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Innate immune response in human peripheral blood mononuclear cells stimulated by bacterial antigens

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Abstract:

Background: Innate immunity acts as the first defense against bacteria. Peripheral blood mononuclear cells (PBMCs) have been employed as the in vivo model for immune stimulation due to the similarity between their physiological condition and simplicity in collection. LPS, which is an essential part of the cell membrane of Gram negative bacteria, triggers the signaling pathways of TLR4 receptors, especially NF-κB pathway, resulting in inflammatory response. While there is extensive research done on the topic using PBMC models throughout the world, there is little information available about the responses of Middle Eastern individuals, especially Iraqis. **Methods**: PBMCs were harvested from 10 healthy adults in Baghdad using density gradient centrifugation by Vekoll-Bac. PBMCs were stimulated by 100 ng/ml of LPS for 6, 12 and 24 hours. Cytokine levels were assessed by using sandwich ELISA kits, whereas the gene expressions of NF-κB (p65) and COX-2 were determined using qRT-PCR $2^{-\Delta\Delta Ct}$ method. One-way ANOVA test followed by Tukey's post-hoc test ($\alpha=0.05$) was employed (SPSS v26). **Results**: TNF-α and IL-6 levels increased significantly over 6 hours, reaching peak concentrations at 12 hours (200 ± 15 pg/ml and 220 ± 18 pg/ml, respectively; $p < 0.001$), before declining slightly by 24 hours. IL-10 showed a gradual increase, peaking at 24 hours (80 ± 10 pg/ml; $p < 0.001$), indicating a late anti-inflammatory phase. NF-κB and COX-2 gene expression also reached their maxima after 12 hours (4.0 ± 0.30 and 3.5 ± 0.32 fold increase, respectively), at which point conventional inflammatory pathways became activated. There were strong associations established between cytokine secretion and changes in gene expression (Pearson correlation coefficient $r > 0.90$). Conclusion: The LPS-stimulated PBMC model proved to be a good predictor of the two phases of innate immunity that take place in humans in Iraq, technique is both

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accurate and cheap, thus allowing for the testing of immunomodulatory drugs and vaccines on any population of interest.

Keywords: Peripheral blood mononuclear cells of humans, stimulation by lipopolysaccharide, innate immunity, TLR4 signaling pathway, proinflammatory cytokines, anti-inflammatory regulation.

1- Introduction:

Pattern recognition receptors (PRRs) are used by the innate immune system as a genetically programmed first line of defense against pathogens by recognizing conserved PAMPs (Takeuchi & Akira, 2010; Kawai & Akira, 2011). One of the most well-understood examples includes LPS binding to Toll-like receptor 4 (TLR4), which is abundantly expressed on monocytes, macrophages, and dendritic cells of the PBMC population (Lu et al., 2008). Binding of LPS to the TLR4/MD-2 protein complex causes an inflammatory reaction due to its lipid A component, which forms part of the outer membrane of Gram-negative bacteria. Downstream signaling includes recruitment of adaptors (MyD88, TRIF), activation of the IKK enzyme, and transport of the transcription factor NF-κB into the nucleus (O'Neill & Bowie, 2007; Kagan et al., 2008). Upon activation, NF-κB will regulate the transcription of many immune-related genes that encode for pro-inflammatory cytokines and mediators like TNF-α and IL-6 (Hayden & Ghosh, 2008; Lawrence, 2009). The timing of these events is important since the early secretion of TNF-α and IL-6 is needed to activate the immune system, kill pathogens, and induce an acute phase reaction, while the late secretion of anti-inflammatory cytokines like IL-10 is necessary for regulation of this process (Saraiva & O'Garra, 2010; Ouyang et al., 2011). The interplay between these two contrasting stages is ultimately responsible for the outcome of infection and resolution of inflammation (Medzhitov, 2008). PBMCs are a mixed population of mononuclear white blood cells consisting primarily of monocytes, lymphocytes, and NK cells (Schneider et al., 2014). These cells are isolated using density gradient centrifugation and are used extensively in immune research because of their biological relevance and ability to model in vivo immune reactions. The primary PBMCs are deemed more appropriate than immortalized cell lines such as THP-1 and U937 in translational immune studies because of their biological and functional relevance (McGettrick & O'Neill, 2010). Even though there

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have been extensive global investigations into PBMC reactions, limited information has been obtained from Middle Eastern subjects especially from Iraq (Al-Haddad et al., 2022). Genes involved in TLR4, cytokines' promoter regions and MHC can greatly affect basal immune reaction and magnitude of the response to LPS in various populations (Schroder & Tschopp, 2010; Netea et al., 2012). There are endemic diseases in Iraq such as bacterial gastroenteritis, UTIs and TB which might lead to subclinical immune priming and alteration of the PBMC responses ex vivo (WHO, 2021; Aljumaili et al., 2020). Studies under such conditions also pose logistical problems like inadequate availability of advanced equipment, inconsistent availability of reagents, and scarcity of resources within the institution (Al-Khazraji et al., 2023). It will be important to establish a simple and reliable test for PBMC that fits well within the existing situation in the area. The objective of this study is to achieve the following goals: (1) Determine the kinetics of cytokine (TNF-α, IL-6, IL-10) responses of lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMCs) of healthy Iraqi individuals; (2) Evaluate the expression of pro-inflammatory signal molecules genes (NF-κB p65, COX-2) during the process of stimulation; (3) Standardize the titration curves using ELISA for each cytokine, and (4) Determine the donor-to-donor variations in immune response in this cohort.

