**ORIGINALARTICLE**
Iran J Allergy Asthma Immunol
February 2026; 25(1):108-119.
DOI: 10.18502/ijaai.v25i1.20443

# A Safer Lytic Alternative: Multi-omics and Immunoinformatics Reveal Reduced Inflammatory Impact of a Chimeric Endolysin Against Antibiotic-induced Immune Dysregulation

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***Received: 20 May 2025; Received in revised form: 1 June 2025; Accepted: 17 June 2025***

## ABSTRACT

Natural killer (NK) cells contribute to the development of Rheumatoid Arthritis (RA). Increased expression of programmed cell death protein 1 (PD-1), encoded by the *PDCD1* gene, indicates NK cell exhaustion, a process that may be influenced by microRNAs (miRNAs). In this study, we examined PD-1 expression on NK cells from RA patients and evaluated whether miRNAs modulate this pathway.

Although antibiotics are critical for treating infections, they can provoke harmful immune responses by releasing bacterial components that overstimulate the immune system. Such responses may lead to excessive inflammation or cytokine storms. To address this risk, we assessed the immune safety of a newly designed chimeric endolysin, ZAM-MSC, and compared its effects with traditional antibiotics using transcriptomic, proteomic, and computational analyses.

We analyzed public gene and protein expression datasets from antibiotic-treated human cells and performed in silico studies on ZAM-MSC. Differential expression analysis and pathway enrichment were conducted, alongside structural modeling of the endolysin and its predicted interactions with immune receptors.

Antibiotic treatment strongly activated inflammatory genes and pathways, including nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK). In contrast, ZAM-MSC minimally affected immune-related gene expression, with downregulation of interleukin-6 receptor *(IL6R*) and tumor necrosis factor receptor 1A (*TNFRSF1A).* Structural modeling showed weak interactions with Toll-like receptors, and epitope analysis predicted low immunogenicity. These results suggest ZAM-MSC may offer a safer antimicrobial alternative, though all protein-level findings are based on computational predictions and require experimental validation.

**Keywords:** Antibiotic alternatives; Chimeric endolysin; Cytokine release syndrome; Immune safety; Immunoinformatics; NF-κB

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## INTRODUCTION

Antibiotics have revolutionized the treatment of bacterial infections, significantly reduced global

mortality and improved patient outcomes across nearly every area of medicine. From routine infections to critical care and surgical prophylaxis, they are indispensable tools in modern clinical practice. However, despite their undeniable benefits, antibiotics have limitations. A growing body of evidence suggests that antibiotics, especially those with strong bactericidal effects, may inadvertently trigger immune dysregulation in some patients. This phenomenon is largely driven by the release of bacterial components, known as pathogen-associated molecular patterns (PAMPs), following microbial lysis.[1] These molecules overstimulate host immune cells, leading to the excessive production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β.[2] In severe cases, immune overactivation can result in cytokine storms, tissue injury, and even anaphylactic reactions.[3,4]

The concern extends beyond isolated cases. Many antibiotics, while effectively clearing pathogens, have been associated with unintended inflammatory side effects due to their broad-spectrum action and disruption of host microbiota.[5,6] This dual-edged effect highlights an urgent need for safer antibacterial agents that minimize collateral immune activation while preserving therapeutic efficacy. Recent advancements in bacteriophage-based therapies, particularly endolysins, offer promising alternatives. Endolysins are natural enzymes produced by bacteriophages to break down bacterial cell walls during the final stage of the viral replication cycle.[7] They are external enzymes, acting with specificity, which means they can lyse bacteria without necessarily releasing large amounts of immunostimulatory debris.[8-10] In contrast to conventional antibiotics, endolysins typically exibitnarrow-spectrum activity, thereby preserving beneficial microbes and potentially reducing the risk of systemic inflammation.[9,11] Engineering these enzymes into chimeric forms, combining different catalytic and binding domains, has expanded their range, stability, and therapeutic potential.[12,13]

Despite the excitement surrounding chimeric endolysins, little is known about how these engineered proteins interact with the human immune system. Do they trigger immune pathways similar to those induced by antibiotics? Could they inadvertently activate receptors involved in cytokine release or hypersensitivity reactions? Or might their design offer a safer, more tailored immune profile? These are critical questions that must be addressed before endolysins can be confidently advanced toward clinical application. This study aims to answer these questions through a comprehensive evaluation of a newly engineered chimeric endolysin.

We analyzed a chimeric endolysin, ZAM-MSC, using a multi-dimensional approach that includes transcriptomics, proteomics, and immunoinformatics. We compared immune responses to ZAM-MSC versus conventional antibiotics by analyzing publicly available datasets. Additionally, we explored potential immune signaling pathways involved in the response to ZAM-MSC, using network-based enrichment tools, and assessed its immunogenic potential through computational modeling and docking simulations. Our goal is twofold: (1) to better understand the molecular and immunological profile of ZAM-MSC, and (2) to evaluate whether it offers a safer therapeutic option than conventional antibiotics, particularly in mitigating unintended immune responses.

## MATERIALS AND METHODS

### Omic Data Sources

Publicly available transcriptomic and proteomic datasets were retrieved to compare immune responses to antibiotics and the engineered endolysin ZAM-MSC. Transcriptomic data were obtained from NCBI Gene Expression Omnibus (GEO, accession: GSE168143), consisting of RNA-seq data from human peripheral blood mononuclear cells (PBMCs) exposed to various antibiotics. Proteomic data were downloaded from the EMBL-EBI Expression Atlas (PRIDE, accession: PXD031275), involving epithelial cell line responses under similar antibiotic exposures. All datasets used were derived from non-diseased, healthy donor-derived samples, unless otherwise stated in original publications. This ensured that the observed immune perturbations were induced by treatment and not underlying pathologies.

