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## Human monocytes tolerant to LPS retain the ability to phagocytose bacteria and generate reactive oxygen species

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# Human monocytes tolerant to LPS retain the ability to phagocytose bacteria and generate reactive oxygen species

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## Abstract

Tolerance to lipopolysaccharide (LPS) occurs when animals or cells exposed to LPS become hyporesponsive to a subsequent challenge with LPS. This mechanism is believed to be involved in the down-regulation of cellular responses observed in septic patients. The aim of this investigation was to evaluate LPS-induced monocyte tolerance of healthy volunteers using whole blood. The detection of intracellular IL-6, bacterial phagocytosis and reactive oxygen species (ROS) was determined by flow cytometry, using anti-IL-6-PE, heat-killed *Staphylococcus aureus* stained with propidium iodide and 2',7'-dichlorofluorescein diacetate, respectively. Monocytes were gated in whole blood by combining FSC and SSC parameters and CD14-positive staining. The exposure to increasing LPS concentrations resulted in lower intracellular concentration of IL-6 in monocytes after challenge. A similar effect was observed with challenge with MALP-2 (a Toll-like receptor (TLR)2/6 agonist) and killed *Pseudomonas aeruginosa* and *S. aureus*, but not with flagellin (a TLR5 agonist). LPS conditioning with 15 ng/mL resulted in a 40% reduction of IL-6 in monocytes. In contrast, phagocytosis of *P. aeruginosa* and *S. aureus* and induced ROS generation were preserved or increased in tolerant cells. The phenomenon of tolerance involves a complex regulation in which the production of IL-6 was diminished, whereas the bacterial phagocytosis and production of ROS was preserved. Decreased production of proinflammatory cytokines and preserved or increased production of ROS may be an adaptation to control the deleterious effects of inflammation while preserving antimicrobial activity.

Key words: IL-6, ROS; Phagocytosis; MALP-2; Flagellin; LPS tolerance

## Introduction

Challenge with lipopolysaccharide (LPS) induces a wide range of biological responses in susceptible animals, including the synthesis and release of inflammatory cytokines, reactive oxygen species (ROS) and reactive nitrogen species (1). The biological activity of LPS may be modulated *in vivo*, and hypersensitivity and tolerance may be induced under experimental conditions (2).

The pre-exposure of an animal to low doses of LPS inhibits the biological response to a re-exposure to high doses of LPS, reducing the pathophysiological changes that are observed in normal hosts. Similar effects are also observed in cell cultures by re-exposure to LPS *in vitro*. This phenomenon is called endotoxin tolerance, although other terms such as reprogramming, adaptation, anergy, or refractoriness are used in the literature (3).

Tolerance to endotoxin has been examined in a series of elegant experiments. It has been reported that periph-

eral blood cells of healthy humans exposed to LPS *in vivo* showed reduced production of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$ , IL-6, and IL-10 after further stimulation *in vitro* (4,5). Tominaga et al. (6) found a lower expression of mRNA for TNF- $\alpha$  and IL-6 in macrophages of tolerant mice in response to re-stimulation with LPS. However, other *in vivo* and *in vitro* studies evaluating endotoxin tolerance have shown that the phenomenon is not a global down-regulation of macrophages, but a complex modulatory response. The re-exposure of monocytes and macrophages previously exposed to LPS showed lower gene expression of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-12, and Jun B, but other genes, including IL-10, IL-1R and tumor necrosis factor receptor II (TNFR2), are normally or highly expressed (for a review, see Ref. 7).

Studies on circulating leukocytes of septic patients have shown reduced production of inflammatory cytokines after

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*in vitro* stimulation, similar to what is found in cells made tolerant to LPS. Ertel et al. (8), using whole blood from septic patients, found a smaller production of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 after stimulation with LPS. This reduced cytokine production was extended to IL-4, IL-10, IL-12, interferon-gamma (IFN- $\gamma$ ), and transforming growth factor-beta (TGF- $\beta$ ) (9). A study conducted by our group using a similar model confirmed the decreased production of TNF- $\alpha$  and IFN- $\gamma$  *in vitro*; however, we found preserved production of IL-10 in patients with severe sepsis and septic shock (10). Brunialti et al. (11) demonstrated a positive regulation of the production of inflammatory cytokines at the onset of sepsis, followed by a prominent decline in more severe stages of the disease, severe sepsis and septic shock, showing that the cellular response may be modulated according to the stages of sepsis.