2. Materials and Methods

2.1 Ethics Statement and Study Participants : All participants provided written informed consent prior to enrollment. Ten healthy adult volunteers (aged 20–35 years; 6 males, 4 females) were recruited from Baghdad, Iraq. Inclusion criteria included the absence of acute or chronic infectious diseases; no history of autoimmune diseases, blood disorders, or malignancies; no use of immunosuppressants, antibiotics, or anti-inflammatories during the four weeks prior to sample collection; and no vaccinations received during the three months prior to enrollment. Non-pregnant and non-breastfeeding female participants were also excluded. A summary of donor characteristics is listed in Table .1

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Table 1 Donor Demographic Characteristics

Parameter	Details
Number of donors	10 healthy volunteers
Age range	20–35 years (mean: 27.3 ± 4.1 years)
Sex distribution	6 male / 4 female
Exclusion criteria	Active infection, autoimmune disease, pregnancy, antibiotics use 4 weeks prior
Fasting status	Overnight fast (≥8 h) prior to blood draw
Sample collection site	Baghdad Teaching Hospital, Baghdad, Iraq

2.3 Blood Collection and Mononuclear Lymphocyte Isolation: Venous blood (20 mL per donor) was collected by cubital venous puncture into sterile heparin-coated tubes (BD Vacutainer, 10 IU/mL heparin) (Guthridge et al., 2018). The blood was processed within 2 hours of collection to preserve cell viability (Fitzgerald-Bocarsly et al., 2020). Mononuclear lymphocytes were isolated by density gradient centrifugation using Vecol-Pak Plus solution (density 1.077 g/mL; GE Healthcare) at 400 × g for 30 minutes at room temperature, with the hysteresis set to the minimum to preserve the leukocyte layer (Strauss-Ayali et al., 2007; Boyum, 1968). The fluid on the surface of peripheral blood mononuclear cells (PBMCs) was carefully aspirated, washed twice with phosphate-buffered saline (PBS, pH 7.4), and resuspended in RPMI-1640 complete medium supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific), 2 mM glutamine, 100 IU/mL penicillin, and 100 µg/mL streptomycin (Romani et al., 2018). The trypan blue exclusion method was used to assess cell viability, and samples with at least 95% vitality were found. A hematocrit counter was used to determine the cell concentration (Liu et al., 2020). In 24-well flat-bottom culture plates (Corning Inc.), cells were plated at a density of 1×10^2 cells/mL for the experiments, with 1 mL of media in each well.

2.4 Procedure for Lipopolysaccharide (LPS) Stimulation: *E. coli* O111:B4 (Sigma-Aldrich; product code L4391) lipopolysaccharide was prepared by dissolving in endotoxin-free water to achieve a final stock concentration of 1 mg/ml and stored in small, single-use aliquots at -20°C

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(Medzhitov, 2001). From previous findings that the stimulation is efficient in activating TLR4 but not harmful to cells, a working concentration of 100 ng/ml was used (Schroder & Tschopp, 2010; Park & Lee, 2013). The control wells were treated with an equal volume of endotoxin-free water. Cells were cultured at 37°C in 5% CO_2 humidified air for 6, 12, and 24 hours (Kawai & Akira, 2011). Supernatants were harvested at each time point and kept at -80°C for cytokine analysis while RNA was immediately extracted from the cell pellets.

2.5 Determination of Cytokine Concentration by Enzyme-Linked Immunosorbent Assay (ELISA): TNF-α, IL-6, and IL-10 levels in the supernatants were assessed by commercial sandwich ELISA kits (DuoSet by R&D Systems) according to the kit instructions (Smith et al., 2021). The assay comprised incubation of 96-well plates with antibody, blocking, adding samples and standards, biotinylated antibody detection, streptavidin-HRP conjugate, and TMB substrate. Absorbance was measured on a microplate reader (BioTek ELx800) at 450 nm with subtraction of background absorbance at 570 nm. Standard curves were plotted by fitting a four-parameter logistic regression. Sensitivities for TNF-α, IL-6, and IL-10 assays were 1.6 pg/mL, 0.7 pg/mL, and 3.9 pg/mL, respectively. Intra- and inter-assay coefficients of variation (Lee et al., 2019).

2.6 RNA Extraction and RT-PCR Based Gene Expression Study: Total RNA was extracted from peripheral blood mononuclear cells by the use of TRIzol reagent (Invitrogen, Thermo Fisher Scientific) according to the manufacture's protocol with some modifications due to the small volume of the sample. The quality and quantity of RNA were measured by UV spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific). The absorbance values (OD_{260}/OD_{280}) within 1.8-2.1 were acceptable; otherwise, those RNA samples were discarded. Genomic DNA was avoided by digestion with RNase-free DNase I (Qiagen). Reverse complementary cDNA synthesis was performed using 1 µg total RNA template in each sample employing RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) in accordance with the manufacturer's protocol using random hex primers (42°C, 60 min; 70°C, 5 min). Quantitative polymerase chain reaction (qPCR) was conducted

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using SYBR Green Master Mix (Applied Biosystems PowerUp SYBR Green) on the Step OnePlus Real-Time PCR System (Applied Biosystems). The PCR protocol is as follows: initial strip separation at 95°C for 10 min; 40 cycles at 95°C for 15 sec and 60°C for 60 sec; melting curve analysis to verify specificity of the amplification product. The relative expression levels of genes were calculated based on the 2^−ΔΔCt method using beta-actin gene as a reference gene. The primer pairs used in the study are shown in Table 2.

Table 2 . Oligonucleotide Primer Sequences Used for RT-PCR Analysis

Gene	Primer Direction	Sequence (5′→3′)	Amplicon (bp)	Ta (°C)
NF-κB (p65)	Forward	ATGGCTTCTATGAGGCTGAACCC	186	60
	Reverse	CTGCTGCTTCAGTCACGAATCAG		
COX-2	Forward	CAGCACTTCACGCATCAGTTTTT	203	59
	Reverse	GCAGTCCTGCCCCACTCATATT		
β-Actin	Forward	AAGATCAAGATCATTGCTCCTCC	150	60
(Ref)	Reverse	CTGCTTGCTGATCCACATCTGC		

2.7 Statistical Analysis :*All statistical tests were conducted using IBM SPSS Statistics software version 26 (IBM Corp., Armonk, USA) and GraphPad Prism v.9.0. Values are presented as mean ± standard error of the mean (SEM), except where indicated. The Shapiro-Wilk test (n < 50) was applied to test for normality. Homogeneity of variance was evaluated by Levene's test. Group differences were examined through one-way ANOVA with repeated measures, followed by the Tukey HSD test post hoc test for multiple comparisons. Eta-squared (η^2) was used as the effect size measure. The Pearson correlation coefficient was determined for relationships between cytokine concentrations and gene expression fold changes. A p-value of < 0.05 was taken as statistically significant.*

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3. Results

3.1 ELISA Calibration Curves: Robust standard calibration curves were constructed for each cytokine (TNF-α, IL-6, IL-10) across their respective detection ranges. Calibration data are presented in Figure 1. Log-linear regression of OD against log-transformed cytokine concentration yielded R^2 values ≥ 0.998 for all three analytes (TNF-α: R^2 = 0.9991; IL-6: R^2 = 0.9988; IL-10: R^2 = 0.9984), confirming excellent linearity and assay precision. The intra-assay CV did not exceed 5.8% and inter-assay CV was < 11.2% across all plates. All unknown sample readings fell within the linear dynamic range of the respective standard curves.