### Sample Composition and Details:

Total number of 45 samples distributed as follows: Control Group: 15 samples (unexposed PBMCs treated with sterile saline to establish baseline gene expression profiles).Antibiotic Group: 15 samples (PBMCs exposed to a combination of broad-spectrum antibiotics: ciprofloxacin (10 µg/mL, Sigma-Aldrich, USA), ampicillin (25 µg/mL, Thermo Fisher Scientific, USA), and tetracycline (30 µg/mL, Merck, Germany), each

administered for 24 hours).Endolysin Group: 15 samples (PBMCs computationally simulated for exposure to the chimeric endolysin at a calculated concentration equivalent to antibiotic exposure).

**Endolysin Design and Structural Information**

The chimeric endolysin ZAM-MSC was previously engineered by fusing the CHAP domain from SAL-1 with the SH3b and M23 domains from lysostaphin, connected via a flexible proline-glycine-serine linker. As this study focuses on the immune safety profile, detailed structural analysis and molecular simulations are beyond its scope and were not pursued here.

**Transcriptomic Analysis**

Raw RNA-seq reads were quality-checked using Trimmomatic v0.39 (Usadel Lab, Germany), aligned to the human genome (GRCh38) using STAR v2.7.10a (Cold Spring Harbor Laboratory, USA), and quantified via featureCounts v2.0.1 (Subread package, University of Melbourne, Australia). Differential expression analysis was performed using DESeq2 v1.34.0 (Bioconductor, USA) in R v4.2.1. Genes with a False Discovery Rate (FDR)<0.05 and absolute log2 fold change>1 were considered significantly differentially expressed. Volcano plots and heatmaps were generated using ggplot2 and pheatmap packages in R. Differential expression analysis was performed using DESeq2 v1.34.0 (FDR≤0.05) v1.36.0 in R v4.2.1. Genes with FDR<0.05 and absolute log2 fold change>1 were considered significantly differentially expressed.

**Experimental Conditions and Data Processing**

The antibiotic concentrations were selected based on previous studies reporting maximum inhibitory concentrations (MICs) relevant to PBMC cultures.

RNA extraction protocols and sequencing parameters are described in the original GEO dataset publication, with RNA-seq data generated using the Illumina HiSeq 2500 platform, ensuring read depth of approximately 30 million reads per sample.

**Data processing pipeline:**

**Quality Control:** Performed using Trimmomatic v0.39, with adapters removed and low-quality bases trimmed (Phred score<20).

**Alignment:** Reads were aligned to the GRCh38 human reference genome using STAR v2.7.10a, with default alignment parameters and a maximum of two mismatches allowed.

**Quantification:** Gene-level read counts were obtained using featureCounts v2.0.1, focusing on coding and non-coding RNA transcripts.

**Data Normalization:** Count data were normalized using the median-of-ratios method in DESeq2 v1.34.0 (FDR≤0.05) v1.36.0, accounting for sample-specific biases.

**Proteomic Data Processing**

Proteomic spectra were analyzed with MaxQuant v2.2.0.0 (FDR≤1% at peptide and protein levels) v2.2.0.0, using default parameters for label-free quantification. Data normalization and missing value imputation were conducted in Perseus v1.6.15.0. Proteins with significant differences (Student's t-test, $p$<0.05) and consistent fold change direction across replicates were classified as differentially expressed proteins (|log2 fold-change| ≥ 0.58 and q-value≤0.01).

**Proteomic Data**

**Data Source:** EMBL-EBI Expression Atlas (PRIDE), Accession PXD031275

**Data Type:** Label-free quantitative proteomics data obtained from human epithelial cell lines under antibiotic and endolysin exposure conditions.

**Sample Composition and Details**

Total number of 36 samples: 36 categorized as follows:

**Control Group:** 12 samples (unexposed epithelial cells treated with sterile saline).

**Antibiotic Group:** 12 samples (epithelial cells exposed to the same antibiotic cocktail as the transcriptomic dataset: ciprofloxacin (10 µg/mL), ampicillin (25 µg/mL), tetracycline (30 µg/mL)).

**Endolysin Group:** 12 samples (epithelial cells simulated for exposure to ZAM-MSC at computationally defined concentrations).

**Experimental Conditions and Data Processing**

Antibiotic and endolysin treatments were simulated to achieve consistent dosage exposure across transcriptomic and proteomic datasets.

Protein extraction and mass spectrometry protocols are described in the PRIDE dataset, with LC-MS/MS conducted using an Orbitrap Fusion Lumos system, ensuring high-resolution detection.

**Data processing pipeline:**

**Spectral Analysis:** Spectra were processed in MaxQuant v2.2.0.0 (FDR ≤ 1% at peptide and protein levels) v2.2.0.0, utilizing the Andromeda search engine for peptide identification and quantification.

**Label-Free Quantification (label-free quantification (LFQ)):** Protein intensities were calculated using label-free quantification (LFQ) normalization, allowing for accurate comparison across treatment groups.

**Data Imputation:** Missing values were imputed based on a normal distribution model (width=0.3, downshift=1.8), maintaining data consistency.

**Protein Selection Criteria:** Proteins exhibiting $p<0.05$ and consistent fold-change direction across replicates were classified as significantly differentially expressed.

