Lanthanum Inhibited the Binding of LPS with Monocyte and CD14 Expression Upregulation

Yang Wang¹,³, Fei Guo², Feng Hu¹, Gongxian Wang¹ and Guohui Li²

To investigate the effects of lanthanum chloride on binding of LPS to monocyte and CD14 expression upregulation induced by LPS, human monocytes were analyzed by flow cytometry (FCM). The results indicated that lanthanum chloride could decrease the binding rate of LPS with monocyte significantly. LPS upregulated the expression of CD14 on monocyte in dose dependant manner, however, lanthanum chloride could inhibit the increase of CD14 expression on monocytes by halves. Cellular & Molecular Immunology. 2004;1(5):392-394.

Key Words: LPS, lanthanum chloride, CD14, monocyte

Introduction

Lipopolysaccharide (LPS, endotoxin), a major component of the outer cell wall of Gram-negative bacteria, is a key factor in eliciting Gram-negative bacteria sepsis. LPS exerts many of its biological effects by binding to specific cell surface receptors. Recently, it has been progressing rapidly in investigating the molecular mechanism of signal transduction induced by LPS. In addition, the method using LPS receptor inhibitor to modulate the expression of cytokines to improve sepsis/shock, has provided new insights into clinic therapy. Lanthanum is one of rare earth with extremely active chemical property and our previous studies showed that lanthanum chloride could bind LPS, reduce its toxicity, greatly decrease the secretion of TNF-α and expression of TNF-α mRNA in mice challenged with LPS and inhibit LPS-induced apoptosis of thymocyte and damage of liver and lungs (1). In this study, we explored effects of lanthanum chloride on binding of monocyte with LPS and CD14 expression on monocyte induced by LPS.

Materials and Methods

Reagents

The following materials were purchased from Sigma (USA): LPS (E.coli. serotype O55:B5), lanthanum chloride (LaCl₃·7H₂O, hallmark 99.9%) and fluorescein isothiocyanate (FITC)-labelled LPS (FITC-LPS, 3 µg FITC/mg LPS). Phycoerythrin (PE)-conjugated mouse anti-human CD14 and isotype control mouse IgG2b were from Diacclone (France). Fetal bovin serum (FBS, LPS < 0.03 U/ml) was from Gibco (USA).

Flow cytometric analysis

Blood samples were obtained from healthy volunteers. After discarding the first 2 ml, the subsequent whole blood was collected in heparinized tubes. (1) The binding rate of LPS and monocyte assay (according to previous protocol (2)): Immediately after sampling, blood was divided into two groups: LPS group and LaCl₃ group. There were 6 tubes with 100 µl whole blood in each group. Then the LPS group was treated with 2 µg/ml FITC-LPS (final concentration) and LaCl₃ group was done with the FITC-LPS which had been pre-incubated with 17 µmol/L LaCl₃ (final concentration) for 15 min. The two groups were incubated at room temperature in the dark for 30 min. In addition, the blood without adding anything as autofluorescence control was run in parallel. After lysis of erythrocytes, samples were measured by flow cytometry (FCM). (2) CD14 expressing assay (according to previous protocol (3)): Immediately after sampling, blood was treated with increasing concentrations of LPS (final LPS concentrations 1, 10, 50, 100 ng/ml) for 60 min at 37°C. Then they were washed in phosphate-buffered saline (PBS) 3 times for analysis. In addition, four groups were made to observe the effect of LaCl₃ on CD14 expression: LPS group, LaCl₃ + LPS group, LaCl₃ control group and NS control group. There were 6 repeat tubes with 100 µl whole blood in each group. LPS group was treated with 50 ng/ml LPS, LaCl₃ + LPS group was treated with 50 ng/ml LPS and 17 µM LaCl₃ at the same time, LaCl₃ control group

Abbreviations: LPS, lipopolysaccharide; LaCl₃, lanthanum chloride; FCM, flow cytometry; FITC, fluorescein isothiocyanate; MFI, mean fluorescence intensity.

Received for publication Sep 13, 2004. Accepted for publication Oct 21, 2004.

Copyright © 2004 by The Chinese Society of Immunology.
was done with 17 μM LaCl₃ and the NS control group was done with NS. All of them were incubated at 37°C for 60 min and then washed 3 times in PBS. For analysis of CD14 expression, all above blood samples were incubated with PE-labeled anti-CD14 at 4°C in the dark for 30 min. At the same time, autofluorescence and isotype controls were run in parallel. For lysis of erythrocytes, 1 ml of lysis buffer (NH₄Cl 155.5 mmol/L, NaHCO₃ 1 mmol/L, EDTA-Na₂ 0.109 mmol/L) was applied for about 10 min at room temperature in the dark. The samples were then centrifuged at 1,500 rpm for 5 min at 4°C in PBS, and prepare for flow cytometric analysis. A FACSort flow cytometer equipped with a 488 nm Argon ion laser (Becton Dickinson) supplied with CellQuest software was used. Antibody binding was shown as positive percentage and mean fluorescence intensity (MFI).