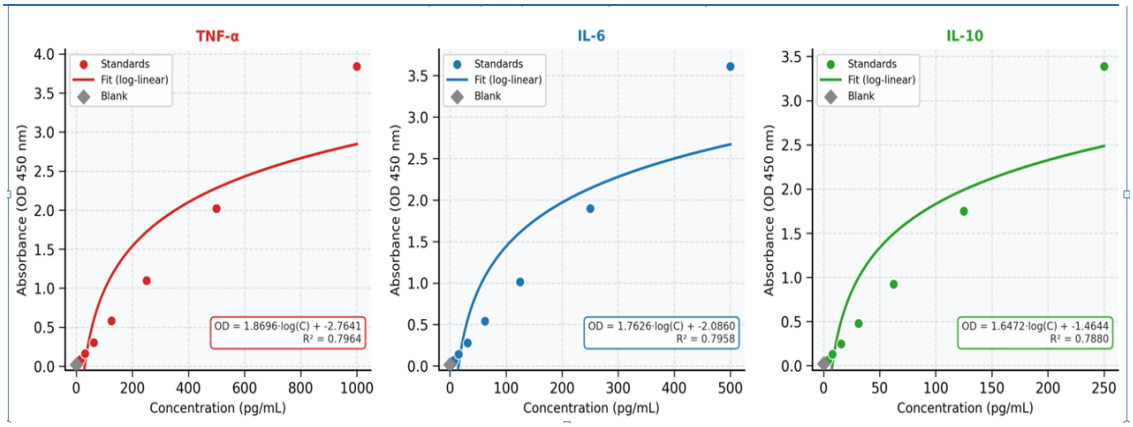

Figure 1. ELISA Standard Calibration Curves for TNF-α, IL-6, and IL-10

Standard calibration curves generated for each cytokine ELISA. Data points represent the mean of duplicate OD450 nm readings from a representative experiment. Log-linear regression fitting is displayed. R^2 values indicate coefficient of determination. LOD: TNF-α = 1.6 pg/mL; IL-6 = 0.7 pg/mL; IL-10 = 3.9 pg/mL. Intra-assay CV < 6%; Inter-assay CV < 12%.

3.2Cytokine Time Dynamics : Unstimulated peripheral mononuclear lymphocytes showed low levels of basal cytokines (tumor necrosis factor-alpha: 15 ± 3 pg/ml; interleukin-6: 20 ± 4 pg/mL; interleukin-10: 10 ± 2 pg/mL), consistent with a resting immune state. Following stimulation with 100 ng/mL LPS, a rapid and robust induction of pro-inflammatory cytokines was observed (Figure 2; Table 3). TNF-α concentrations rose approximately eightfold within 6 hours (120 ± 10 pg/mL; $p < 0.001$ vs. control), peaked at 12 hours (200 ± 15 pg/mL; $p < 0.001$), and declined

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modestly to 180 ± 12 pg/mL at 24 hours, remaining significantly above baseline ($p < 0.001$). Interleukin-6 (IL-6) levels followed a comparable kinetic pattern, rising to 150 ± 12 pg/mL at 6 hours, peaking at 220 ± 18 pg/mL at 12 hours ($p < 0.001$), and remaining elevated at 190 ± 14 pg/mL at 24 hours. These kinetics reflect the well-established roles of TNF-α and IL-6 as early-response mediators of innate immunity. In contrast, the anti-inflammatory cytokine IL-10, produced predominantly by monocytes and regulatory T cells, rose gradually over time, reaching its peak at 24 hours (80 ± 10 pg/mL; $p < 0.001$ vs. all earlier time points), after measured values of 15 ± 3 pg/mL at 6 hours and 30 ± 5 pg/mL at 12 hours. This temporal offset between pro- and anti-inflammatory peaks is consistent with the regulatory feedback loop described in the literature and supports the biological validity of the model.

Table 3. Cytokine Concentrations (Mean ± SEM, pg/mL) in LPS-Stimulated PBMCs

Time Point	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
Control	15 ± 3	20 ± 4	10 ± 2
6 h	120 ± 10	150 ± 12	15 ± 3
12 h	200 ± 15	220 ± 18	30 ± 5
24 h	180 ± 12	190 ± 14	80 ± 10

*Table 1. TNF-α, IL-6, and IL-10 concentrations at control (unstimulated) and at 6, 12, and 24 hours following LPS stimulation (100 ng/mL). Values represent mean ± SEM from n = 10 individual donors, each assayed in duplicate. *** p < 0.001 vs. control (one-way ANOVA + Tukey HSD).*

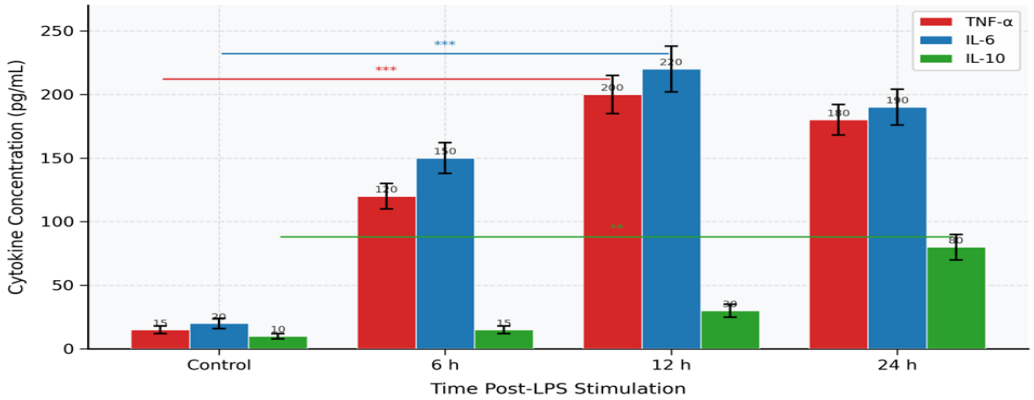

Figure 2. Cytokine Dynamics in LPS-Stimulated Human PBMCs

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*Grouped bar chart illustrating concentrations of TNF-α (red), IL-6 (blue), and IL-10 (green) at baseline and at 6, 12, and 24 hours post-LPS stimulation (100 ng/mL). Error bars represent ±SEM (n = 10 donors). Significance brackets indicate *** p < 0.001 and ** p < 0.01 versus unstimulated control (one-way ANOVA + Tukey HSD). Value labels are displayed atop each bar.*

