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MicroRNA-mediated post-transcriptional regulation of inflammatory cytokine expression

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Abstract

What determines whether an inflammatory response protects the host or spirals into tissue damage? Growing evidence points to microRNAs (miRNAs) as post-transcriptional regulators that calibrate cytokine output during innate immune activation. This research examined five candidate miRNAs—miR-146a, miR-155, miR-21, miR-223, and miR-125b—and their capacity to modulate TNF- α , IL-6, and IL-1 β in LPS-challenged THP-1 human monocytes. Cells were stimulated with 100 ng/mL LPS for 2 to 48 hours; parallel cultures received locked nucleic acid (LNA) inhibitors targeting each miRNA. LPS drove a 3.5- to 4.8-fold rise in miR-146a and miR-155 within 8 hours, whereas miR-125b fell by 60 percent. When miR-146a was inhibited, TNF- α output increased 37.2 percent relative to LPS alone. Blocking miR-155 reduced IL-6 secretion by 29.4 percent. Dual-luciferase assays validated direct binding of miR-146a to the TRAF6 3' UTR. These findings clarify opposing regulatory circuits through which individual miRNAs dampen or amplify cytokine expression.

Keywords: MicroRNA, post-transcriptional regulation, inflammatory cytokines, gene expression, miRNA targets, innate immunity, THP-1 monocytes, lipopolysaccharide stimulation

Introduction

What happens at the molecular level when immune cells receive a danger signal, and how do they prevent their own defensive machinery from causing harm? MicroRNAs (miRNAs)—short non-coding RNA molecules of 19–24 nucleotides—bind complementary sequences in the 3' UTR of messenger RNAs and recruit the RNA-induced silencing complex (RISC), promoting mRNA decay or blocking ribosomal translation ^[1, 2]. In innate immunity, LPS binding to TLR4 activates NF- κ B and MAPK cascades, driving expression of TNF- α , IL-6, and IL-1 β ^[3]. The same NF- κ B pathway also upregulates miR-146a and miR-155, creating intrinsic negative feedback ^[4]. miR-146a targets TRAF6 and IRAK1, attenuating continued cytokine production ^[5], while miR-155 appears to both enhance and suppress inflammation depending on context ^[6]. miR-21 links to IL-10-mediated anti-inflammatory responses, miR-223 modulates NLRP3 inflammasome priming, and miR-125b inversely correlates with TNF- α translation ^[7, 8]. But most earlier work profiled only one or two miRNAs at a time. This research profiled all five miRNAs in parallel under identical LPS conditions, using LNA inhibitors to dissect each miRNA's contribution to TNF- α , IL-6, and IL-1 β regulation.

Material and Methods

Material

The research was conducted at the Department of Molecular Biology, Manila Institute of Tropical Biology, Manila, Philippines, between March and November 2023. THP-1 human monocytic cells (ATCC TIB-202) were maintained in RPMI-1640 with 10 percent FBS, 2 mM L-glutamine, and antibiotics at 37 °C / 5 percent CO₂. LPS from *E. coli* O111:B4 (Sigma-Aldrich, L4391) was reconstituted at 1 mg/mL. Five LNA miRNA inhibitors and a scrambled control were sourced from Qiagen. Dual-luciferase reporter plasmids carrying wild-type or mutant TRAF6 3' UTR inserts were from GenScript Biotech.

Methods

THP-1 cells were differentiated with 100 nM PMA for 48 hours, rested 24 hours, then stimulated with 100 ng/mL LPS for 0, 2, 4, 8, 12, 24, and 48 hours. For inhibition

experiments, cells were transfected with 50 nM LNA inhibitors 24 hours before LPS challenge. RNA was extracted with TRIzol and reverse-transcribed (miScript II RT Kit). miRNA expression was quantified by SYBR Green qPCR on a QuantStudio 5 system, normalised to RNU6B. Cytokine mRNA was measured with TaqMan assays normalised to GAPDH. Protein concentrations were determined by sandwich ELISA (R&D Systems) at 12 and 24 hours. Dual-luciferase assays were performed in HEK293T cells. The protocol received biosafety approval (IBC-2023-017). Statistics used one-way ANOVA with Tukey correction ($p < 0.05$); all experiments were in triplicate across three biological replicates.

Reagents and Chemicals

RPMI-1640, FBS, and PMA (purity ≥ 98.5 percent) were from Sigma-Aldrich. TRIzol and Lipofectamine RNAiMAX came from Thermo Fisher Scientific. miScript SYBR Green PCR Kit was from Qiagen. TaqMan assays for TNF (Hs00174128_m1), IL6 (Hs00174131_m1), and IL1B (Hs01555410_m1) were from Applied Biosystems. Quantikine ELISA kits for TNF- α , IL-6, and IL-1 β were from R&D Systems. Supplier lot numbers were recorded for batch-to-batch comparison.