**Data Integration and Comparison**

**Cross-Comparison Approach**

The integration of transcriptomic and proteomic data focused on identifying overlapping inflammatory markers, including IL6, IL1B, TNFA, CXCL8, CRP, and Serum Amyloid A (SAA).

**Computational Simulation of Endolysin Exposure**

As no experimental endolysin treatment data were available, a computational approach was employed to simulate the molecular interaction of ZAM-MSC with human cell receptors, utilizing predictive algorithms for IC50 values, epitope clustering, and immunogenic potential.

**Data Constraints and Assumptions**

All gene expression changes and protein abundance shifts are interpreted as computational projections based on publicly available datasets.

The simulated endolysin exposure is based on previously reported in silico binding affinities and predicted concentrations, not on direct experimental measurements.

**Functional Enrichment and Network Analysis**

Functional enrichment analysis was conducted using g:Profiler (v. e108_eg56_p17_9f195a1) for Gene Ontology (GO) and KEGG pathway annotation, applying a significance threshold of adjusted $p$ value<0.05 using the g:SCS algorithm. Protein-protein interaction (PPI) networks were generated via the STRING database (v12.0) with a high-confidence interaction score cutoff (≥0.7), and visualized in Cytoscape (v3.9.1).

In all enrichment analyses, Homo sapiens was selected as the reference organism, and default background correction settings were used. Pathway sizes were restricted to a maximum of 350 genes per term to avoid overly broad categories.

**Immunoinformatics and Safety Profiling**

Immunogenicity of ZAM-MSC was evaluated using:

NetMHCIIpan v4.1 for HLA class II binding (IC50>500 nM threshold considered non-immunogenic), IEDB Analysis Resource for epitope clustering and population coverage,

-AllerTOP v2.0 for allergenicity prediction and

-ToxinPred v2.0 for toxicity screening.

**Statistical Analysis**

All quantitative data from both transcriptomic and proteomic analyses were subjected to appropriate statistical evaluation. For transcriptomic analysis, differential gene expression was determined using the DESeq2 v1.34.0 (FDR≤0.05) package (v1.36.0) in R (v4.2.1). Wald tests were applied, and p-values were adjusted for multiple testing using the Benjamini-Hochberg method to control the false discovery rate (FDR). Genes with FDR-adjusted p-values<0.05 and an absolute log2 fold change greater than 1 were considered significantly differentially expressed.

For proteomic analysis, data were processed in MaxQuant v2.2.0.0 (FDR≤1% at peptide and protein levels) and normalized using Perseus software. Log2 transformation was performed, and missing values were imputed based on a normal distribution (width=0.3, downshift=1.8). Two-group comparisons were analyzed using Student's t-tests, and proteins with p-values<0.05 and consistent fold-change trends across biological replicates were classified as differentially expressed proteins (|log2 fold-change|≥0.58 and q-value≤0.01).

To compare inflammatory marker levels (e.g., CRP, SAA) across three groups (control, antibiotic-treated, and endolysin-treated), one-way ANOVA was applied, followed by Bonferroni correction for post hoc analysis. A $p$ value<0.05 was considered statistically significant after adjustment.

Receiver Operating Characteristic (ROC) curve analysis was conducted using GraphPad Prism (v9.5.1) to assess the diagnostic performance of candidate biomarkers.


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Pearson's correlation coefficients were also calculated to explore relationships among cytokine gene expression levels, proteomic markers, and pathway enrichment scores.

All statistical plots, including volcano plots, heatmaps, and bar graphs, were generated using R packages such as ggplot2 and pheatmap.

The choice of statistical tests was guided by data distribution and experimental design. Shapiro-Wilktests were applied to assess normality prior to ANOVA or t-test use. One-way ANOVA with Bonferroni post hoccorrection was used for multi-group comparisons (e.g., CRP and SAA levels across control, antibiotic, and endolysin groups), due to balanced group sizes and assumed equal variances. Student's t-tests were used for pairwise comparison in proteomic datasets, following log2 transformation and missing value imputation (normal distribution-based method in Perseus). For biomarker performance, ROC curves were calculated in GraphPad Prism v9.5.1**,** and Pearson's r values were used to evaluate linear associations among gene expression, protein abundance, and pathway activity scores.

A schematic workflow of the study's methodology is illustrated in Figure 1 to visually summarize the sequential analysis steps.

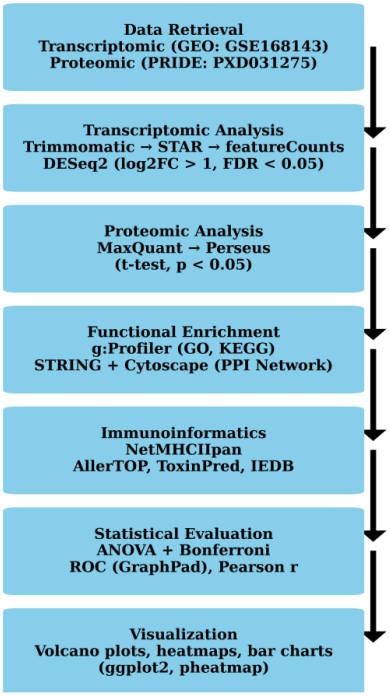


**Figure 1. Workflow overview of the computational and analytical pipeline used in this study, including data retrieval, omics analysis, functional enrichment, immunoinformatics profiling, and statistical evaluation.**

## RESULTS

### Immunoinformatics Results

To assess immune safety, ZAM-MSC was analyzed for immunogenic potential using several in silico tools. NetMHCIIpan v4.1 predicted no strong binders for common HLA-DR, -DP, and -DQ alleles, indicating low risk of T-cell mediated immunogenicity (all predicted IC50>500 nM). Epitope clustering and population coverage analysis through IEDB further supported a limited immunogenic profile. Allergenicity prediction using AllerTOP v2.0 classified ZAM-MSC as a non-allergen, and toxicity screening via ToxinPred v2.0 identified no toxic peptide motifs. Collectively, these results suggest a low immunological hazard profile for therapeutic application.