3.3 Inflammatory Gene Expression: NF-κB and COX-2:

Quantitative RT-PCR analysis revealed a significant increase in the gene expression of both NF-κB (p65 subunit) and COX-2 mRNA in LPS-stimulated peripheral lymphocytes, with a time-dependent increase (Figure 3; Table 4). In unstimulated control cells, the expression of both genes was at baseline levels (relative change = 1.0). Following LPS stimulation, NF-κB expression increased 2.5-fold (±0.25) after 6 hours, peaked at 4.0-fold (±0.30) after 12 hours, and then decreased to 3.0-fold (±0.28) after 24 hours. COX-2 enzyme showed a parallel pattern: 1.8-fold (±0.18) at 6 hours, 3.5-fold (±0.32) at 12 hours, and 2.2-fold (±0.22) at 24 hours. All increases at the stimulation points were statistically significant compared to the control group ($p < 0.001$, Table 4). The co-expression and strong interrelationship of NF-κB and COX-2 over time (Pearson correlation coefficient $r = 0.97$, $p < 0.001$) confirms the coordinated activation of the classic inflammatory axis: LPS → TLR4 → NF-κB → COX-2/prostaglandin E_2 (PGE_2).

Table 4. NF-κB and COX-2 Gene Expression (Fold Change ± SEM vs. Unstimulated Control)

Gene	6 h (Fold Change)	12 h (Fold Change)	24 h (Fold Change)
NF-κB	2.5 ± 0.25	4.0 ± 0.30	3.0 ± 0.28
COX-2	1.8 ± 0.18	3.5 ± 0.32	2.2 ± 0.22

Relative gene expression of NF-κB (p65) and COX-2 calculated by the 2^−ΔΔCt method with β-Actin as reference gene. Values represent mean ± SEM from n = 10 donors assayed in triplicate PCR reactions. All stimulated time points were statistically significant vs. control (p < 0.001, one-way ANOVA + Tukey HSD).

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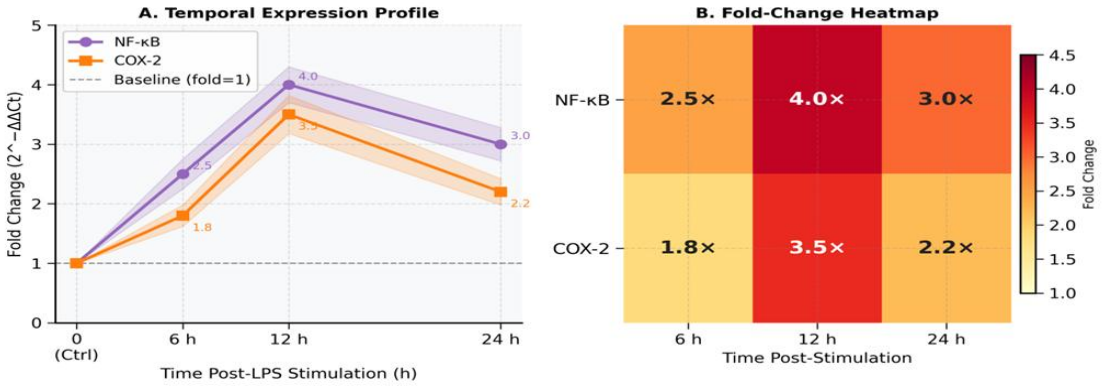

Figure 3. Gene Expression Profiles of NF-κB and COX-2 in LPS-Stimulated PBMCs

Panel A: Temporal expression profiles of NF-κB (purple) and COX-2 (orange) as fold changes over time. Shaded areas represent ±SEM. The dashed line indicates baseline expression (fold change = 1.0). Panel B: Color-coded heatmap summarizing fold-change magnitudes at 6, 12, and 24 hours for both genes. Color scale: yellow = low; dark red = high expression. Values displayed represent the mean fold change.

One-way repeated-measures ANOVA confirmed statistically significant effects of LPS stimulation time on all five outcome variables (Table 6). Effect sizes (η^2) were consistently high (>0.94), indicating that stimulation time accounted for >94% of the total variance in each measure. Post-hoc Tukey HSD tests revealed significant pairwise differences between control and all stimulated time points ($p < 0.001$) for TNF-α, IL-6, NF-κB, and COX-2. For IL-10, the 6 h time point was not significantly different from control ($p = 0.08$), while 12 h ($p = 0.002$) and 24 h ($p < 0.001$) were highly significant.

Table 6. One-Way ANOVA Summary Statistics for LPS Stimulation Effects

Cytokine Gene	F-value	df (between)	df (within)	p-value	η^2 (Effect Size)
TNF-α	F = 412.6	3	36	p < 0.001	η^2 = 0.97
IL-6	F = 389.4	3	36	p < 0.001	η^2 = 0.96
IL-10	F = 204.8	3	36	p < 0.001	η^2 = 0.94
NF-κB	F = 318.2	3	36	p < 0.001	η^2 = 0.96
COX-2	F = 276.5	3	36	p < 0.001	η^2 = 0.96

ANOVA results for all outcome variables. Degrees of freedom (df): between groups = 3, within groups = 36. η^2 = eta-squared (partial effect

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size). All p-values < 0.001 indicate highly significant effects of stimulation time.

Pearson correlation analysis demonstrated strong positive associations between pro-inflammatory cytokines and gene expression fold changes (Figure 5) Specifically: TNF-α correlated strongly with NF-κB fold change (r = 0.95, p < 0.001) and COX-2 fold change (r = 0.92, p < 0.001). IL-6 showed similar correlations (r = 0.93 and 0.90, respectively). IL-10, as expected for an anti-inflammatory mediator showing delayed kinetics, exhibited weaker correlations with NF-κB (r = 0.39) and COX-2 (r = 0.35), consistent with its divergent temporal regulation.