The induction of the phenotype of tolerance to endotoxin may be triggered by other family members of Toll-like receptors (TLR)/IL-1R ligands, in addition to LPS and TLR4. The ability of a stimulus other than LPS to initiate the phenotype of tolerance is called cross-tolerance (12). Medvedev et al. (13) showed that pretreatment with IL-1 $\beta$  resulted in inhibition of activation of NF- $\kappa$ B and AP-1 in response to LPS. The conditioning of macrophages with other bacterial products such as lipoteichoic acid resulted in inhibited secretion of LPS-induced TNF- $\alpha$  (14).

Invading pathogens are phagocytized and killed by a powerful arsenal of enzymes, cationic proteins, ROS, and reactive nitrogen species produced by the cell and released into the phagosomes (1). In animal models, *in vivo* and *in vitro* experiments have shown preserved phagocytosis and bacterial clearance in tolerant cells (15,16), while both reduced (17) and enhanced (18,19) nitric oxide (NO) generation has been reported.

In a previous study by our group involving patients with severe sepsis and septic shock, we observed lower production of inflammatory cytokines by mononuclear cells after *in vitro* stimulation with LPS (11). However, the production of ROS in the same patients was increased in monocytes under basal conditions and after stimulation with PMA, fMLP, LPS, and *Staphylococcus aureus* compared to healthy volunteers (20). This finding revealed a dichotomy of the monocyte response in septic patients with down-regulation of the production of inflammatory cytokines and up-regulation of ROS.

Since there are similarities between the functional modulations found in leukocytes of septic patients and in cells made tolerant to LPS, we investigated whether the same phenomenon occurs in tolerance induced in human whole blood *in vitro*.

## Material and Methods

### Healthy volunteers

The study was approved by the Ethics Research Com-

mittee of Universidade Federal de São Paulo, Hospital São Paulo (protocol #0121/06). Written informed consent was obtained from all volunteers. Blood samples from 17 healthy volunteers (mean age, 29  $\pm$  8 years; 82% females) were drawn into heparin-treated vacuum tubes (Becton Dickinson, UK). All volunteers reported being healthy and none were taking medication.

### Reagents

LPS from *Salmonella abortus equi*, prepared by the phenol-water method and further purified with phenol-chloroform-petroleum ether as previously described (21), was a gift from C. Galanos (Max-Planck Institute of Immunobiology, Germany). Macrophage-activating lipopeptide-2 (MALP-2) and flagellin were purchased from Alexis Biochemicals, Germany. *Pseudomonas aeruginosa* (ATCC 27853) was purchased from Chrisope Technologies, UK, and *S. aureus* (ATCC 25923) was purchased from Difco, USA. The following monoclonal antibodies were used: CD66b-fluorescein isothiocyanate (FITC), clone G10F5; CD14-peridinin-chlorophyll-protein (PerCP), clone M $\Phi$ P9; anti-IL-6-phycoerythrin (PE), clone AS14 (BD Bioscience, USA).

### Induction of tolerance by LPS pretreatment

Heparinized blood was diluted 1:2 in RPMI (Sigma, USA) and divided into 2-mL aliquots. Samples were pretreated with increasing concentrations of LPS: 1, 7.5, 15, and 30 ng/mL, or without LPS. After 18 h of incubation at 5%CO<sub>2</sub> at 37°C, the samples were mixed and divided into 4 aliquots (500  $\mu$ L each) and then challenged with different TLR agonists (LPS, MALP-2, flagellin) or Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria (Figure 1).

### Intracellular detection of cytokines in monocytes in whole blood

After challenge with different TLR agonists, samples were transferred to 5-mL propylene tubes and incubated at 37°C in the presence of 5% CO<sub>2</sub> for 5 h for intracellular detection of IL-6. After 30 min, 2  $\mu$ L 1 mM monensin was added to the samples. Two hundred microliters of whole blood was washed in 2 mL phosphate-buffered saline (PBS) and centrifuged at 2500 *g* for 5 min at 4°C. Red blood cells were ruptured with 2 mL lysis solution (FACS lysing solution, BD Bioscience) for 10 min in the dark at room temperature followed by centrifugation at 2500 *g* for 5 min at 4°C. After washing with 2 mL PBS, samples were incubated with fluorochrome-conjugated monoclonal antibodies, CD66b-FITC and CD14-PerCP, for surface staining for 15 min in the dark at room temperature. After centrifugation, the samples were washed in 2 mL PBS, centrifuged, and fixed with 1 mL fixation buffer (PBS 4% paraformaldehyde) for 20 min in the dark at 4°C. After centrifugation, the supernatants were discarded, and 50  $\mu$ L permeabilization buffer was then added (PBS 1% FCS; 0.1% sodium azide; 0.1% saponin)

plus anti-IL-6-PE. The tubes were incubated for 30 min in the dark at 4°C. After this time, the samples were washed with 2 mL permeabilization buffer and centrifuged. Supernatants were discarded and cells were suspended in 0.3 mL PBS 1% sodium azide.