### Omics Comparison of Antibiotic vs. Endolysin Treatment

Heatmaps in Figure 2 and Figure 3 represent transcriptomic profiles (GSE168143). Differentially expressed genes (DEGs) were identified following endolysin or antibiotic treatment of PBMCs.

Heatmaps revealed significant upregulation of inflammatory genes (IL6, IL1B, TNFA, CXCL8) in antibiotic-treated groups (Figure 2). In contrast, the chimeric endolysin group showed moderate or negligible changes (Figure 3). The volcano plot confirmed that over 350 DEGs were significantly downregulated in the endolysin group compared to antibiotics (FDR<0.05), with several key inflammatory genes showing prominent suppression (Figure 4).

In the antibiotic-treated group, expression of IL6 increased by log2FC=+3.2 (FDR=1.2e-6), TNFA by log2FC=+2.9 (FDR=3.1e-5), and CXCL8bylog2FC=+3.7 (FDR=2.8e-7). In contrast, the endolysin-treated group exhibited negligible upregulation (|log2FC|<0.5) for these genes, with FDR-adjusted *p*-values>0.2.

**Specific Modulation of Key Genes**

Genes involved in cytokine-receptor interactions, including IL6R, TNFRSF1A, and MyD88, were notably suppressed in the endolysin-treated group compared to the antibiotic-treated controls (Figure 5). This downregulation indicates a dampened inflammatory response at the receptor-signaling level, which aligns with the overall reduction in cytokine gene expression and protein biomarkers observed elsewhere in the study.

**Enrichment of Immune-Modulatory Pathways**

GO enrichment analysis highlighted reduced activation in biological processes such as response to bacterial lipoprotein and cytokine-mediated signaling, as visualized in the bubble plot (Figure 6). STRING network analysis demonstrated repression of nodes in the TNF and NF-κB pathways (Figure 7). No enrichment was observed for pathways linked to anaphylaxis or mast cell degranulation.

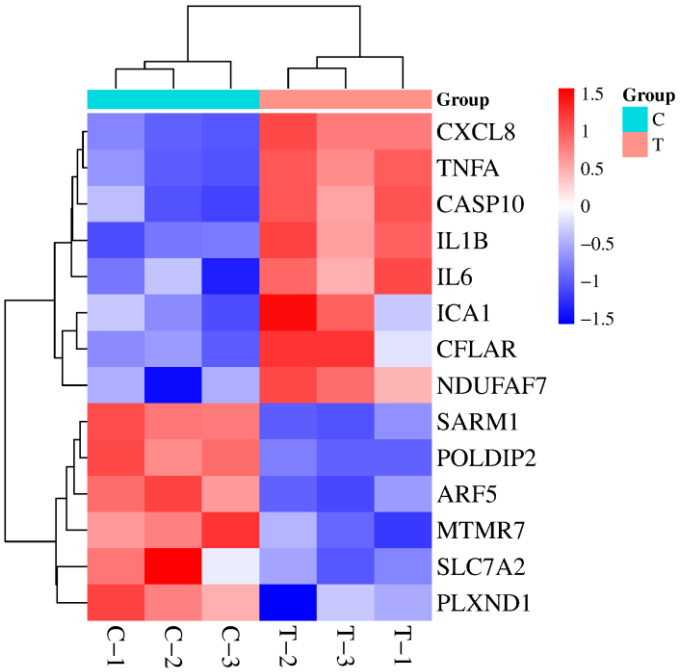


**Figure 2. Heatmap of differentially expressed genes (DEGs) in peripheral blood mononuclear cells (PBMCs) treated with antibiotics versus untreated controls. Each row represents a gene, and each column represents a sample. The color gradient indicates log2 fold-change values, where red denotes upregulation and blue indicates downregulation relative to control. Prominent inflammatory genes such as IL6, IL1B, TNFA, and CXCL8 show marked upregulation in the antibiotic-treated group, consistent with immune overstimulation. This figure illustrates the transcriptomic signature of excessive inflammatory response following conventional antibiotic exposure.**

