

PATHOGENESIS

Magnesium Affects the Cytokine Secretion of CD4⁺ T Lymphocytes in Acute Asthma

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Introduction. Magnesium (Mg) administration has been shown to promote bronchodilation and to improve lung function in asthma. It also plays an additional role in modulating the immune responses. This study was initiated to explore if Mg supplementation could affect the secretion of cytokines in acute asthmatic CD4⁺ T cells. **Methods.** Total serum Mg concentrations of the acute asthmatic patients and healthy controls were determined. CD4⁺ T cells were isolated from the blood of the acute asthmatic patients. They were cultured in various concentrations of Mg-supplemented (0.8, 5, 10, 15, and 20 mmol/l) medium. Cytokine (IL-5, IL-13, and IFN- γ) levels were determined by Enzyme-Linked Immunosorbent Assay (ELISA). **Results.** Serum Mg concentration was lower in the acute asthmatic patients than that in the healthy controls ($p < .05$). The secretion of IL-5 and IL-13 was decreased, while the acute asthmatic CD4⁺ T cells were cultured in 10 and 15 mmol/l Mg-supplemented medium, respectively, as compared to the 0.8 mmol/l Mg group ($p < .05$). The secretion of IFN- γ increased in the 10 mmol/l Mg group ($p < .05$). **Conclusion.** Mg supplementation was able to modulate the immune responses of acute asthmatic CD4⁺ T cells and decrease the secretion of type 2 CD4⁺ T lymphocytes cytokines.

Keywords acute asthma, CD4⁺ T cell, IFN- γ , IL-5, IL-13, immune response, magnesium, regulation

INTRODUCTION

Magnesium (Mg) administration has been shown to promote bronchodilation and to improve lung function in asthmatic patients and can be one of the treatments of asthma (1). Additionally, Mg also plays an essential role in controlling immune phenomena (2). It has been shown that Mg exerts a marked influence on immunoglobulin synthesis, immune cell adherence, antibody-dependent cytotoxicity, Immunoglobulin M (IgM) lymphocyte binding, macrophage response to lymphokines, and T helper–B cell adherence (3). A study showed that the deficiency of Mg in diet was able to increase the total leukocytes in rats and increase the subpopulation counts of lymphocytes, neutrophils, monocytes, and eosinophils. B-cells decreased significantly as a percentage of lymphocytes when dietary Mg was decreased. As total counts in blood, B-cells, CD4, and CD8 cells were significantly increased in the rats consuming the 30 ppm Mg diet (4). Another study demonstrated in the high-dose Mg-treated rats that the mitogen response of lymphocytes to lipopolysaccharide (LPS) in the spleen was significantly reduced, the reactive oxygen species level in macrophages was decreased, and the nitric oxide production was decreased. In addition, tumoricidal activities of splenic NK cell and peritoneal macrophage in high Mg exposed rats were significantly increased (5). All of these suggest a crucial role of Mg in

maintaining an optimum immune response. The measurement of serum Mg concentration is the most available and commonly applied test to assess Mg status, which is generally stable in the range of 0.8–1.2 mmol/l. Serum values that are under 0.8 mmol/l usually indicate a severe Mg deficiency.

The contribution of CD4⁺ T helper cells in controlling airway inflammation in asthma is well established (6). Asthma is now increasingly being recognized as a heterogeneous disorder and a consequence of differential roles of type 1 CD4⁺ T lymphocytes (Th1) and type 2 CD4⁺ T lymphocytes (Th2) immune reactions. The Th1/Th2 imbalance, reflected by the predominance of Th2 cells and their cytokines, is important in the pathogenesis of allergic asthma (7). The Th2 cells, through the secretion of their cytokines (IL-3, IL-5, IL-9, IL-13, etc.), are assumed to contribute to various pathological features of asthma (8). The Th1 cells and their cytokines (such as IFN- γ and IL-2) are also involved. However, the CD4⁺ T lymphocyte subpopulations have not been investigated for their Th1/Th2 status in Mg supplementation.

We initiated this study to investigate the association between serum Mg concentration and acute asthma and to explore the impacts of Mg supplementation on the levels of Th1/Th2 cytokines (IL-5, IL-13, and IFN- γ) produced by the acute asthmatic CD4⁺ T cells.

MATERIALS AND METHODS

Participants

All experiments were performed on human CD4⁺ T lymphocytes isolated from the peripheral blood of the acute

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asthmatic patients. The study was approved by the Institute Ethics Committee of Sun Yat-sen University. A written informed consent was obtained from all the participants.