Event acquisition and analyses were performed using the CellQuest software (BD Bioscience) and a FACSCalibur four-color flow cytometer (BD Bioscience). For each condition 5000 events were acquired using forward- and side-scatter parameters combined with CD14-positive cells and CD66b-negative cells. All events were acquired and stored.

### Analysis of phagocytosis in tolerant and non-tolerant monocytes in whole blood

Phagocytosis was detected using heat-killed *S. aureus* stained with propidium iodide (PI; Sigma). Briefly, after inactivation at high temperature, the bacteria were incubated with PI (5% concentrated) for 30 min in the dark at room temperature. The bacteria suspension was washed, resuspended in glycoside PBS solution and stored in aliquots of  $2.4 \times 10^9$  mL at -20°C until use. An aliquot (100 µL) of PI-labeled *S. aureus* was added to 200 µL whole blood, pretreated or not with LPS for 18 h, and 700 µL PBS (Figure 1). The samples were incubated at 37°C in a shaking water bath for 30 min. Thereafter, 2 mL PBS 3 mM EDTA (Sigma) was added to each tube followed by centrifugation at 250 *g* for 5 min at 4°C. Hypotonic lyses in saline (0.2 and 1.6%, Merck, Germany) were performed followed by centrifugation. Finally, the supernatant was discarded and pellets were stained with 6 µL CD14-PerCP. After centrifugation, pellets were suspended in 0.3 mL PBS 3 mM EDTA for flow cytometry analysis.

Monocytes were identified by side scatter and forward scatter parameters combined with positive staining for CD14. Monocyte phagocytosis was shown as the per-

centage of PI-stained cells and as the geometric mean of fluorescence intensity (GMFI).

### Analysis of monocyte oxidative burst in whole blood after pretreatment with LPS and challenge with LPS, *S. aureus* or *P. aeruginosa*

Oxidative burst was quantified by measuring the oxidation of 2',7'-dichlorofluorescein diacetate (DCFH-DA, Sigma) in whole blood after pretreatment and challenge (Figure 1). Tubes containing 200 µL whole blood, stimuli, 200 µL 0.3 mM DCFH-DA and PBS to complete 1 mL were incubated at 37°C in a shaking water bath for 30 min. The stimuli added were LPS (250 ng/mL), *S. aureus* ( $24 \times 10^6$ /mL) or *P. aeruginosa* ( $12 \times 10^6$ /mL). The samples were processed as described above.

Monocytes were identified by side scatter and forward scatter parameters and positive staining for CD14. ROS generation was shown in histograms and reported as the GMFI.