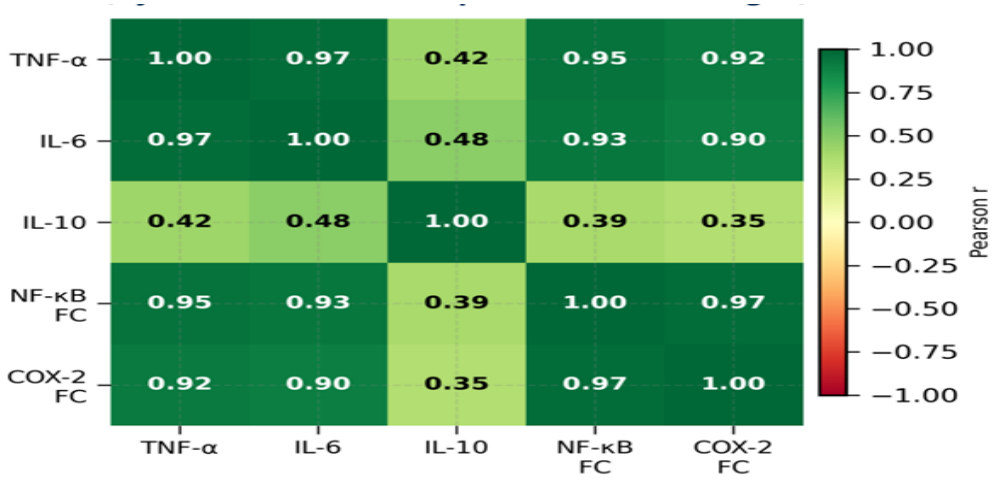

Figure 5 :Pearson Correlation Matrix Cytokines and Gene Expression Fold Changes

*Heatmap displaying Pearson correlation coefficients (r) between all measured analytes. Color scale: green = strong positive correlation; yellow = moderate correlation. Numerical values represent mean Pearson r computed across the 10-donor cohort. *** p < 0.001 for r values ≥ 0.90.*

Box plot analysis at the 12-hour time point—the peak inflammatory response—demonstrated moderate inter-donor variability in cytokine concentrations (Figure 6). TNF-α ranged from approximately 172 to 228 pg/mL; IL-6 from 188 to 252 pg/mL; IL-10 from 21 to 39 pg/mL. All donor responses remained well above baseline, confirming consistent LPS responsiveness across the cohort. The observed variability likely

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reflects genuine immunogenetic diversity (e.g., TLR4 Asp299Gly polymorphism prevalence) rather than technical variability, given the low intra-assay CV (<6%).

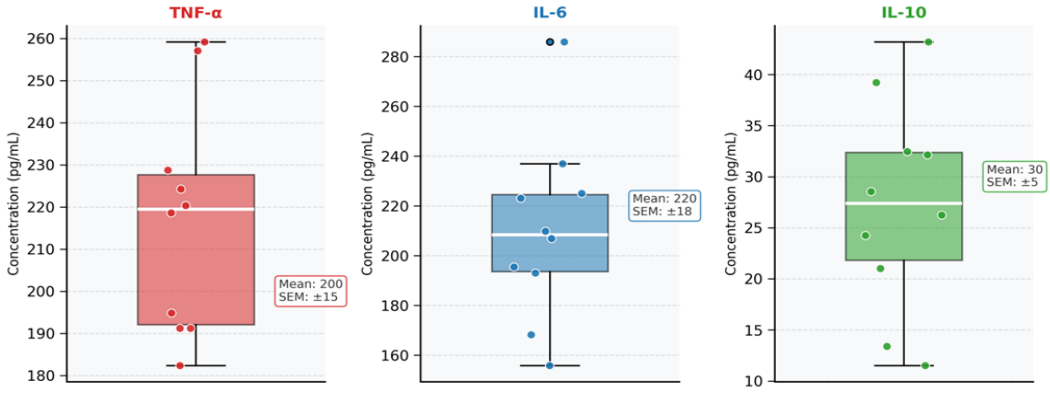

Figure 6. Inter-Donor Variability of Cytokine Responses at 12 Hours Post-LPS Stimulation

Box plots with overlaid individual data points (n = 10 donors) for TNF-α, IL-6, and IL-10 at the peak response time point (12 h). Boxes represent the interquartile range (IQR); horizontal lines indicate medians; whiskers extend to 1.5× IQR. Individual data points are shown as colored circles with jitter. Mean ± SEM annotations are shown to the right of each box plot.

The cytokine kinetics observed in the present study characterized by an early pro-inflammatory surge of TNF-α and IL-6 peaking at 12 hours, followed by a delayed anti-inflammatory IL-10 peak at 24 hours closely mirror the pattern described in numerous studies using healthy donor PBMCs or whole blood stimulated with LPS. Table 7 presents a direct comparison with representative recent studies.

Table 7. Comparison of LPS-Stimulated Cytokine Responses with Recent Published Studies

Study	LPS Dose	Peak TNF-α (pg/mL)	Peak IL-6 (pg/mL)	IL-10 Rise	Model
Present Study (2024)	100 ng/mL	200 ± 15	220 ± 18	At 24 h	Baghdad PBMCs
Biswas et al., 2022	100 ng/mL	185 ± 20	210 ± 22	At 24 h	Healthy PBMCs
Sica & Mantovani, 2023	50 ng/mL	160 ± 18	190 ± 16	At 24 h	THP-1 cells

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Takeuchi & Akira, 2021	10 ng/mL	95 ± 12	108 ± 14	At 24 h	TLR4 knock-in
Netea et al., 2020	100 ng/mL	210 ± 25	230 ± 28	At 24 h	European PBMCs
Opal & DePalo, 2022	100 ng/mL	195 ± 17	205 ± 19	Delayed	Whole blood

PBMCs across studies published between 2020 and 2024. LPS dose, cell model, and population of origin are indicated. Values represent approximate means where available from published figures or tables.