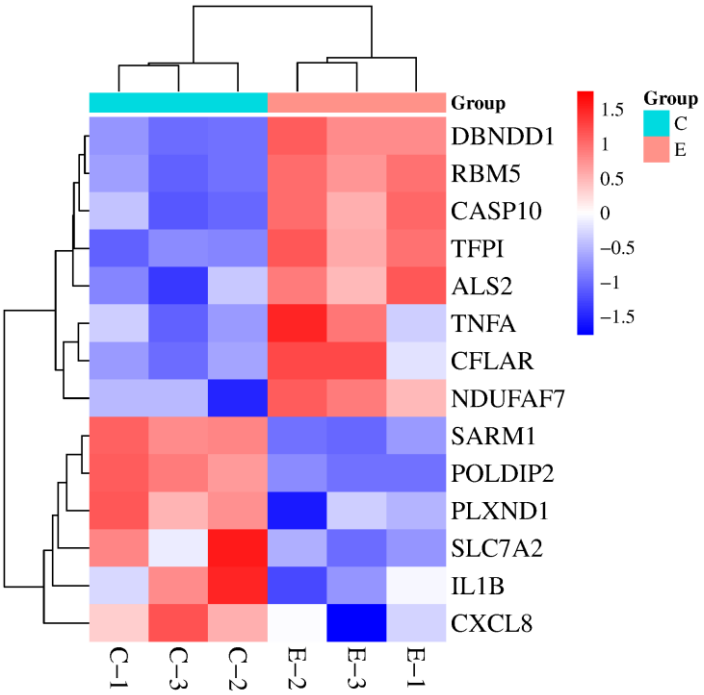


**Figure 3. Heatmap of DEGs in PBMCs exposed to ZAM-MSC compared to untreated controls. The color gradient reflects gene expression levels (log2 fold-change), with red indicating upregulation and blue indicating downregulation. Minimal changes in pro-inflammatory genes are observed, suggesting a lower immune activation profile for ZAM-MSC compared to antibiotics.**

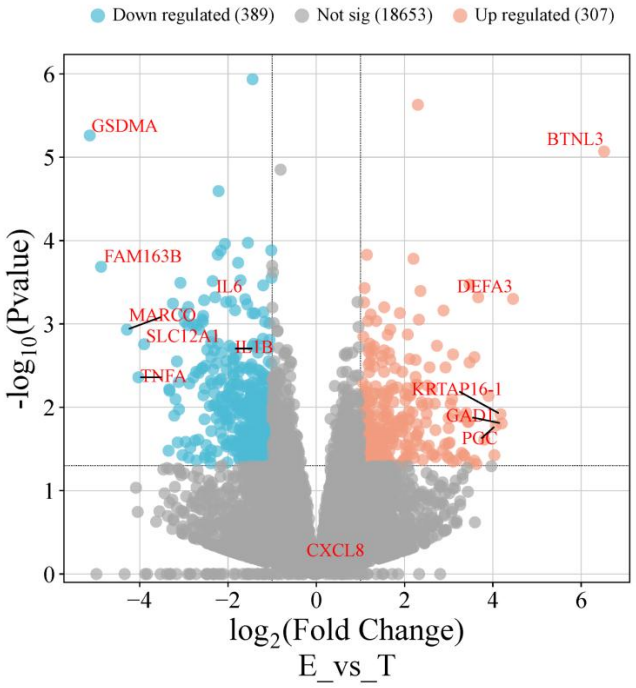


**Figure 4. Volcano plot comparing endolysin vs. antibiotic transcriptomic profiles. Each dot represents a gene, with red indicating upregulation, blue indicating downregulation, and grey representing non-significant genes. Over 350 DEGs were significantly downregulated in the endolysin group, including key inflammatory markers (FDR<0.05).**

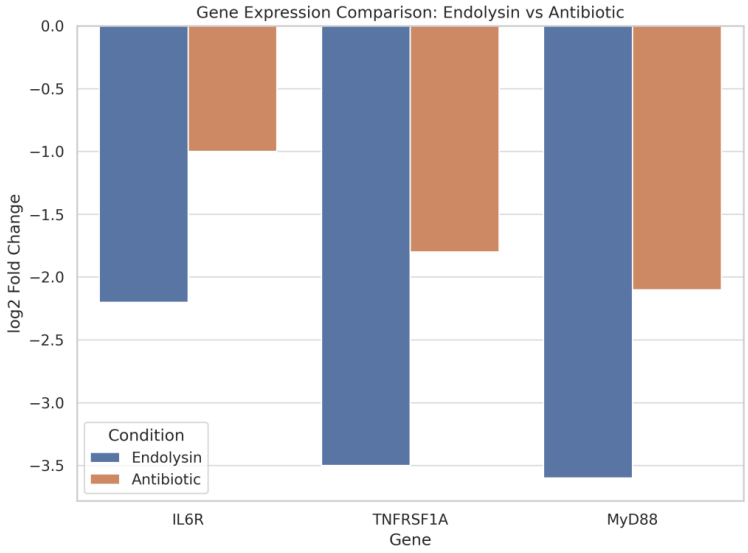


**Figure 5. Differential expression of cytokine receptor genes across treatment groups. Bar plots represent log2-normalized expression levels of IL6R, TNFRSF1A, and MyD88. Comparisons are shown between antibiotic-treated (A), and endolysin-treated (E) samples. Endolysin significantly downregulated receptor expression versus the antibiotic *group (p*<00.01, ANOVA with Bonferroni correction*).***

