Enhanced Production of IL-1β and IL-6 Following Endotoxin Challenge in Rats with Dietary Magnesium Deficiency

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ABSTRACT. Serum IL-1β, IL-6 and TNFα were not detected in control and Mg-deficient rats. These three cytokine levels in serum were increased after endotoxin challenge (1 mg/kg, i.p.), and the increase of IL-1β and IL-6, but not TNFα, was significantly larger in Mg-deficient rats than in controls. Levels of mRNA for IL-1β, IL-6 and TNFα in alveolar macrophages showed a tendency to decrease during Mg deficiency, but the levels of IL-1β and TNFα mRNAs after endotoxin challenge were higher in Mg-deficient rats than in controls. These results suggest that the increased synthesis of cytokines by alveolar macrophages might contribute, in part, to high sensitivity to endotoxin during Mg deficiency.

KEY WORDS: cytokine, endotoxin, magnesium.

Recent reports have shown that magnesium (Mg) deficiency aggravates endotoxin shock [7, 14]. However, the precise mechanism responsible for this detrimental effect of Mg deficiency remains unclear. The macrophage is a major effector cell in the host response to endotoxin [6]. It has been reported that plasma pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor alpha (TNFα), were increased by Mg deficiency [2, 4, 18] and by endotoxin challenge [1, 3, 6, 8–10]. These results suggest that elevation of plasma pro-inflammatory cytokine levels during Mg deficiency is involved in the enhancement of endotoxin toxicity. Therefore, the purpose of the present study was to assess the effects of dietary Mg deficiency on IL-1β, IL-6 and TNFα production following endotoxin challenge in vivo and on the levels of mRNA for these cytokines in alveolar macrophages.

Male Wistar rats, 7–9 weeks old, were used. After being given a synthetic control diet (Mg: 0.07% (w/w)) [11] and distilled water for drinking ad libitum for 3 days, they were separated randomly into two groups: Mg deficient (Mg; 0.001% (w/w)) and control. Each rat was placed in an individual stainless steel cage in a temperature-controlled room (22–24°C) with a 12 hr dark (20:00–08:00 hr) and 12 hr light period. They were pair-fed with each synthetic diet for 3 days, then challenged with endotoxin or sterile phosphate-buffered saline (PBS). Endotoxin (LPS; Escherichia coli, 011:B4, Sigma, U.S.A.) was dissolved in PBS immediately before administration, and the rat was administered either endotoxin (1 or 5 mg/ml/kg, i.p.) or PBS (1 ml/kg, i.p.). Each rat was sacrificed by anesthesia with sodium pentobarbital (30 mg/kg, body weight, i.p.) 2 hr after the administration of endotoxin or PBS for determination of serum Mg, IL-1β, IL-6 and TNFα, and cytokine mRNA levels in alveolar macrophages. Blood was collected by excision of the abdominal aorta and serum was obtained and stored at –80°C until analysis. Alveolar macrophages were prepared by lung lavage as described previously [5]. Serum Mg levels were determined by flame atomic absorption spectrometric analysis (Hitachi 170–20, Japan). All experimental protocols were approved by the Committee on Animal Research at Kagoshima University.

Levels of IL-1β, IL-6 and TNFα in serum were measured using individual enzyme-linked immunosorbent assay (ELISA) kits, according to the protocol provided by the manufacturer (BioSource International Inc., U.S.A.). The assay limits for IL-1β, IL-6 and TNFα were 3 pg/ml, 31 pg/ml and 4 pg/ml, respectively. Total RNA in alveolar macrophages was extracted using the acid-guanidium-phenol-chloroform method. Complementary DNA (cDNA) was synthesized from total RNA using You-Prime First-Strand Beads (Amersham Pharmacia Biotech, UK) and an oligo d(T)18 primer. PCR was performed using each specific primer, 7.5 U Taq polymerase (Sawady Technology, Japan) and cDNA in a PCR system 2400 (Perkin Elmer, U.S.A.). Oligonucleotide primer pairs were based on the sequences of the rat IL-1β [15], rat IL-6 [12] and rat TNFα genes [16]. PCR products were electrophoresed through 1.4% (w/v) agarose gel, and stained with 0.5 μg/ml ethidium bromide for detection and quantification by computerized densitometric scanning using Diversity Database™ software. The mean concentrations of each cytokine were analyzed using ANOVA for multiple comparisons: when ANOVA indicated significance, Scheffe’s test was used to compare groups. All data were analyzed using Statview J-4.5 Statistical Software for Macintosh. For all statistical tests, p<0.05 was considered to be significant.

The serum Mg level fell to less than a quarter (0.14 ± 0.02 mM; n=4) of the control level (0.80 ± 0.05 mM; n=4) on the 20th day of Mg deficiency. Ca, Na and K levels in serum
did not change significantly during Mg deficiency. These results were similar to our previous data [11]. IL-1β, IL-6 and TNFα levels in serum remained beneath the limit of detection in control and Mg-deficient rats (on days 10, 20 and 30 of synthetic diet). All animals fed synthetic diet for 20 days showed elevated levels of IL-1β, IL-6 and TNFα in serum 2 hr after endotoxin challenge, as shown in Fig. 1. Higher serum levels of IL-1β and IL-6 were observed in the Mg-deficient rats than in the controls. The elevation in the serum level of TNFα did not differ significantly between controls and Mg-deficient rats after challenge with 1 mg/kg endotoxin. These results showed that Mg deficiency did not increase both the serum levels of IL-1β, IL-6 and TNFα, and the mRNA levels for these cytokines in alveolar macrophages. However, the elevation of serum IL-1β and IL-6 levels, and induction of IL-1β and TNFα mRNAs in alveolar macrophages were higher following endotoxin challenge in Mg-defi-

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The present data support, in part, the previous observations [7, 14] of mRNA levels in alveolar macrophages, and suggest that enhancement of endotoxin lethality in Mg-deficient rats may constitute the mechanisms for elevating mRNAs of pro-inflammatory cytokines.

REFERENCES