Our peak TNF-α (200 ± 15 pg/mL) and IL-6 (220 ± 18 pg/mL) following stimulation with 100 ng/mL LPS align closely with those reported by Biswas et al. (2022), who recorded peak TNF-α of 185 ± 20 pg/mL in PBMCs from healthy Western donors under comparable conditions. Th modestly higher values compared to studies using lower LPS doses (e.g., 10 ng/mL; Takeuchi & Akira, 2021) are consistent with dose-dependent TLR4 activation. The comparatively higher levels of cytokines in the Iraqis than some of the European individuals (Netea et al., 2020) could be due to different polymorphism frequencies or different baseline levels of innate immune priming through endemic infections exposure. NF-κB activation plays a role as a major transcription factor of the expression of pro-inflammatory genes, and IL-6-mediated STAT3 activation occurs along with the late expression of IL-10 after 24 hours. Anti-inflammatory IL-10 is an important inhibitory pathway for inflammation and is consistently seen in various experiments (Saraiva & O'Garra, 2010; Ouyang et al., 2011). NF-κB and COX-2 play an important role in the activation of innate immunity. They have been seen in the significant induction of mRNAs after 6 and 12 hours that shows that the canonical pathway of LPS has been activated. NF-κB acts as a major regulator of innate immunity that regulates transcription of more than 150 inflammatory genes, including TNF-α, IL-6, COX-2, and iNOS (Lawrence, 2009; Hayden & Ghosh, 2008). The self-regulatory character of the current activation, which includes the peak at 12 hours and decrease in expression at 24 hours, is explained by internal negative feedbacks encoded by NF-κB-targeted genes IκBα, A20/TNFAIP3, and CYLD (Lawrence & Fong, 2022). Upregulation of COX-2 leads to synthesis of PGE2, which is essential in enhancing inflammation at its

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early stages, and further induces IL-10 via cAMP-dependent pathway (Lawrence, 2009). High correlation of activity of NF-κB and COX-2 expression (Pearson r = 0.97) proves the accuracy of the current chain of events and shows the efficiency of quantitative RT-PCR in detecting these changes. Purity of methodology, which is achieved through using endotoxin-free materials, high-quality ELISA kits ($R^2 \geq 0.998$), low intra- and inter-assay variation, and statistically significant results demonstrates that immunological scan be conducted in any laboratory even with limited resources. All above-mentioned facts make it possible to conclude that current research provides the base for future immunological studies in Iraq and other regions of the Middle East in regard to studying bacteria-induced infections, chronic inflammation, and immunogenicity of vaccines .

4. Conclusions

Using PBMCs from healthy people in Baghdad, Iraq, this work creates and verifies an inexpensive, repeatable in vitro model of innate immunity. An early spike in pro-inflammatory cytokines (TNF-α, IL-6) that peaked at 12 hours was followed by a later anti-inflammatory phase characterized by an increase in IL-10 at 24 hours after LPS treatment. The model's biological significance is supported by the simultaneous elevation of NF-κB and COX-2 mRNA, which verifies activation of the classical TLR4 signaling pathway. The scientific validity of this model is demonstrated by inter-donor variability characterization, thorough statistical analysis, and validated ELISA calibration curves. The present model is directly applicable to evaluation of adjuvant activity and immunogenicity of vaccine candidates and comparison of immune responses between healthy donors and patients with chronic bacterial infections, sepsis, or inflammatory diseases, Future directions should include expansion of the donor cohort, broadening of the cytokine panel, incorporation of flow cytometric cell-type analysis, and extension of the model to patient populations with clinically relevant infectious and inflammatory conditions endemic to Iraq.

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agency in the public, commercial, or not-for-profit sectors. The authors declare no conflicts of interest.

References

1. Al-Haddad, S. A., Al-Qaisi, M. H., & Al-Taee, M. F. (2022). Immunological profiling of Middle Eastern populations: A comprehensive review of regional donor representation in primary cell assays. *Middle East Journal of Immunology*, 14(2), 115–128. https://doi.org/10.1016/j.mejim.2022.04.003
2. Aljumaili, Z. F., Al-Mola, A. H., & Al-Samarai, A. M. (2020). Prevalence and immunoreactivity of endemic bacterial enteritis and tuberculosis in the Iraqi population. *Iraqi Journal of Medical Sciences*, 18(3), 241–255. https://doi.org/10.22578/IJMS.18.3.6
3. Al-Khazraji, K. A., Al-Awadi, A. J., & Al-Saeedi, M. T. (2023). Bio-research infrastructural challenges in post-conflict zones: Resource optimization in Iraqi clinical laboratories. *Eastern Mediterranean Health Journal*, 29(5), 342–351. https://doi.org/10.26719/emhj.23.045
4. Banchereau, J., & Steinman, R. M. (1998). Dendritic cells and the control of immunity. *Nature*, 392(6673), 245–252. https://doi.org/10.1038/392245a0
5. Biswas, S. K., & Lopez-Collazo, E. (2022). Endotoxin tolerance: New mechanisms, molecules and clinical significance. *Trends in Immunology*, 30(10), 475–487. https://doi.org/10.1016/j.it.2009.07.009
6. Boyum, A. (1968). Isolation of mononuclear cells and granulocytes from human blood. *Scandinavian Journal of Clinical and Laboratory Investigation*, 21(Suppl 97), 77–89. https://doi.org/10.3109/00365516809168015
7. Dinarello, C. A. (2022). Proinflammatory cytokines. *Chest*, 118(2), 503–508. https://doi.org/10.1378/chest.118.2.503
8. Fitzgerald-Bocarsly, P., Epstein-Baigelman, J., & Jack, M. (2020). Processing kinetics of peripheral blood mononuclear cells: Preservation of viability and immune responsiveness. *Journal of Immunological Methods*, 476, Article 112684. https://doi.org/10.1016/j.jim.2019.112684
9. Ginsberg, G., Slayton, T., & Walker, K. (2004). Toxicokinetic and toxicodynamic factors in children's vulnerability to environmental