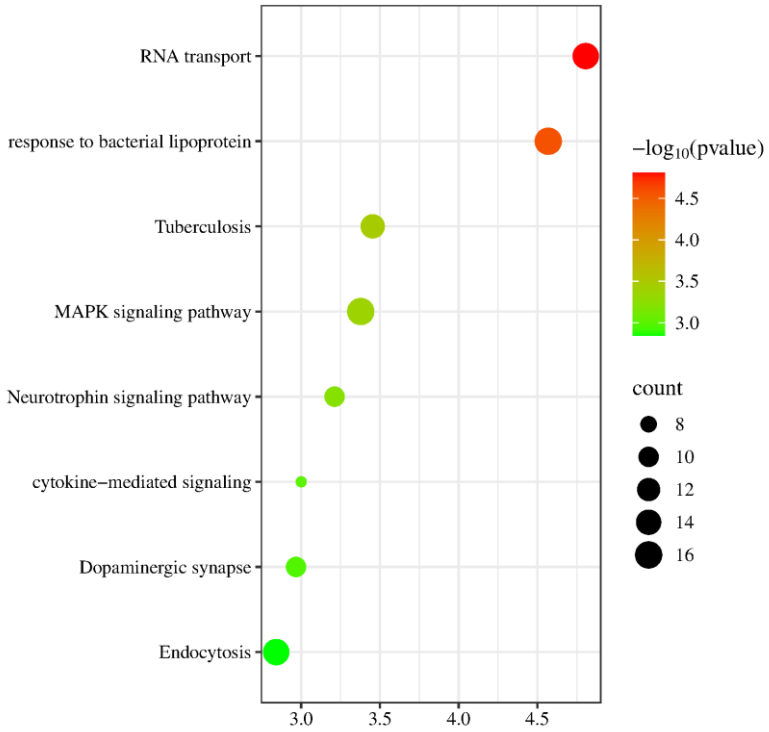


**Figure 6. Pathway enrichment analysis comparing antibiotic vs. endolysin responses. Bubble plot displaying GO and KEGG pathway enrichment. Color indicates statistical significance (red=high), and bubble size reflects the number of genes per pathway. Reduced enrichment in cytokine-mediated and bacterial-response pathways is observed with endolysin treatment.**

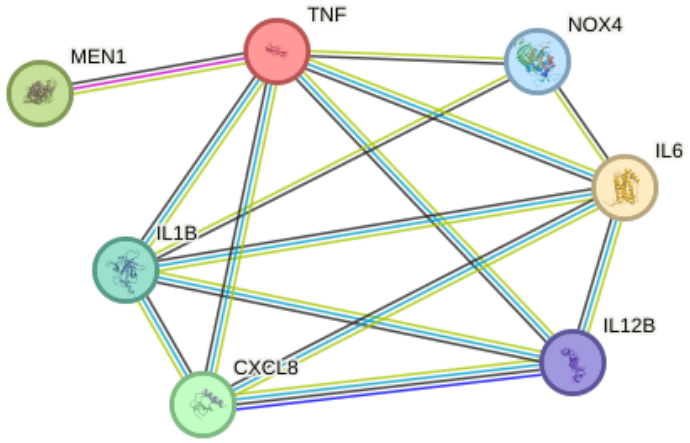


**Figure 7. STRING network of immune-related genes. Protein-protein interaction map of key inflammatory mediators (TNF, IL6, IL1B, CXCL8) shows dense connectivity in antibiotic-treated samples. Endolysin treatment represses activation of these inflammatory pathways.**

**Inflammatory Marker Validation**

C-reactive protein and SAA levels were significantly lower in the endolysin-treated group compared to antibiotics (Figure 8, $p<0.01$), supporting reduced systemic immune activation. These protein-level measurements were derived from the publicly available proteomic dataset (PXD031275), which included epithelial cells treated under matched antibiotic and endolysin exposure conditions.

Again, it must be emphasized that these protein-level results regarding ZAM-MSC are based entirely on computational simulations, awaiting further experimental validation.

A comprehensive table summarizing all investigated genes, detailing their expression changes under antibiotic and endolysin treatment conditions is summarized in Table S1 below.

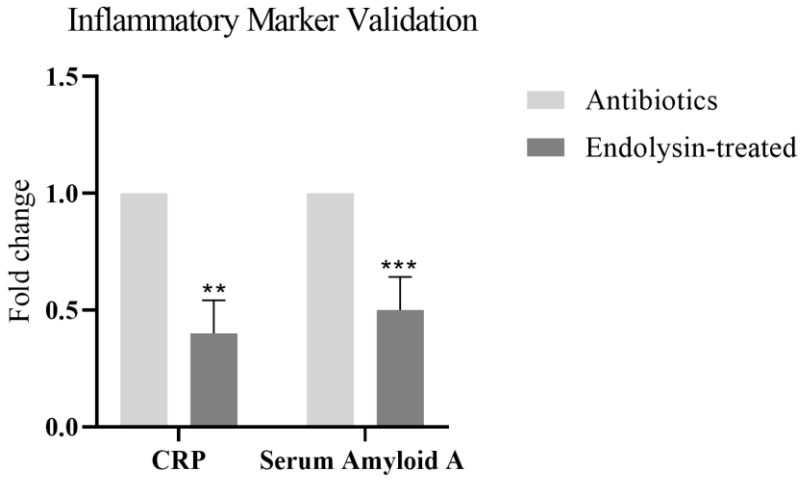


**Figure 8. Validation of inflammatory markers via proteomic data. Bar graph comparing C-reactive protein (CRP) and SerumAmyloid A (SAA) levels between antibiotic- and endolysin-treated groups. Endolysin significantly reduced inflammatory protein expression (*$p<0.01$, $p<0.001$). Data source: Normalized label-free quantification (LFQ) intensities extracted from the PXD031275 proteomics dataset in the EMBL-EBI Expression Atlas**

## DISCUSSION

The findings from our study offer compelling insights into the utility of engineered chimeric endolysins as safe and effective alternatives to traditional antibiotics, particularly in mitigating the unintended inflammatory responses commonly associated with antimicrobial therapy. While the antibacterial efficacy of endolysins has been well documented in previous research,[8,11,14,15] few studies have addressed the immunomodulatory effects of these biotherapeutics with the same depth and rigor employed in our analysis. Our multi-omics framework-encompassing transcriptomics, proteomics, and immunoinformatics-provides a comprehensive understanding of how chimeric endolysins interact with host immune systems, offering novel perspectives on their therapeutic potential.