Statistical analysis
Data were presented as mean ± standard error of the mean (SEM). Software SPSS 10.0 was used to evaluate the statistical significance of the results.

Results

Binding rates of LPS with monocytes
The binding rate of LPS group was 56.31 ± 8.67%, significantly higher than that of LaCl₃ group (13.51 ± 1.37%), p < 0.001. The MFI of LPS group was 28.07 ± 12.24, higher than that of LaCl₃ group (14.73 ± 0.67), p < 0.05 (Table 1). All these results showed that LPS could bind monocytes and the binding ability of LPS pretreated with lanthanum chloride was significantly lower, suggesting that lanthanum chloride could inhibit the bioactivity of LPS and affect the binding of LPS with monocytes (Figure 1 and Table 1).

CD14 expression on monocytes induced by LPS and the effect of lanthanum chloride on it

The positive percentages of CD14 on monocytes stimulated with 1, 10, 50, 100 ng/ml LPS were 12.06 ± 1.05%, 36.59 ± 2.75%, 57.67 ± 5.06% and 33.26 ± 2.3%, respectively. The results showed that LPS could upregulate the expression of CD14 on the surface of monocytes, and in the dosage of 1-50 ng/ml, the expression level increased dose-dependently. However, when the dosage of LPS was 100 ng/ml, CD14 expression decreased (Figure 2).

In the experiments of the effect of lanthanum chloride on CD14 expression, the data showed that the positive percentage of CD14 in LPS group was 58.79%, significantly higher than that of NS control group (1.35%, p < 0.001); the rate of LPS + LaCl₃ group was 34.94%, significantly lower than that of LPS group, but higher than those of NS and LaCl₃ control groups (1.26%), all with p < 0.001. Therefore, lanthanum chloride could inhibit the increase of CD14 expression on the surface of monocytes induced by LPS incompletely (Table 2).

Discussion

CD14, a major receptor of LPS, mainly locates at monocytes. Many studies show that mCD14 plays an important role in inducing monocytes to produce proinflammatory cytokines (such as TNF-α, IL-1 and IL-6) (4-6). As a receptor of LPS,
mCD14 might be regulated directly by LPS or indirectly by various mediators induced by LPS. *In vitro* experiment was the most popular way to observe the effect of LPS on expression of CD14. However, the results were always controversial but most of the reports showed that LPS increased CD14 expression. Our results indicated that LPS could upregulate the expression of CD14 on the surface of monocytes, and to some degree, the expression level was in a dose dependent manner, 1 ng/ml LPS could increase CD14 expression and 50 ng/ml had the best effect, similar to Marchant’s report (3). Lanthanum chloride was able to inhibit the increase of CD14 expression on monocytes induced by 50 ng/ml LPS to a certain degree. Probably lanthanum chloride could partly bind LPS and inhibit LPS to upregulate CD14 incompletely. Rare earth compounds possess broad pharmacological activities. As early as 1947, rare earth elements with the least toxicity, usually exists as trivalence cation with extremely active chemical property and can form compounds with various molecules easily (7). Our previous studies proved that lanthanum chloride could bind to LPS and inhibit LPS from inducing macrophages of mice to secrete TNF-α and express TNF-α mRNA (8). The present study further demonstrated that LPS could bind to monocytes and lanthanum chloride could inhibit the binding and that LPS could upregulate the expression of CD14 on the surface of monocytes in human whole blood, and the expression level was in a dose dependent manner, but when LPS surpassed a certain quantity, the expression of CD14 decreased; meanwhile, lanthanum chloride could inhibit the increase of CD14 expression on monocytes induced by LPS incompletely. In order to block inflammatory cascade elicited by LPS and to cure sepsis, scholars have been searching for LPS antagonists and observing the effects of hundreds of medicaments in the recent 30 years. Medicaments, including lipid A inhibitor, antibody of LPS, proteins that can bind to or counteract with LPS (e.g., BPI, rHDL and LALF, etc.), anti-CD14, inhibitor of endocellular signal transduction (such as protein tyrosine kinase inhibitor, etc.), antibodies and inhibitors to cytokines, can not be applied to clinic therapy extensively because of limitation in antagonism, toxicity or side effects, etc. Our research, summing up domestic and overseas experience, suggests lanthanum has potentially applicable value in the prevention and cure of endotoxic sepsis.

Acknowledgements

This work was supported by National Natural Science Foundation of China (30260107) and the Natural Science Foundation of Jiangxi province (024008).

References