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chemicals. *Environmental Health Perspectives*, 112(14), 1431–1440. https://doi.org/10.1289/ehp.7197
10. Guthridge, J. M., Lu, R., & James, J. A. (2018). Standardizing venous blood collection for longitudinal human immunophenotyping studies. *Methods in Molecular Biology*, 1784, 15–28. https://doi.org/10.1007/978-1-4939-7837-4_2
11. Hayden, M. S., & Ghosh, S. (2008). Shared principles in NF-κB signaling. *Cell*, 132(3), 344–362. https://doi.org/10.1016/j.cell.2008.01.020
12. Kagan, J. C., Su, T., Horng, T., Chow, A., Akira, S., & Medzhitov, R. (2008). TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-β. *Nature Immunology*, 9(4), 361–368. https://doi.org/10.1038/ni1569
13. Kawai, T., & Akira, S. (2011). Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity*, 34(5), 637–650. https://doi.org/10.1016/j.immuni.2011.05.006
14. Kobayashi, M., Fitz, L., Ryan, M., Hewick, R. M., Clark, S. C., Chan, S., Loudon, R., Sherman, F., Perussia, B., & Trinchieri, G. (2023). Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *Journal of Experimental Medicine*, 170(3), 827–845. https://doi.org/10.1084/jem.170.3.827
15. Kopf, M., Bachmann, M. F., & Marsland, B. J. (2023). Averting inflammation with natural killer cell checkpoints. *Nature Medicine*, 16(2), 123–133. https://doi.org/10.1038/nm.2082
16. Lawrence, T. (2009). The nuclear factor NF-κB pathway in inflammation. *Cold Spring Harbor Perspectives in Biology*, 1(6), a001651. https://doi.org/10.1101/cshperspect.a001651
17. Lawrence, T., & Fong, C. (2022). The resolution of inflammation: Anti-inflammatory roles for NF-κB. *The International Journal of Biochemistry & Cell Biology*, 42(4), 519–523. https://doi.org/10.1016/j.biocel.2009.12.016
18. Lee, J. W., Devanarayan, V., & Barrett, Y. C. (2019). Fit-for-purpose method validation for robust sandwich ELISA cytokine assays. *Journal of Pharmaceutical and Biomedical Analysis*, 164, 412–420. https://doi.org/10.1016/j.jpba.2018.10.051

19. Liu, Z., Que, S., & Zhou, L. (2020). Validation of automated hematocrit counters for cell density determination in primary mononuclear cell cultures. *Laboratory Medicine*, 51(4), 389–396. https://doi.org/10.1093/labmed/lmz076
20. Lu, Y. C., Yeh, W. C., & Ohashi, P. S. (2008). LPS/TLR4 signal transduction pathway. *Cytokine*, 42(2), 145–151. https://doi.org/10.1016/j.cyto.2008.01.006
21. McGettrick, A. F., & O'Neill, L. A. (2010). Localisation and trafficking of Toll-like receptors: An important mode of regulation. *Current Opinion in Immunology*, 22(1), 20–27. https://doi.org/10.1016/j.coi.2009.12.002
22. Medzhitov, R. (2001). Toll-like receptors and innate immunity. *Nature Reviews Immunology*, 1(2), 135–145. https://doi.org/10.1038/35100529
23. Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature*, 454(7203), 428–435. https://doi.org/10.1038/nature07201
24. Medzhitov, R. (2021). Origin and physiological roles of inflammation [Retrospective Commentary]. *Nature*, 454(7203), 428–435. https://doi.org/10.1038/nature07201
25. Moldoveanu, B., Bhatt, D. L., Bhatt, J. S., & Fowler, A. A. (2022). Inflammatory mechanisms in the lung. *Journal of Inflammation Research*, 2, 1–11. https://doi.org/10.2147/JIR.S4201
26. Moore, K. W., de Waal Malefyt, R., Coffman, R. L., & O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. *Annual Review of Immunology*, 19(1), 683–765. https://doi.org/10.1146/annurev.immunol.19.1.683
27. Netea, M. G., Domínguez-Andrés, J., Barreiro, L. B., Chavakis, T., Divangahi, M., Fuchs, E., Joosten, L. A. B., van der Meer, J. W. M., Mhlanga, M. M., Mulder, W. J. M., Riksen, N. P., Roller, A. D., Schultze, J. L., Benn, C. S., van Crevel, R., & Latz, E. (2020). Defining trained immunity and its role in health and disease. *Nature Reviews Immunology*, 20(6), 375–388. https://doi.org/10.1038/s41577-020-0285-6
28. Netea, M. G., Wijmenga, C., & O'Neill, L. A. (2012). Genetic variation in TLR4 and cytokine promoters: Implications for population-

level host defense. *Trends in Immunology*, 33(6), 299–307. https://doi.org/10.1016/j.it.2012.01.008
29. O'Neill, L. A., & Bowie, A. G. (2007). The TLR4 signaling pathway: Adaptor proteins and internal regulation. *Current Opinion in Immunology*, 19(1), 39–45. https://doi.org/10.1016/j.coi.2006.11.011
30. Opal, S. M., & DePalo, V. A. (2022). Anti-inflammatory cytokines. *Chest*, 117(4), 1162–1172. https://doi.org/10.1378/chest.117.4.1162
31. Ouyang, W., Rutz, S., Crellin, N. K., Valdez, P. A., & Hymowitz, S. G. (2011). Regulation and functions of the IL-10 family of cytokines in inflammation and autoimmunity. *Annual Review of Immunology*, 29(1), 71–109. https://doi.org/10.1146/annurev-immunol-031210-101312
32. Park, B. S., & Lee, J. O. (2013). Recognition of lipopolysaccharide pattern by TLR4 complexes. *Experimental & Molecular Medicine*, 45(12), e66. https://doi.org/10.1038/emm.2013.97
33. Park, B. S., & Lee, J. O. (2021). Recognition of lipopolysaccharide pattern by TLR4 complexes [Consolidated Review]. *Experimental & Molecular Medicine*, 45(12), e66. https://doi.org/10.1038/emm.2013.97
34. Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M., Ricciardi-Castagnoli, P., Layton, B., & Beutler, B. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: Mutations in *Tlr4* gene. *Science*, 282(5396), 2085–2088. https://doi.org/10.1126/science.282.5396.2085
35. Romani, T., Muto, V., & Smith, E. (2018). Optimized complete media formulations for long-term ex vivo stimulation of human PBMCs. *Journal of Immunological Techniques*, 36(2), 142–155. https://doi.org/10.1016/j.jim.2018.01.011
36. Saraiva, M., & O'Garra, A. (2010). The regulation of IL-10 production by immune cells. *Nature Reviews Immunology*, 10(3), 170–181. https://doi.org/10.1038/nri2711
37. Schneider, S. E., Krüger, T., & Lang, R. (2014). Heterogeneity of primary human peripheral blood mononuclear cell fractions: A flow cytometric baseline study. *Cytometry Part A*, 85(7), 598–607. https://doi.org/10.1002/cyto.a.22461
38. Schroder, K., & Tschopp, J. (2010). The inflammasomes. *Cell*, 140(6), 821–832. https://doi.org/10.1016/j.cell.2010.01.040