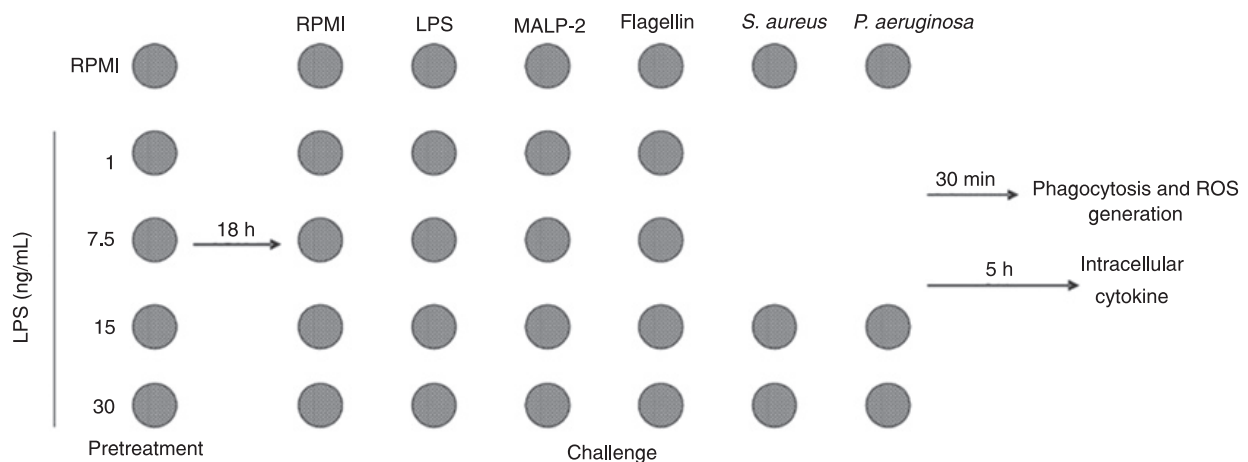
### Statistical analysis

The Kolmogorov-Smirnov test was applied to determine whether distribution was normal. Continuous variables were described as median and range and represented in box-plot graphs. Differences among groups were tested by ANOVA plus the Bonferroni's *post hoc* test. A P value of  $\leq 0.05$  was considered to be significant. Analysis was performed using the SPSS package, version 13.0 (USA).

## Results

### Production of IL-6 by monocytes conditioned with increasing concentrations of LPS and challenged with different TLR agonists and whole bacteria

Peripheral blood cells were conditioned with small doses



**Figure 1.** Schedule for the induction of tolerance *in vitro*. Heparinized blood was diluted 1:2 in RPMI and divided into aliquots. To induce tolerance, the samples were incubated with low concentrations of lipopolysaccharide (LPS) or without LPS (control). After 18 h of incubation, samples were re-stimulated with different TLR agonists. Then, production of interleukin-6, phagocytosis and reactive oxygen species (ROS) generation in monocytes was measured by flow cytometry. MALP-2 = macrophage-activating lipopeptide-2.



of LPS and challenged with different TLR agonists: LPS (TLR4), MALP-2 (TLR2/6), flagellin (TLR5), and whole Gram-negative (*P. aeruginosa*) and Gram-positive (*S. aureus*) killed bacteria.

Detection of IL-6 in monocytes in whole blood was modulated by conditioning with low LPS concentrations ( $P < 0.001$ ). Pre-exposure of peripheral blood to the concentrations of 7.5 and 30 ng/mL LPS resulted in a lower detection of intracellular IL-6 in monocytes after challenge with 100 ng/mL LPS compared to the detection in non-conditioned monocytes ( $P < 0.05$ ; Figure 2A). Pre-exposure to LPS also led to a decreased IL-6 production by monocytes after challenge with a TLR2/6 agonist (MALP-2;  $P < 0.001$ ). Conditioning with 15 and 30 ng/mL LPS resulted in the detection of less intracellular IL-6 in monocytes after challenge with MALP-2 compared to control non-conditioned cells ( $P < 0.05$ ; Figure 2B). Increasing concentrations of LPS used for pre-exposure resulted in greater inhibition of IL-6 production. Accordingly, a decrease in the detection of the cytokine was found at a dose of 30 ng/mL in comparison to conditioning with 7.5 ng/mL LPS ( $P < 0.05$ ; Figure 2B). When the TLR5 agonist flagellin was used as the challenge stimulus on monocytes conditioned with different concentrations of LPS no decrease in the production of IL-6 was observed ( $P = 0.946$ ; Figure 2C).

Pre-exposure of monocytes to LPS modulated the induction of IL-6 by *S. aureus* ( $P = 0.001$ ) and *P. aeruginosa* ( $P < 0.001$ ). Conditioning with concentrations of 15 and 30 ng/mL LPS resulted in a lower detection of intracellular IL-6 in monocytes after challenge with *S. aureus* ( $P < 0.05$ ; Figure 2D) and *P. aeruginosa* ( $P < 0.05$ ; Figure 2E) compared to the detection in cells without conditioning.

#### Analysis of phagocytosis in tolerant and non-tolerant monocytes in whole blood

No difference in phagocytosis of PI-labeled *S. aureus* was found between monocytes conditioned with 7.5 and 15 ng/mL LPS and without pre-exposure to LPS in the percentage of positive-stained monocytes ( $P = 0.956$ ; Figure 3A, left) or in the intensity of fluorescence of *S. aureus* phagocytosing cells ( $P = 0.263$ ; Figure 3A, right).

#### ROS production by tolerant and non-tolerant monocytes in whole blood

ROS generation after LPS challenge (250 ng/mL) was similar in monocytes conditioned with 7.5 and 15 ng/mL (GMFI: median = 27.8, range = 19.7-35.8, and median = 40.0, range = 28.2-68.4, respectively) compared to non-conditioned monocytes (GMFI: median = 28.5, range = 16.5-39.9;  $P = 0.162$ ). Pre-exposure to LPS and challenge with *S. aureus* ( $P = 0.05$ ; Figure 3B, left) resulted in increased ROS generation in monocytes while no difference was found with challenge with *P. aeruginosa* ( $P = 0.217$ ; Figure 3B, right).