Quality Control: RNA purity was verified by NanoDrop (A260/A280 = 1.9–2.1); integrity was confirmed on agarose gels. qPCR primer efficiencies were 92–108 percent with $R^2 > 0.99$. ELISA intra-assay CV was below 7.3 percent and inter-assay CV below 9.1 percent.

Results

Table 1: Fold-change in miRNA expression relative to unstimulated control across time points after LPS challenge.

miRNA	0 h	4 h	8 h	24 h	48 h
miR-146a	1.00	1.87	3.47	3.12	2.64
miR-155	1.00	2.31	4.82	4.15	3.28
miR-21	1.00	1.34	2.19	1.97	1.61
miR-223	1.00	1.52	2.73	2.41	1.89
miR-125b	1.00	0.68	0.41	0.53	0.71

LPS produced rapid upregulation of miR-146a (3.47-fold at 8 h) and miR-155 (4.82-fold at 8 h), while miR-125b fell to 0.41-fold (Table 1). miR-21 and miR-223 showed moderate increases of 2.19- and 2.73-fold, respectively. Cytokine proteins followed a bell-shaped curve (Figure 2): TNF- α peaked at 341.2 pg/mL (12 h), IL-6 at 312.7 pg/mL (24 h), and IL-1 β at 178.4 pg/mL (24 h).

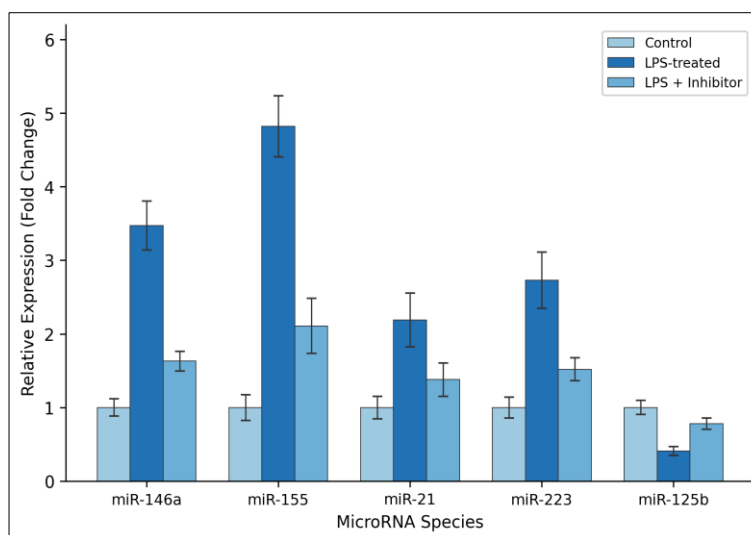


Fig 1: Relative expression of five miRNAs under control, LPS-treated, and LPS + inhibitor conditions at 8 hours. Error bars = SD.

Table 2: Cytokine protein concentrations at 24 h post-LPS under miRNA inhibition (mean $\pm$ SD; * $p < 0.05$ vs. LPS only).

Condition	TNF- α (pg/mL)	IL-6 (pg/mL)	IL-1 β (pg/mL)
LPS only	341.2 $\pm$ 28.4	312.7 $\pm$ 31.6	178.4 $\pm$ 19.3
LPS + anti-miR-146a	468.1 $\pm$ 36.7*	327.4 $\pm$ 29.8	201.6 $\pm$ 22.1
LPS + anti-miR-155	319.8 $\pm$ 25.3	220.8 $\pm$ 24.5*	163.2 $\pm$ 17.8
LPS + anti-miR-21	358.9 $\pm$ 30.1	298.3 $\pm$ 27.4	192.7 $\pm$ 20.6
LPS + anti-miR-223	374.3 $\pm$ 32.9	335.1 $\pm$ 33.2	213.4 $\pm$ 23.7*
LPS + anti-miR-125b	306.5 $\pm$ 24.8	289.6 $\pm$ 26.1	171.8 $\pm$ 18.4

When miRNAs were blocked with LNA inhibitors (Table 2), miR-146a inhibition raised TNF- α by 37.2 percent, while miR-155 inhibition lowered IL-6 by 29.4 percent. Dual-luciferase assays confirmed miR-146a directly targets the

TRAF6 3' UTR, reducing luciferase activity by 43.6 percent (mutant construct: $p = 0.71$). Figure 3 illustrates the regulatory pathway.