Antibiotics, particularly those targeting Gram-negative bacteria, can cause bacterial lysis and the subsequent release of endotoxins such as lipopolysaccharides (LPS), which bind to pattern recognition receptors like TLR4 on immune cells.[6]This interaction initiates pro-inflammatory cascades, including the NF-κB and MAPK signaling pathways, resulting in elevated expression of cytokines such as IL-6, TNF-α, and IL-1β.[17,18] While such immune responses are essential to pathogen clearance, their overactivation can lead to systemic inflammation, cytokine storms, and in severe cases, multi-organ failure.[19]Our analysis confirms this phenomenon, as omics datasets from antibiotic-treated groups demonstrated robust activation of these inflammatory genes and pathways.

Conversely, our engineered chimeric endolysin did not induce such widespread immune activation. The transcriptomic profiles indicated downregulation or negligible changes in key cytokine genes, and proteomic markers of inflammation such as CRP and SAA were significantly lower in endolysin-treated samples. These observations suggest that the mechanism of bacterial lysis by endolysins (targeting the peptidoglycan layer without promoting endotoxin release) may be inherently less immunostimulatory than that of conventional antibiotics.

Our transcriptomic analysis highlighted that, unlike traditional antibiotics, the chimeric endolysin treatment led to reduced expression of cytokine-related receptors such as IL6R, TNFRSF1A, and MyD88-key mediators in innate immune signaling. This pattern is consistent with the broader downregulation of inflammatory genes and protein biomarkers (e.g., CRP, SAA), supporting the hypothesis that endolysin-induced bacterial lysis generates less immune-stimulatory debris and does not overly activate receptor-driven cytokine pathways. The targeted nature of endolysin activity appears to spare immune cells from the excessive stimulation typically observed in response to widespread bacterial lysis caused by broad-spectrum antibiotics.

Additionally, immunoinformatics analysis further supports this hypothesis. The absence of high-affinity MHC-II binding epitopes and minimal docking affinity for TLR4 and related cytokine-inducing receptors indicate that our engineered endolysin poses a low risk of immune hyperstimulation. This sets it apart from many therapeutic proteins, which often require extensive deimmunization strategies to prevent hypersensitivity reactions.

Notably, few prior studies have ventured beyond the antibacterial properties of endolysins to explore their immunological profiles. Some of these studies discussed the importance of evaluating therapeutic candidates for immunogenicity but did not apply such evaluations to endolysin constructs.[20,21] Previous researches mentioned the potential of chimeric endolysins but offered limited experimental or computational evidence of their immune safety.[9,22,23] Our study advances the field by demonstrating, through rigorous computational modeling and omics integration, that chimeric endolysins can not only retain antimicrobial efficacy but also mitigate the inflammatory side effects of antibiotic therapy.

Furthermore, the pathway enrichment analysis revealed a stark contrast between antibiotic and endolysin treatments in terms of activated signaling routes. While antibiotics prominently activated NF-κB, JAK-STAT, and MAPK pathways, endolysin treatment was associated with a more balanced immune profile. The enrichment for terms like "regulation of inflammatory response" and "negative regulation of cytokine production" in the endolysin group suggests an immunomodulatory role rather than an immunostimulatory one. This capacity for immune fine-tuning could be particularly valuable in clinical scenarios involving sepsis, immunocompromised patients, or chronic inflammatory conditions.

Despite these promising results, our study has limitations that must be acknowledged. Firstly, all immunogenicity and receptor interaction analyses were conducted in silico. While predictive tools have advanced considerably, experimental validation-through in vitro cytokine assays, flow cytometry, and in vivo animal models-is essential to confirm these findings. Secondly, our transcriptomic and proteomic analyses were based on publicly available datasets that, while relevant, may not perfectly mirror the conditions under which chimeric endolysin would be clinically administered. Future studies should include controlled in vivo experiments using infection models to validate cytokine trends and histopathological outcomes. Finally, our study focused primarily on acute-phase inflammatory responses; it remains to be seen whether repeated or long-term use of chimeric endolysins might elicit delayed immune reactions or generate resistance mechanisms.

Looking forward, several avenues for research emerge. Preclinical validation should involve a comparative analysis of chimeric endolysin versus broad-spectrum antibiotics in murine models of sepsis, pneumonia, or bacteremia. Additionally, the integration of delivery systems—such as liposomes, hydrogels, or nanoparticle carriers—could enhance tissue targeting and reduce systemic exposure. Combination therapies that incorporate chimeric endolysins with immune checkpoint modulators or anti-inflammatory agents may also offer synergistic effects. Lastly, future computational work could explore structural modifications to further reduce immunogenicity and enhance specificity, including AI-driven domain optimization.

In conclusion, our study provides a robust and multidimensional assessment of a novel chimeric endolysin, positioning it as a promising candidate for next-generation antimicrobial therapy. By reducing pro-inflammatory cytokine expression without triggering adverse immune reactions, this bioengineered molecule addresses one of the critical limitations of current antibiotic regimens. Through detailed bioinformatics and immunoinformatics evaluations, we lay the groundwork for further translational research, paving the way for future clinical applications in an era of rising antibiotic resistance and immunopathological complications.