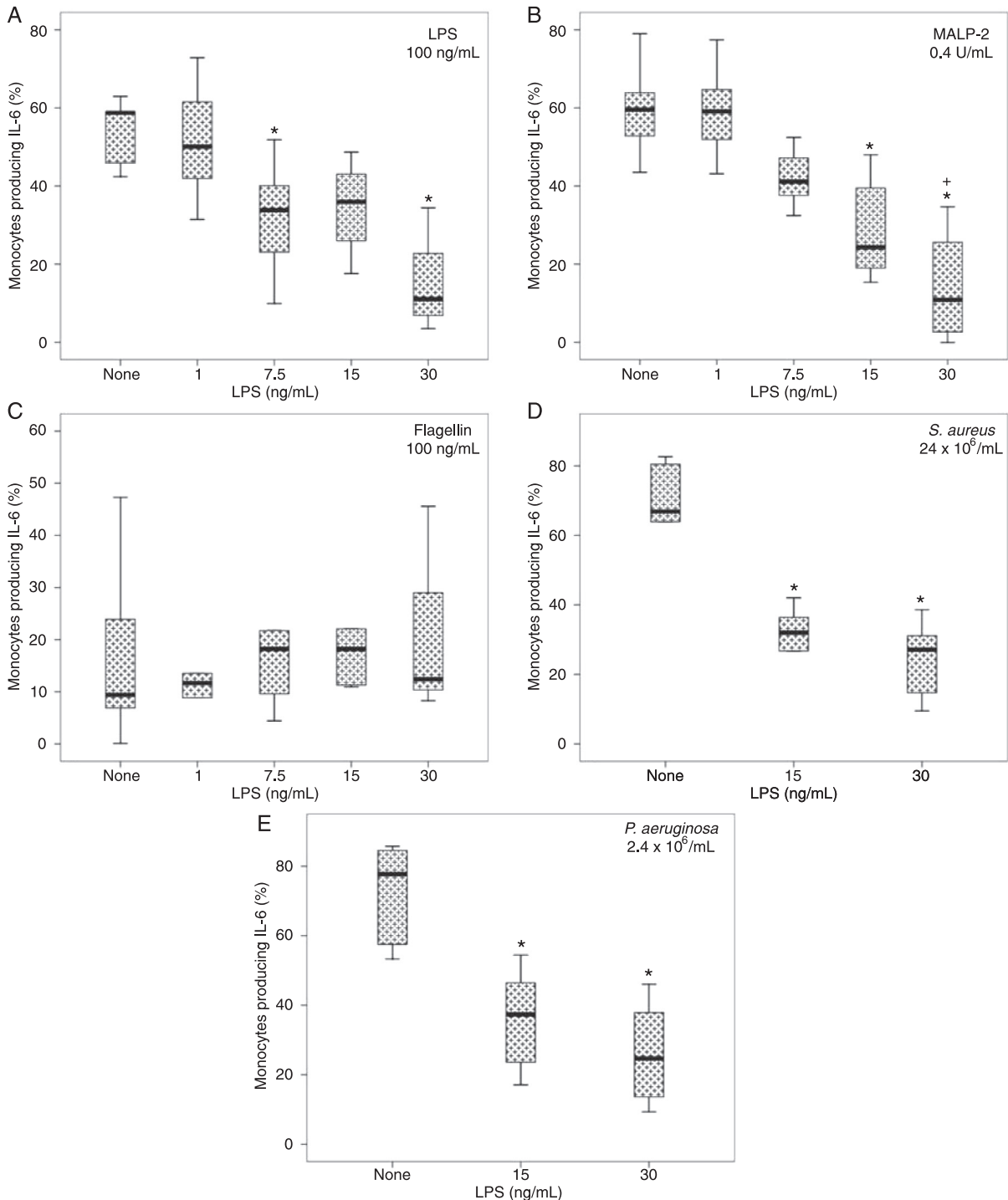
## Discussion

We evaluated the tolerance to LPS and cross-tolerance to other TLR agonists in monocytes present in human whole blood, thus mimicking the microenvironment of LPS-cell interaction as it occurs *in vivo*. Information about the effects of tolerance at the cellular level, measured by production of IL-6, phagocytosis and ROS generation by monocytes, was obtained using flow cytometry. Previous studies of induction of *in vitro* tolerance have evaluated macrophages and cells of different mouse tissues (6,8), and peripheral blood mononuclear cells, alveolar macrophages or blood from healthy individuals (4,5,11) exposed *in vivo* and *in vitro* to LPS.

