Vol. 1, No. 1: 14-18.

WORLD JOURNAL OF EXERIMENTAL BIOSCIENCES

Research article

# Thermoregulation of IL-1α production and phagocytic activity of *Escherichia coli* Lipopolysaccharide-induced mononuclear cells

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**ABSTRACT**

Peripheral blood mononuclear cells were stimulated with lipopolysaccharide and incubated at different temperatures (34, 37 and 39 °C). Interleukin-1 alpha (IL-1α) was measured. Phagocytic activity of LPS-stimulated monocytes in terms of bacterial uptake and intracellular bacterial killing was checked at different temperatures and time intervals *in vitro*. Early elevation IL-1α was found in LPS-stimulated monocytes that incubated at 39 °C followed by cells that incubated at 37 °C and lowest level was detected at 34 °C. Similar trend was reported in the phagocytic activity in terms of bacterial uptake and intracellular bacterial killing. The sharp decrease in IL-1α was observed 12 h post exposure to LPS in LPS-stimulated monocytes that incubated at 39 °C only. While, the decrease of IL-1α levels in other incubated temperatures (34 and 37 °C) was seen later than incubated at 39 °C. This report describes the striking effect of incubation temperature on activity of LPS-stimulated monocytes. This result explains clearly the important role of elevation temperature in modulating the immune response against external pathogens.

**Keywords: IL-1α, E. coli, Lipopolysaccheride, Momocytes, Phagocytosis.**

**Citation: Hassan SA, Zgair AK.** (2013) Thermoregulation of IL-1α production and phagocytic activity of *Escherichia coli* Lipopolysaccharide-induced mononuclear cells. *World J Exp Biosci*. **1**: 14-18.

**Received March 29, 2013; Accepted April 28, 2013; Published May 5, 2013.**

## INTRODUCTION

Monocytes play a central role in host defense by engulfing pathogens, presenting antigens to immune cells and producing numerous inflammatory mediators [1]. Pro-inflammatory cytokines are important mediators in immune regulation and are produced during immune and host defense response [2-4].

Interleukin 1 alpha (IL-1α) is recognized as a central mediator of inflammation, produced mainly by activated macrophages [5]. IL-1α has a wide range

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of target cells such as fibroblasts, endothelial cells, mature T cells, mature B cells, monocytes and neutrophils (induces secretion of several cytokines, including Interleukin 8 (IL-8) and IL-1 itself). Recognition of LPS by the innate immune system cells especially by monocytes and macrophages leads to immediate cell activation and release of pro-inflammatory cytokines (IL-1 α and TNF-α) and NO [6]. LPS recognition is facilitated by a cluster of differentiation 14 (CD14), which exists in both soluble and membrane-anchored forms [7]. The bound LPS then associates with Toll-like receptor 4 (TLR4), a signaling receptor of the Toll receptor family [8]. As a consequence, the transcription factor nuclear factor kappa B (NF-ĸB) is activated, leading to transcriptional stimulation of pro-inflammatory genes [9]. IL-1 is carried by the blood stream to the thermoregulatory center in the hypothalamus that results in fever and this phenomenon plays an important role in immune response [10]. Sutcliffe et al. [11] reported that hypothermia reduced the immune response mediated by pro-inflammatory cytokines [11]. In contrary, a previous study by Fairchild et al. [12] was shown that febrile range temperature (FRT) (40, 37, 32 $^{o}$C) modified cytokine production by adult macrophages obtained from adult and newborns after exposing to LPS [12]. The prolonged presence of pro-inflammatory cytokines (IL-1) leads to excess inflammation and ultimately resulting tissue damage [13]. Therefore, regulation of leukocytes activity is important in developing the best immune response against infectious agents. In the present study we investigated the thermoregulation of IL-1α production by LPS-stimulated monocytes and phagocytic activity of these cells. This may help in explanation of the role of elevation of temperature (in period of infection) in modulating the immune response against pathogens and reducing the side effect of immune response.

## MATERIALS AND METHODS

### Human monocyte isolation

Human peripheral blood monocytes were isolated from buffy coats from healthy donors (Central public health laboratories, Baghdad, Iraq) by centrifugation (800 g for 15 min) over Histopaque (Sigma) as described previously [14]. The standard method of Lee et al (1996) was followed to isolate peripheral blood monocytes. The number of viable monocytes was estimated using trypan blue exclusion assay [15].

### Treatment of cells

Number of monocytes was adjusted to $10^6$ viable cells/ml and re-cultured under standard cell culture condition [6]. The cell culture divided into three groups of test tubes and each group was incubated at different temperatures (34, 37, 39 $^{o}$C). Each group divided into two subgroups, test subgroup A (test) was exposed to 1 µg/ml of LPS (Escherichia coli O127:B8; Difco, Detroit, Mich. USA) and control subgroup was incubated with PBS instead of LPS. Samples were collect at different time points to estimate the level of IL-1α and phagocytic activity in terms of bacterial uptake.