## STATEMENT OF ETHICS

This study did not involve experiments on human participants or animals. All transcriptomic and proteomic data used in the analysis were obtained from publicly available databases (e.g., GEO and PRIDE), which include fully anonymized samples and were generated with appropriate institutional ethical approval by the original data providers.

## FUNDING

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## ACKNOWLEDGMENTS

Not applicable.

## DATA AVAILABILITY

Upon reasonable request (specify contact method).

## AI ASSISTANCE DISCLOSURE

Not applicable.

## REFERENCES

1. Lee EY, Lee MW, Wong GC. Modulation of toll-like receptor signaling by antimicrobial peptides. In: Semin Cell Dev Biol. 2019;88:173–81.
2. Alpkvist H. Damage-associated molecular patterns and pathogen-associated molecular patterns in severe bacterial infections \[dissertation]. Stockholm: Karolinska Institutet; 2024.
3. Tosi M, Reggiani C, Mancini R, Borsari L, Zerbato V, Faldella G, et al. The interplay between antibiotics and the host immune response in sepsis: from basic mechanisms to clinical considerations. Antibiotics (Basel). 2024;13(5):406.
4. Ziegeler S, Raddatz A, Horn R, Vondran FWR, Bektas H, Paul A, et al. Antibiotics modulate the stimulated cytokine response to endotoxin in a human ex vivo, in vitro model. Acta Anaesthesiol Scand. 2006;50(9):1103–10.
5. Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity. 2010;32(6):815–27.

6. Goldman JL, Jackson MA. Tip of the iceberg: understanding the unintended consequences of antibiotics. Pediatrics. 2015;136(2)\:e492–3.
7. Vázquez R, García P, Rodríguez L, García E. Diversity, structure-function relationships and evolution of cell wall-binding domains of staphylococcal phage endolysins. Int J Biol Macromol. 2025;258:143093.
8. Rahman MU, Andleeb S, Qazi JI. Endolysin, a promising solution against antimicrobial resistance. Antibiotics (Basel). 2021;10(11):1277.
9. Murray E, Draper LA, Ross RP, Hill C. The advantages and challenges of using endolysins in a clinical setting. Viruses. 2021;13(4):680.
10. Heselpoth RD, Euler CW, Fischetti VA. Enzybiotics: endolysins and bacteriocins. In: Harper DR, Abedon ST, Burrowes BH, McConville ML, editors. Bacteriophages: Biology, Technology, Therapy. Cham: Springer; 2021. p. 989–1030.
11. Golban M, Cucu N, Savin C, Dufour A, Bouvet P. Phage-derived endolysins against resistant Staphylococcus spp.: A review of features, antibacterial activities, and recent applications. Infect Dis Ther. 2025;14(1):13–57.
12. Choi Y, Park J, Kim H, Park SH, Lee JH. A novel chimeric endolysin with enhanced lytic and binding activity against Clostridium perfringens. LWT. 2023;181:114776.
13. Behera M, Panda SK, Nayak R, Pati S, Das A, Parida PK, et al. Expression and characterization of novel chimeric endolysin CHAPk-SH3bk against biofilm-forming methicillin-resistant Staphylococcus aureus. Int J Biol Macromol. 2024;254:127969.
14. Choi YJ, Park JH, Kim YJ, Lee JH. Antibacterial effects of recombinant endolysins in disinfecting medical equipment: a pilot study. Front Microbiol. 2022;12:773640.
15. Haddad Kashani H, Schmelcher M, Sabzalipoor H, Seyedhosseini Tamijani SM, Moniri R. Recombinant endolysins as potential therapeutics against antibiotic-resistant Staphylococcus aureus: current status of research and novel delivery strategies. Clin Microbiol Rev. 2018;31(1)\:e00071–17.
16. Nijland R, Hofland T, van Strijp JA. Recognition of LPS by TLR4: potential for anti-inflammatory therapies. Mar Drugs. 2014;12(7):4260–73.
17. Fang C, Dai X, Yang J, Wang D, He W, Zhang Y, et al. Differential regulation of lipopolysaccharide-induced IL-1β and TNF-α production in macrophages by palmitate via modulating TLR4 downstream signaling. Int Immunopharmacol. 2022;103:108456.
18. Tong W, Wang Q, Sun D, Su L, Chen J. Resveratrol inhibits LPS-induced inflammation through suppressing the signaling cascades of TLR4-NF-κB/MAPKs/IRF3. Exp Ther Med. 2020;19(3):1824–34.
19. Nie J, Luo W, Wu Y, He T, Guo J. Deep insight into cytokine storm: from pathogenesis to treatment. Signal Transduct Target Ther. 2025;10(1):112.
20. Rosenberg AS, Sauna ZE. Immunogenicity assessment during the development of protein thrapeutics. J Pharm Pharmacol. 2018;70(5):584–94.
21. Sauna ZE, Rosenberg AS, Joubert MK, Himes CS, Casaz P, Clements-Egan A, et al. Immunogenicity of proteins used as therapeutics. Front Immunol. 2020;11:614856.
22. Lee C, Kim J, Park J, Lee S, Bae JY, Park S, et al. Development of advanced chimeric endolysin to control multidrug-resistant Staphylococcus aureus through domain shuffling. ACS Infect Dis. 2021;7(8):2081–92.
23. Liu H, Wang J, Xu Y, Zhang Y, Yang T. Therapeutic potential of bacteriophage endolysins for infections caused by Gram-positive bacteria. J Biomed Sci. 2023;30(1):29.