Studies with monocytes and macrophages of patients with severe sepsis and septic shock showed reduced production of inflammatory cytokines after *in vitro* stimulation with LPS (8-10), a phenomenon that has similarities to tolerance induced by pre-exposure to LPS (3).

One hypothesis to explain this phenomenon would be the modulation of soluble proteins or cell surface receptors that are responsible for LPS carrying and cell recognition. Accordingly, previous reports have shown changes in plasma concentrations of proteins regulating the biological action of LPS (22) and decreased expression of CD14 on the surface of monocytes (23), among others. Nomura et al. (24) studied the expression of TLR4 in macrophages of mice tolerant to LPS and found a correlation between the reduction in the expression of TLR4 and the fall in production of IL-6, indicating a modulation at the cell membrane level. A previous study by our group showed no difference in the binding of LPS to the cell membrane of monocytes of septic patients compared to healthy individuals. However, there was a significant impairment of TNF- $\alpha$  production, detected intracellularly in monocytes by flow cytometry, suggesting that regulation occurs in the cell signaling cascade (25,26).

Our results show that pre-exposure to small amounts of LPS was able to induce tolerance in peripheral blood monocytes in whole blood of human volunteers challenged with high concentrations of LPS. Other studies using human peripheral blood cells showed a reduced production of inflammatory cytokines (4,5). The decreased response to LPS can be explained by the presence of several intracellular proteins that suppress the inflammatory response, regulating the cascade of negative signaling, and by different combinations of NF- $\kappa$ B hetero- and homodimers that modulate gene expression under inflammatory or endotoxin-tolerant conditions (for a review, see Ref. 27). In addition to the idea of blocking the signaling and gene transcription, it has been proposed that the tolerance phenotype is caused by increased production of anti-inflammatory factors (28). Recently, it was demonstrated that another mechanism is involved in tolerance, comprising epigenetic changes able to regulate the expression of isolated genes from a pathway (29).