We recruited 36 patients diagnosed with asthma with mild to moderate acute attack according to the Global Initiative for Asthma (2006 criteria) (9) from August 2007 to March 2008 at the outpatient clinic of the Fifth Affiliated Hospital of Sun Yat-sen University. Patients with mild to moderate acute exacerbations of asthma attending the outpatient clinic were informed of the choice of participation in the study as well as the risks, benefits, and alternatives. Patients who consented to participate were enrolled consecutively. All these patients were receiving conventional treatment for acute exacerbations before the blood samples were taken. The treatment included glucocorticoid and bronchodilators. Treatment of diuretics has been terminated for more than 2 weeks before the blood samples were taken. A group of 20 healthy individuals were enrolled concurrently in the study. Those who had any history of cardiac, renal, or hepatic dysfunction were excluded. In addition, participants with family history of diabetes mellitus or receiving any Mg-wasting drug, such as diuretics, were not included in this study. Only one blood sample of 10 ml was needed from each participant. The blood sample was drawn from the antebachial basilic vein between 8:00 am and 10:00 am after overnight fasting. The baseline demographics of the participants are shown in Table 1.

Laboratory Analysis

All chemicals were purchased from Sigma Chemical Co., Shanghai, China unless otherwise indicated. Roswell Park Memorial Institute (RPMI) medium 1640 and modified Hanks' balanced salt solution lacking Mg were also purchased from Sigma.

Each blood sample was labeled with a code and made blind to the laboratory personnel. Each sample from the acute asthmatic patients was split into two parts. Two milliliters of each sample was then centrifuged and the serum was used for determining the Mg concentration. The remaining 8 ml of each sample was used for isolating the CD4⁺ T lymphocytes. The blood samples from the healthy donors were only used to determine the serum Mg concentration. Total serum Mg concentration was determined using flame atomic absorption spectrometric analysis

(Perkin Elmer 400, Analyst 400 Atomic Absorption Spectrometer, PerkinElmer Inc., Massachusetts, USA).

Preparation and Culture of Human CD4⁺ T Lymphocytes

Eight milliliter of total blood from each acute asthmatic patient was used for peripheral blood mononuclear cells (PBMCs) isolation. PBMCs were isolated by density gradient centrifugation (10) (Jingmei Biotech Co. Ltd., Shanghai, China) and cultured in RPMI 1640 complete medium (supplemented with 2 mmol/l L-glutamine, 10 mmol/l 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1 mmol/l sodium pyruvate, 100 units/ml penicillin G, and 100 mg/ml streptomycin). CD4⁺ T cells were isolated from the gradient purified PBMCs by negative selection using antibodies for CD8⁺ T cells. Briefly, the isolated PBMCs (5×10^6 /ml) were suspended in RPMI 1640 complete medium and incubated with 1 μ g of anti-CD8 antibody (mouse anti-human CD8 antibody, Sigma) for 30 min at 37°C. The mixture was then centrifuged at 1000 rpm for 5 min at room temperature. The supernatant was removed and the cells were resuspended in 3 ml RPMI 1640 complete medium. The magnetic beads (30 μ g) against the anti-CD8 antibody (goat anti-mouse IgG magnetic beads, Sigma) were used for binding the CD8⁺ T cells for 30 min at 37°C. A magnetic isolator was used to precipitate the bead-binding CD8⁺ T cells. The supernatant, which contained mostly CD4⁺ T cells, was transferred to fresh tubes followed by centrifugation. The CD4⁺ T lymphocytes were obtained and resuspended in RPMI 1640 medium. Trypan blue staining revealed no less than 95% of the cells were viable. Immunostaining of CD4 antibody and subsequent analysis by FACScalibur flow cytometry (Becton Dickinson, Franklin Lakes, New Jersey, USA) were performed. The percentage of CD4⁺ T lymphocytes isolated by this method always exceeded 95%.

To assess whether changes in extracellular Mg levels may affect cytokine secretion in the CD4⁺ T lymphocytes, the cells were cultured in various concentrations of Mg-supplemented (0.8, 5, 10, 15, and 20 mmol/l) medium at 37 °C and 5% CO₂ (HERA Cell CO₂ incubator, Heraeus, Hanau, Germany). Cytokine levels were measured at 24, 48, and 72 h.

Quantitation of Cytokine Levels

Cytokine levels (IL-5, IL-13, and IFN- γ) were determined by ELISA using the monoclonal antibodies purchased from Jingmei Biotech Co. Ltd., China. At 24, 48, and 72 h, the supernatants of the culture medium were added to the wells coated with the respective monoclonal antibody against the cytokine of interest. The absorbance at 450 nm was measured (Bio-Tek Instruments Inc., Beijing, China). A standard curve was produced concurrently using the standard protein sample. The assay was performed according to the manufacturer's protocol.