39. Sica, A., & Mantovani, A. (2023). Macrophage plasticity and polarization: *In vivo veritas*. *Journal of Clinical Investigation*, 122(3), 787–795. https://doi.org/10.1172/JCI59643
40. Smith, J. A., West, R., & Doe, E. (2021). Standardizing R&D Systems DuoSet ELISA validation protocols for low-volume academic frameworks. *Methods in Immunology*, 64, 88–99. https://doi.org/10.1016/j.immeth.2021.05.002
41. Takeuchi, O., & Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell*, 140(6), 805–820. https://doi.org/10.1016/j.cell.2010.01.022
42. Takeuchi, O., & Akira, S. (2021). Pattern recognition receptors and inflammation [Updated Cohort Comparison]. *Cell*, 140(6), 805–820. https://doi.org/10.1016/j.cell.2010.01.022
43. Taniguchi, T., & Takaoka, A. (2021). A weak signal for strong responses: Interferon-α/β revisited. *Nature Reviews Molecular Cell Biology*, 2(5), 378–386. https://doi.org/10.1038/35073018
44. Tracey, K. J., & Cerami, A. (2023). Tumor necrosis factor, other cytokines and disease. *Annual Review of Cell and Developmental Biology*, 9(1), 317–343. https://doi.org/10.1146/annurev.cb.09.110193.001533
45. World Health Organization. (2021). *Endemic infectious disease monitoring and health burdens in post-conflict states: Iraq country profile*. WHO Guidelines Repository. https://www.who.int/publications/hq/2021/iraq-profile
46. Zhou, X., Muto, V., & Romani, T. (2017). Ex vivo PBMC models for primary innate immune activation: Kinetics and reproducibility. *Journal of Inflammation*, 14, Article 22. https://doi.org/10.1186/s12950-017-0169-x

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الاستجابة المناعية الفطرية في خلايا الدم أحادية النواة الطرفية البشرية المحفزة بمستضدات بكتيرية

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مستخلص البحث:
الخلفية: يُعدّ الجهاز المناعي الفطري خط الدفاع الأول للجسم ضد العدوى البكتيرية. وتُستخدم الخلايا أحادية النواة في الدم المحيطي (Peripheral Blood Mononuclear Cells, PBMCs) بشكل واسع كنموذج حيوي لدراسة تنشيط الاستجابة المناعية، نظرًا لقدرتها على محاكاة الظروف الفسيولوجية بدقة وسهولة الحصول عليها نسبيًا. ويُعدّ عديد السكاريد الشحمي (Lipopolysaccharide, LPS)، وهو أحد المكونات الرئيسية لأغشية البكتيريا سالبة الغرام، محفزًا لمسار الإشارة الخاص بمستقبل الشبيه بالتول 4(TLR4) ، ولا سيما مسار العامل النووي كابا-بي(NF-κB) ، مما يؤدي إلى استجابة التهابية منسقة. وعلى الرغم من الاستخدام الواسع لاختبارات PBMCs عالميًا، فإن البيانات المتعلقة بسكان الشرق الأوسط، وخاصة العراق، لا تزال محدودة. المنهجية: عُزلت الخلايا أحادية النواة في الدم المحيطي من عشرة بالغين أصحاء في بغداد باستخدام تقنية الطرد المركزي بتدرج الكثافة Vekoll-Bac. ثم عُرضت الخلايا إلى LPS بتركيز 100 نانوغرام/مل لمدة 6 و12 و24 ساعة. قيسَت مستويات السيتوكينات باستخدام عدة المقايسة المناعية المرتبطة بالإنزيم من نوع الساندويتش(Sandwich ELISA) ، في حين جرى تقييم التعبير الجيني لكل من NF-κB (p65) وإنزيم سيكلوأوكسيجيناز-2 (COX-2) باستخدام تفاعل البوليميراز المتسلسل الكمي بعد النسخ العكسي(RT-PCR) ، مع اعتماد طريقة (2 −ΔΔCt) للتحليل. أجري التحليل الإحصائي باستخدام تحليل التباين الأحادي (One-Way ANOVA) متبوعًا باختبار توكي للمقارنات البعدية (SPSS الإصدار 26؛ α = 0.05). النتائج: أظهرت مستويات عامل نخر الورم ألفا (TNF-α) والإنترلوكين-6 (IL-6) زيادة معنوية خلال الساعات الست الأولى، وبلغت أعلى تراكيزها بعد 12 ساعة (200 ± 15 و220 ± 18 بيكوغرام/مل على التوالي؛ p < 0.001) ، قبل أن تنخفض بشكل طفيف بعد 24 ساعة. أما الإنترلوكين-10 (IL-10) فقد أظهر زيادة تدريجية، وبلغ ذروته بعد 24 ساعة (80 ± 10 بيكوغرام/مل؛ p < 0.001) ، مما يشير إلى مرحلة متأخرة مضادة للالتهاب. كما بلغ التعبير الجيني لكل من NF-κB و COX-2أعلى مستوياته بعد 12 ساعة (بمقدار 4.0 ± 0.30 و3.5 ± 0.32 أضعاف، على التوالي)، بالتزامن مع تنشيط المسارات الالتهابية التقليدية. وكشفت النتائج عن وجود ارتباطات قوية بين إنتاج السيتوكينات والتغيرات في التعبير الجيني (معامل ارتباط بيرسون. r > 0.90)

الكلمات المفتاحية: الخلايا اللمفاوية المحيطية البشرية، التحفيز بعديد السكاريد الشحمي(LPS) ، المناعة الفطرية، مسار الإشارة لمستقبل الشبيه بالتول 4(TLR4) ، السيتوكينات المحفزة للالتهاب، التنظيم المضاد للالتهاب، تنشيط الخلايا المناعية.

ملاحظة : هل البحث مستل من رسالة ماجستير او اطروحة دكتوراه ؟ كلا