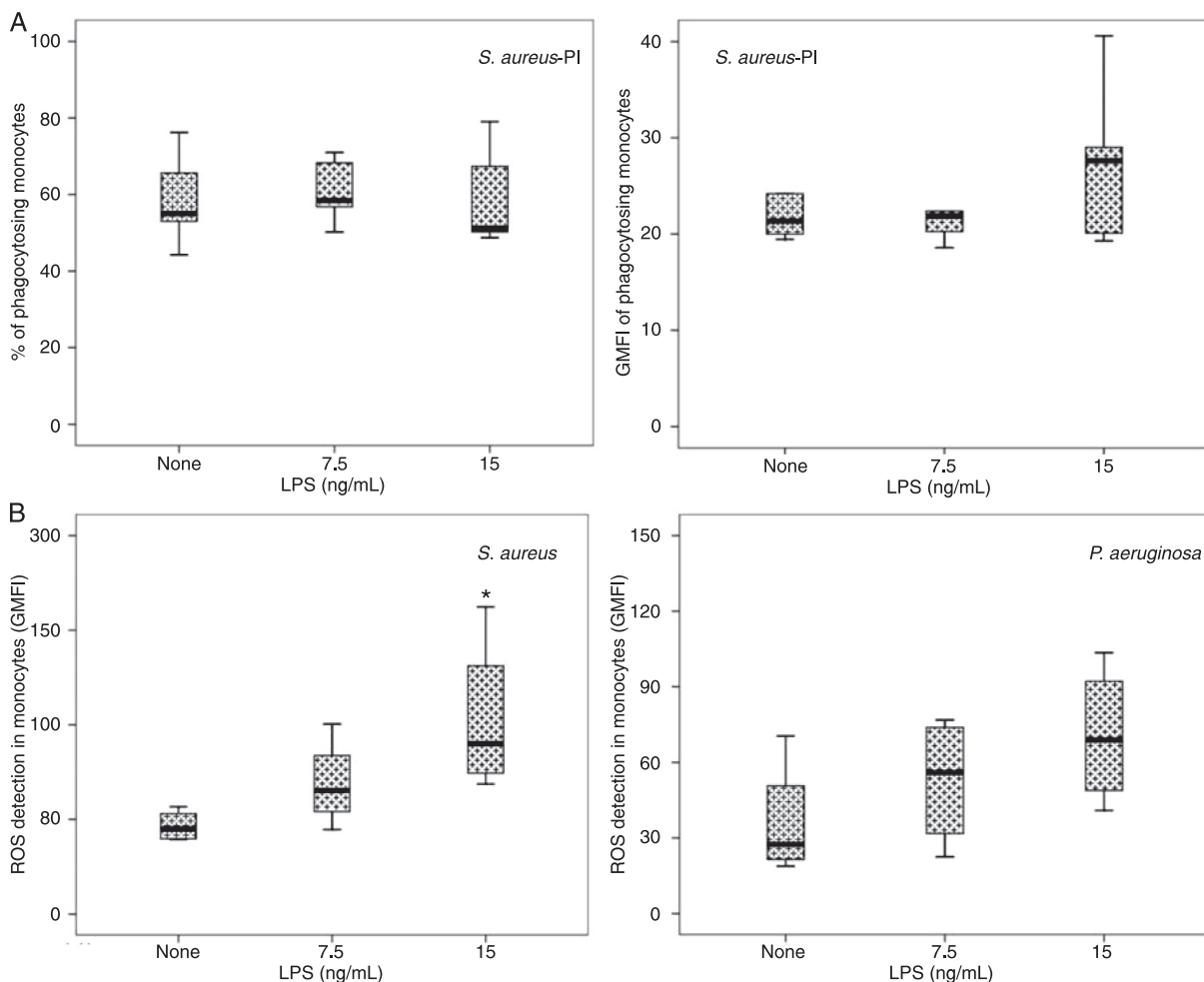


**Figure 2.** Interleukin-6 (IL-6) detection in monocytes conditioned with lipopolysaccharide (LPS) and challenged with Toll-like receptor (TLR) agonists and whole bacteria. Peripheral blood cells were conditioned with low concentrations of LPS for 18 h and challenged with different TLR agonists: LPS (TLR4) (A), macrophage-activating lipopeptide-2 (MALP-2) (TLR2/6) (B), flagellin (TLR5) (C), and whole Gram-positive (*S. aureus*) (D) and Gram-negative (*P. aeruginosa*) (E) killed bacteria. For the detection of intracellular IL-6, whole blood samples were stimulated for 6 h. Monocytes were identified by forward scatter and side scatter parameters, CD14-positive staining and intracellular detection of IL-6 on monocytes performed by flow cytometry. \*P < 0.05 compared to no pretreatment (ANOVA followed by the Bonferroni's test). \*P < 0.05 compared to pretreatment with 7.5 ng/mL LPS (ANOVA followed by the Bonferroni's test). N = 5 for three different experiments.

Once tolerance was demonstrated to LPS, a TLR4 agonist, in the model of whole blood *in vitro*, we tested whether this effect occurs with TLR2/6 and TLR5 agonists. The pre-exposure to LPS led to a reduction of IL-6 production in peripheral blood monocytes after challenge with the TLR2/6 agonist MALP-2. This finding agrees with Sato and colleagues (30), who observed reduced production of TNF- $\alpha$  in peritoneal macrophages of mice pre-exposed to LPS and challenged with MALP-2. However, the production of this cytokine was increased when both MALP-2 and LPS were simultaneously added to the culture of peritoneal macrophages, showing a synergistic effect between these agonists. Conversely, Dobrovolskaia et al. (12) demon-

strated that conditioning with LPS from *Escherichia coli* and challenge with LPS from *Porphyromonas gingivalis* (a TLR2 agonist) were not able to reduce the production of TNF- $\alpha$  in peritoneal macrophages of mice, the same occurring when the cells were conditioned with *E. coli* LPS and challenged with Pam3Cys (a TLR2/1 agonist) (12).

Conditioning with LPS did not change the production of IL-6 after a challenge with flagellin (a TLR5 agonist). A possible explanation for the lack of tolerance is the use of an alternative pathway of cell signaling by flagellin that was not inhibited by pre-exposure to LPS. One possibility that may be considered is the recognition of flagellin directly in the cytoplasm of the cell by Ipaf, a NOD like-receptor,



**Figure 3.** Phagocytosis and reactive oxygen species (ROS) generation by human tolerant monocytes. For monocyte phagocytosis, whole blood was stimulated for 30 min with *S. aureus*-propidium iodide (PI) after pretreatment with or without lipopolysaccharide (LPS; control). Monocyte phagocytosis was shown as the percentage of stained cells (A, left) and as the geometric mean of the fluorescence intensity (GMFI) (A, right). For induction of ROS, whole blood was stimulated for 30 min with  $24 \times 10^6$ /mL *Staphylococcus aureus* (B, left) and  $12 \times 10^6$ /mL *Pseudomonas aeruginosa* (B, right) after pretreatment with or without LPS (control). ROS production was measured by flow cytometry using 2',7'-dichlorofluorescein-diacetate detection. \*P = 0.05 compared to no pretreatment (ANOVA followed by the Bonferroni's test). N = 5 for three different experiments.

which activates the caspase-1 and triggers the release of inflammatory cytokines (31,32). Thus, cross-tolerance seems to depend on the cell type studied, the model used, and the concentration, sequence of conditioning and the stimulus.