### IL-1α measurement

Equal volumes were collected from tissue cultures at different time intervals (4, 12, 24, 48 h) post exposure to 1 µg/ml LPS. The samples were centrifuged at 600 g for 5 min to pellet cells. Supernatants were then collected and stored at -20 ºC until use for ELISA. Level of IL-1α was determined using the human IL-1α ELISA kit (KOMA BIOTECH INC) according to manufacturer's instructions.

### Phagocytosis

Phagocytosis was performed according to the method of Zgair and Chhibber [16]. Bacteria (*E. coli* MTCC- 1687, Institute of Microbial Technology, Chandigarh, India) were harvested and suspended in PBS (0.2 M, pH7.2) so as to obtain the optical density corresponding to $10^5$ c.f.u./ml. Human monocytes were obtained according to the method described by Lee and Rikihisa [15] and their suspension of $10^4$ was made in RPMI 1640 (High Media Laboratories Ltd., Mumbai). For uptake of bacteria, 0.4 ml of normal human serum, 0.5 ml of monocytes suspension ($10^4$ cells/ml) and 0.1 ml of bacterial suspension ($10^5$ c.f.u./ml) were taken, vortexed and incubated at 37 $^{o}$C under 5 per cent CO2 atmosphere. Aliquots were taken regularly after 0 and 60 min of incubation and centrifuged. The viable count of bacteria in the supernatant was determined by plating appropriate serial dilutions on nutrient agar plates. The results were expressed as viable bacteria (c.f.u./ml) taken up by the monocyte at respective sampling time interval [16].

### Statistical analysis

All values were calculated as the means ± SD. Differences between test and control groups were analyzed using Student's t-test. Differences among multiple groups were analyzed by applying the Tukey honestly significant differences test to a one-way ANOVA using Origin version 8.0 software. A value of $p < 0.05$ was considered statistically significant.

## RESULTS

### LPS stimulates human monocytes to produce IL-1α

In current study presence of LPS stimulated the peripheral blood monocytes to produce IL-1α but the production (*in vitro*) affected by incubation temperature. **Fig. 1** shows production of IL-1α in vitro after stimulation with LPS. The significant production of IL-1α in suspension of peripheral blood monocytes post stimulating with LPS was started as early as 4 h post incubation time. The maximum production of IL-1α was observed in suspension of culture of LPS-stimulated monocytes that incubated at 39 $^{o}$C followed by the culture of LPS-stimulated monocytes that incubated at 37 $^{o}$C. While, the lowest level of IL-1α was observed in the culture of LPS-stimulated monocytes that incubated at 34 $^{o}$C. After that, level of the cytokines (IL-1α) declined gradually and no significant difference with control was observed at 24 h and 48 h after addition of LPS (39 $^{o}$C). However, optimum level of IL-1α production was seen at 12 h post addition of LPS in monocytes that cultured at 34 $^{o}$C and 37 $^{o}$C, but this level remained high ($p < 0.05$) up to 48 h. These results showed the significant differences among tested groups as well as control group ($P < 0.05$).

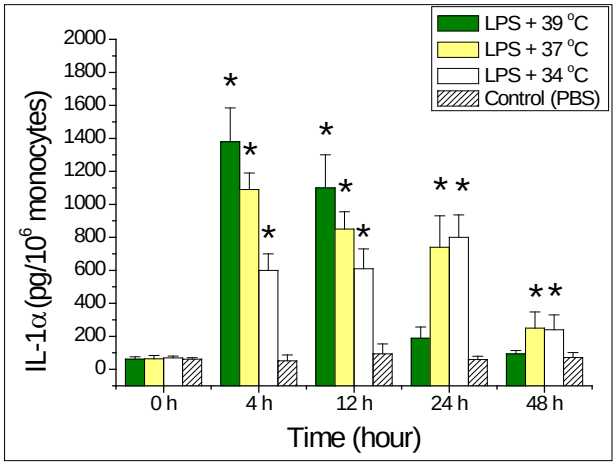


**Fig. 1.** Time-course of changes in IL-1α and TNF-α concentrations (*in vitro*) in peripheral human monocytes cultures that exposed to 1 lg/ml of bacterial LPS and incubated at different temperatures (34, 37, 39 $^{o}$C). *, $p < 0.05$ vs 37 $^{o}$C without LPS (control group).