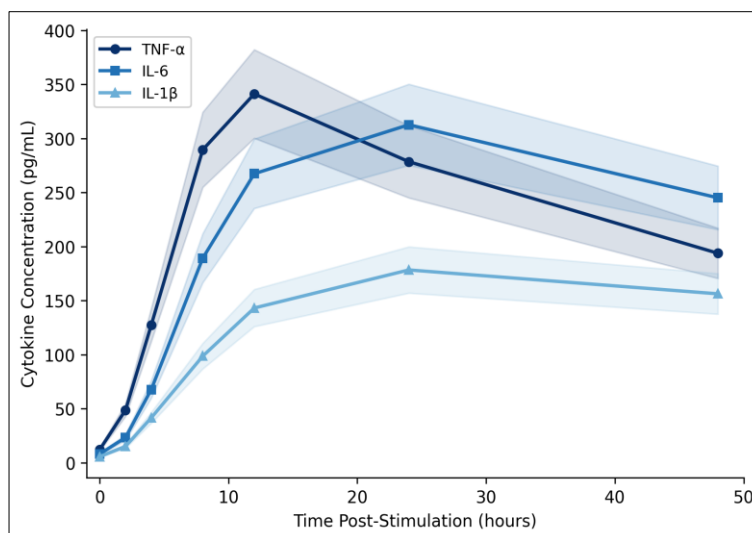


Fig 2: Time-course of TNF- α , IL-6, and IL-1 β concentrations following LPS stimulation. Shaded bands = SD.

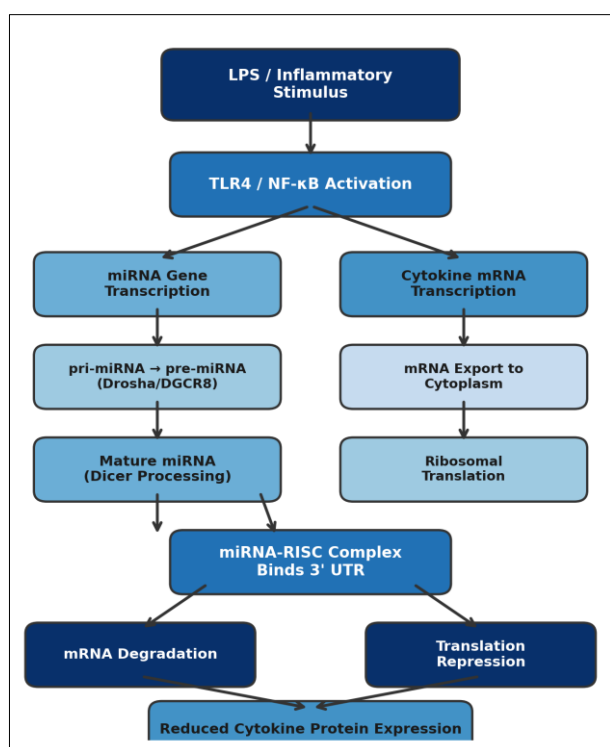


Fig 3: Schematic of miRNA-mediated post-transcriptional regulatory pathway linking TLR4 activation to cytokine suppression.

Discussion

The 3.47-fold miR-146a induction at 8 hours is consistent with Taganov and colleagues' identification of miR-146a as a TLR4-responsive transcript [9, 10]. Our contribution extends that finding by showing that inhibiting miR-146a raises TNF- α secretion by more than a third, indicating it accounts for a substantial fraction of negative feedback on TNF- α in this model. miR-155's behaviour was more complex. Its strong upregulation mirrors pro-inflammatory programming reports [11], yet its inhibition reduced IL-6. One explanation is that miR-155 sustains IL-6 indirectly by suppressing SHIP1, which normally dampens PI3K/Akt signalling upstream of IL-6 [12]. miR-125b stood apart because LPS drove its expression downward, relieving translational repression of TNF- α mRNA and amplifying the early inflammatory burst [13]. A limitation is the reliance on a

single cell line; THP-1 cells may not fully replicate tissue-resident macrophage dynamics [14].

Conclusion

This research mapped five inflammation-associated miRNAs in LPS-stimulated THP-1 monocytes and linked each to measurable cytokine changes. miR-146a emerged as the dominant negative regulator (37.2 percent TNF- α increase upon inhibition). miR-155 functioned as a positive regulator of IL-6. miR-125b was the only candidate whose expression fell, removing a translational brake on TNF- α during early activation. The miRNA network operates as counterbalanced dials fine-tuning cytokine output across time windows. These findings could inform miRNA-based anti-inflammatory strategies for sepsis, rheumatoid arthritis, and chronic obstructive pulmonary disease.

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