After evaluating the modulatory effect of pre-exposure to LPS on the induction of IL-6 by TLR4, TLR2/6 and TLR5 agonists, we assessed the effects of pre-exposure to LPS on the response to Gram-positive and Gram-negative bacteria, where multiple mechanisms of cellular activation are present. We chose to use *S. aureus* (a Gram-positive bacterium) and *P. aeruginosa* (a Gram-negative bacterium), common agents of infections in patients admitted to intensive care units (33).

Conditioning with increasing concentrations of LPS resulted in the detection of lower amounts of IL-6 in monocytes challenged with *Staphylococcus aureus*. This result agrees with Kreutz et al. (34) who showed induction of cross-tolerance between LPS and *S. aureus* in human monocytes by decreasing the production of TNF- $\alpha$ . In contrast, Peck et al. (35) found increased production of TNF- $\alpha$ , IL-6, IL-8, and IL-1 $\beta$  and unchanged production of granulocyte macrophage colony-stimulating factor (GM-CSF) in THP-1 cells conditioned with LPS and challenged with *S. aureus*.

The challenge with *P. aeruginosa* resulted in lower detection of intracellular IL-6 after conditioning with LPS in monocytes of peripheral blood. Corroborating these data, we found a lower production of TNF- $\alpha$  induced by *P. aeruginosa* in peripheral blood cells of septic patients compared to healthy volunteers (10). Thus, the modulation of cells of septic patients during infection again shows similarities with tolerance induced in whole blood *in vitro*.

In previous studies involving patients with severe sepsis and septic shock, we observed a dichotomy in mononuclear cell modulation, with lower production of inflammatory cytokines after stimulation with LPS, and preserved or increased production of ROS (11,20). To check whether this phenomenon was also observed in monocytes of healthy volunteers conditioned *in vitro* with LPS, we assessed phagocytosis and ROS generation induced by challenge with LPS, *P. aeruginosa* and *S. aureus*. Pre-exposure to LPS did not alter the percentage of monocytes phagocytosing bacteria or the GMFI of positively stained cells, indicating that both the proportion of phagocytosing cells and the amount of phagocytosed bacteria are preserved.

Conditioning with LPS did not alter the production of ROS in monocytes challenged with *P. aeruginosa* or LPS, and an even increased production of ROS was detected in monocytes challenged with *S. aureus*. Thus, we demonstrated that monocytes of healthy individuals tolerant to LPS show the same dichotomy that we observed in septic patients. These results in humans are supported by experimental evidence showing that animals tolerant to LPS present preserved bacterial clearance (15), and preserved oxidative stress response and NO production (36). The presence of antimicrobial peptides produced by cells during sepsis or after exposure to LPS *in vitro* could support these findings. It has been shown that pre-incubation with antimicrobial peptides increases respiratory burst in murine (RAW 264.7) and human (THP-1) macrophage-like cell lines, while inhibiting the release of TNF- $\alpha$  and NO induced by LPS (37). Our results also corroborated the findings of del Fresno et al. (38) who showed that human monocytes made tolerant to LPS and monocytes isolated from cystic fibrosis patients had high phagocytic activity and low production of inflammatory cytokines. This dichotomy fits the concept of Foster and co-workers (29) that gene regulation in cells made tolerant to LPS falls into two classes, the tolerizeable (T) and non-tolerizeable (NT) genes. The T genes, which are associated with pro-inflammatory genes, are down-regulated to protect against tissue damage, and the NT genes, which are associated with antimicrobial genes, remain inducible to protect against the microbes (29).

In conclusion, the phenomenon of tolerance in human whole blood monocytes encompasses up- and down-regulation of cellular activity. Decreased production of proinflammatory cytokines and preserved or increased production of ROS may be an adaptation driven to control the deleterious effects of inflammation, while preserving antimicrobial activity, corroborating a previous study on gene expression in tolerant cells (29). The similarities between the changes found in leukocytes of septic patients and in cells made tolerant to LPS *in vitro* indicate that this approach may be valuable for the understanding of the modulation of LPS-induced cell signaling during sepsis.

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