Statistical Analysis

The values are expressed as mean $\pm$ SD. A comparison of the means between indicated groups was carried out using

TABLE 1.—Baseline demographics and serum Mg concentrations in the participants.

	Asthmatic patients	Healthy controls	
	n = 36	n = 20	p-Value
Gender (M/F)	25/11	11/9	.384
Age (years)	31 $\pm$ 3	29 $\pm$ 4	.87
Serum Mg concentration* (mmol/l)	0.78 $\pm$ 0.078	0.88 $\pm$ 0.057	.021

Notes: M, male; F, female.
p-value: *p < .05.

Student's *t*-test. A comparison of the means between multiple groups was performed using one-way analysis of variance (ANOVA). A level of $p < .05$ was considered significant.

RESULTS

Serum Mg Concentration in Acute Asthmatic Patients and Healthy Control Participants

The baseline demographics of the participants are shown in Table 1. The two groups were comparable with regard to the baseline characteristics. The serum Mg concentration of the acute asthma group was 0.78 ± 0.078 mmol/l, while that of the healthy control group was 0.88 ± 0.057 mmol/l ($p < .05$). This indicates that the patients suffering from acute asthma may have lower serum Mg levels as compared to the healthy controls.

Effects of Extracellular Mg Concentration on Cytokine Secretion (IL-5, IL-13, and IFN- γ) of CD4⁺ T Lymphocytes

Levels of cytokines (IL-5, IL-13, and IFN- γ) secreted by the acute asthmatic CD4⁺ T lymphocytes in various concentrations (0.8, 5, 10, 15, and 20 mmol/l) of Mg-supplemented medium are shown in Table 2. We found that the levels of IL-5 and IL-13 decreased gradually when the CD4⁺ T lymphocytes were cultured in 5, 10, and 15 mmol/l Mg-supplemented medium, respectively, as compared to those in the 0.8 mmol/l Mg group at the respective time point (Table 2, Figure 1A and 1B). However, when the Mg concentration increased to 20 mmol/l, the secretion of IL-5 and IL-13 no longer decreased. This suggests that 15 mmol/l Mg may exert an optimal effect for reducing the Th2 cytokines in the acute asthmatic CD4⁺ T lymphocytes. In addition, the secretion of IFN- γ varied with different Mg concentrations. It seems that the 10 mmol/l Mg

could enhance the secretions of IFN- γ at 24 and 48 h, but not at 72 h (Table 2, Figure 1C). Our in vitro data suggest that Mg supplementation may produce immunological modifications in the CD4⁺ T lymphocytes in acute asthma.

DISCUSSION

Through its regulatory effect on sodium-potassium pump and ATPase, Mg directly affects the intracellular concentrations of potassium and calcium and its increased extracellular levels favorably influence the cell tolerance to stress and cell edema (11). Low extracellular Mg concentrations (0–0.4 mmol/l) are associated with moderate and severe human hypomagnesemia (0.01–0.5 mmol/l) (12), and high concentrations (2 and 5.6 mmol/l) are associated with human hypermagnesemia after therapeutic use (3.5–5 mmol/l) (13). A study examined the effects of Mg on the occurrence of apoptosis in primary cultures of rat hepatocytes, which revealed that low extracellular Mg concentrations (0–0.4 mmol/l) enhance lipid peroxidation, but high (2 and 5.6 mmol/l) concentrations of Mg cannot clearly identify a beneficial effect (14). There was other study showing that treatment with more than 7.5 mmol/l Mg gained positive therapeutic effect in acute stroke (15). In this study, we showed that the serum Mg concentration in the acute asthma group was lower than that in the healthy control group. This is consistent with the findings of the study conducted by Kowal et al. (16).

Reduced microbial exposure in early life is responsible for a shift of Th1/Th2 balance in the immune system toward the preallergic Th2 response. The Th1 cells predominantly produce IFN- γ and delayed-type hypersensitivity, while the Th2 cells produce IL-4, IL-5, IL-6, and IL-13 and regulate B cell and eosinophil-mediated responses. Mbugi et al. (17) reported that Mg deficiency was associated with an 80% increase in IL-13 concentrations and seemed to be associated with a 49% increase in IL-5 concentrations (17). This suggests that Mg deficiency is associated with Th2 cytokine production.

