasize the potential utility of targeting epithelial-derived cytokines to mitigate exaggerated inflammatory responses. The dynamic secretion of these cytokines observed at 12, 24, 48, and 72 hours post-treatment underscores the prolonged inflammatory potential of *P. aeruginosa* LPS. Previous research has highlighted that sustained IL-6 signaling in respiratory epithelial cells may contribute to airway remodeling and chronic inflammation, especially in diseases such as asthma and COPD (28). Our results further emphasize the capability of *P. aeruginosa* LPS to perpetuate inflammatory signaling, which may be relevant in the context of recurrent infections or colonization in vulnerable patient populations.

Study Limitations: This study has some limitations. The use of BEAS-2B cells in this study provides a relevant in vitro model for airway epithelial responses. However, future studies incorporating co-culture systems or in vivo models may be necessary to better reflect the complex cellular interactions during pulmonary *P. aeruginosa* infection.

Conclusion

In conclusion, our findings demonstrate that *P. aeruginosa* LPS robustly induces IFN-γ, TNF-α, and IL-6 responses in bronchial epithelial cells in a time-dependent manner without inducing cytotoxicity. Unlike many previous studies that primarily focused on immune cell populations or animal models, our work emphasizes the direct contribution of airway epithelial cells as active participants in initiating and sustaining inflammatory responses. This epithelial-driven mechanism provides a complementary perspective on P. aeruginosa-associated lung diseases and highlights the potential of targeting epithelial signaling pathways in the development of novel anti-inflammatory strategies for respiratory infections.

Conflict of interest: The authors have no conflict of interest regarding this study.

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Author contributions: Concept (AY), Design (AY, UY), Data Collection and Processing (AY, UY), Analysis and Interpretation (AY, UY)

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