### Monocytes activity to engulf bacteria in vitro

The phagocytic activity of peripheral blood monocytes was estimated in terms to ability of this kind of cells to engulf bacteria with limit time**. Fig. 2** shows the mean bacterial uptake (*E. coli* MTCC-1687) in vitro by human monocytes incubated at different temperature (34, 37 and 39 $^{o}$C) and different time intervals (0, 4, 12, 24, 48 h) post exposure to 1 µg/ml of LPS (*E. coli* O127:B8). Significant increase of bacterial uptake by monocytes that incubated at 39 $^{o}$C was seen as early as 4 h post LPS exposure as compared with bacterial engulfment by control cells (monocytes were not exposed to LPS). Maximum activity of monocytes (incubated at 39 $^{o}$C) was observed at 12 h post LPS exposure and this activity declined after this time point gradually. While, significant increase in bacterial uptake was observed in case of monocytes that incubated at 37 $^{o}$C and collected at 4 h with maximum activity at 24 h post LPS exposure. No significant increase observed at 48 h in case of all test groups as compared with control. This result reported no significant activity of monocytes that incubated at low temperature (34 $^{o}$C) was observed in all time points as compared with bacterial engulfment by control cells. The present study proved the role of temperature in modulates the activity of LPS-induced monocytes.

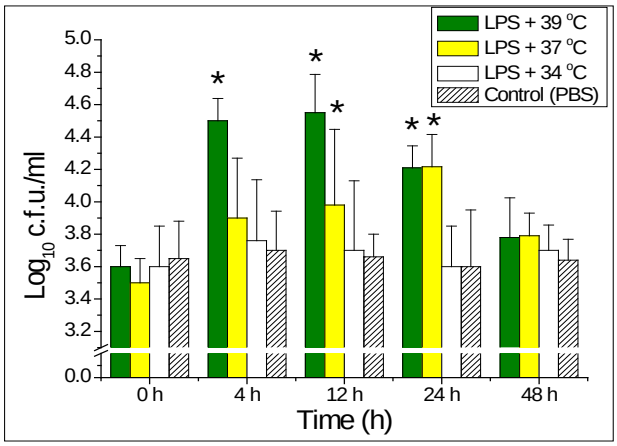


**Fig. 2.** Time course of bacterial uptake by human monocytes incubated at different temperatures (34, 37 and 39 $^{o}$C). The activity was checked at different time intervals (0, 4, 12, 24 and 48 h) post exposure to 1 µg/ml of LPS. Earliest and highest activity of monocytes to engulf was observed in cells that incubated at 39 $^{o}$C at all time points except 48 h post LPS exposure). *, $p < 0.05$ vs 37 $^{o}$C without LPS (control group).

## Discussion

The thermoregulation effect of temperature and LPS together in modulation of production of IL-1α by human monocytes in vitro was checked. Furthermore, the effect of these factors (temperature and LPS) on phagocytic activity of human monocytes in terms of bacterial uptake in vitro. Frankly, LPS plays a crucial role in

pathogenicity and elevation of temperature in host body infected with Gram negative bacteria. In this study, It was proved the fact that the elevation of temperature in presence of LPS make the innate immune response in optimum status and that happened by modulating pro-inflammatory cytokines production (IL-1α) and increasing phagocytic activity. The highest and earliest generation of IL-1α cytokines was detected in culture of human monocytes that incubated at 39 $^{o}$C as compared with IL-1α production by monocytes incubated at normal condition (37 $^{o}$C and PBS), but this level declined gradually to be in normal level within short time. However, in other temperatures (34 and 37 $^{o}$C) the level of IL-1α was produced in moderate to low level and this level continued in significant up to 48 h as compared with IL-1α produced by control cells. Previous study of Nagarsekar et al. [17] reported that the exposing to high FRT accelerated the apoptosis of polymorphoneuclear cells (PMN) [17]. The pro-inflammatory mediators are very important molecules in immune response against infectious agents and that will be very beneficial if they produce early and in high level [18,19]. Production of pro-inflammatory mediators stimulates the innate immune response faster than pathogen adaptation in infected area [20]. The last make the host body more resistant to pathogens. The previous phenomenon happen when the presence of LPS concomitant with high FRT [21]. However, the presence of pro-inflammatory cytokines in tissue for long time will create a lot of pathological problems. Many investigators listed many diseases associated with overproduction of pro-inflammatory cytokines [18, 22]. In current study, the presence of LPS concomitant with high FRT helps rapid reduction of IL-1α levels. That will not give a chance to pro-inflammatory mediators to create a crucial damage in infected area. It is concluded from our study that the elevation of temperature with presence of LPS is very important in modulation of monocytes activity in terms of production of IL-1α and phagocytic activity in terms of bacterial uptake that maybe optimize the innate immune response.

### Conflict of interest

The authors declare that they have no conflict of interests.

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