In our study, we found that the serum Mg concentration in acute asthmatic patients was lower than that in the healthy controls ($p < .05$). The secretion of IL-5 and IL-13 by acute asthmatic CD4⁺ T lymphocytes significantly decreased when the cells were cultured in 5, 10, and 15 mmol/l Mg-supplemented medium, respectively, as compared to those in the physiological condition (Mg concentration of 0.8 mmol/l) ($p < .05$, Figure 1A and 1B). On the other hand, a significant increase in IFN- γ production was detected in the acute asthmatic CD4⁺ T lymphocytes in 10 mmol/l Mg-supplemented medium (Figure 1C). The level of IFN- γ reduced after 72 h, which may be due to the prolonged culture time. We found that 10 mmol/l Mg-supplemented medium decreased the levels of IL-5 and IL-13, yet increased the level of IFN- γ in acute asthmatic CD4⁺ T lymphocytes. Since there is the theory of a shift of Th1/Th2 balance towards the predominant Th2 response in acute allergic asthma, attempts to reverse the shift and modulate the imbalance of Th1/Th2 responses have been

TABLE 2.—Cytokine levels in acute asthmatic CD4⁺ T lymphocytes cultured in various concentrations of Mg.

	Mg (mmol/l)	IL-5 (pg/ml)	IL-13 (pg/ml)	IFN- γ (pg/ml)
24 h	0.8	65.5 $\pm$ 1.2	86.6 $\pm$ 2.7	42.1 $\pm$ 1.6
	5	62.30 $\pm$ 1.1*	81.3 $\pm$ 1.8*	44.1 $\pm$ 1.0*
	10	55.9 $\pm$ 1.3*	75.8 $\pm$ 3.3*	45.1 $\pm$ 1.4*
	15	50.3 $\pm$ 1.1*	81.4 $\pm$ 1.9*	42.2 $\pm$ 1.2**
	20	63.4 $\pm$ 2.2*	87.5 $\pm$ 2.5**	43.7 $\pm$ 1.5*
48 h	0.8	66.6 $\pm$ 1.4	90.2 $\pm$ 1.6	41.0 $\pm$ 0.9
	5	55.6 $\pm$ 1.3*	75.1 $\pm$ 1.8*	40.3 $\pm$ 1.4**
	10	48.8 $\pm$ 2.6*	71.1 $\pm$ 1.3*	43.2 $\pm$ 1.3*
	15	55.1 $\pm$ 1.9*	70.4 $\pm$ 1.5*	39.7 $\pm$ 1.3*
	20	65.5 $\pm$ 1.9**	87.2 $\pm$ 1.5*	39.2 $\pm$ 1.0*
72 h	0.8	69.0 $\pm$ 1.3	88.1 $\pm$ 1.2	36.9 $\pm$ 1.2
	5	45.2 $\pm$ 1.5*	78.4 $\pm$ 1.3*	35.9 $\pm$ 1.4*
	10	39.7 $\pm$ 1.9*	70.3 $\pm$ 1.4*	35.9 $\pm$ 1.2*
	15	44.7 $\pm$ 1.1*	68.8 $\pm$ 1.2*	36.4 $\pm$ 1.4*
	20	66.5 $\pm$ 1.3*	87.0 $\pm$ 1.2*	35.6 $\pm$ 5.5**

Notes. *p*-values: * $p < .05$ as compared to the 0.8 mmol/l Mg concentration group at the reference time point;

** $p > .05$ as compared to the 0.8 mmol/l Mg concentration group at the reference time point.

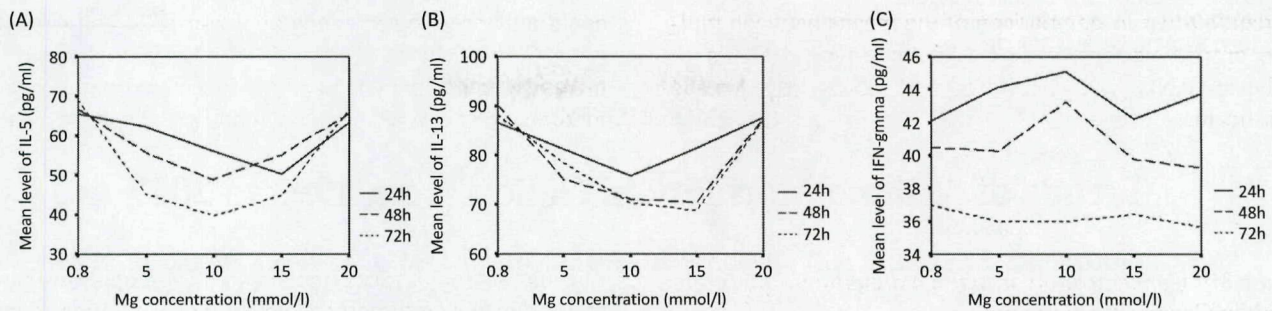


FIGURE 1.—Cytokine levels in asthmatic CD4⁺ T lymphocytes. (A)–(C) Levels of IL-5, IL-13, and IFN-γ secreted by the acute asthmatic CD4⁺ T lymphocytes in various concentrations of Mg-supplemented medium.

made extensively. The results of Mg supplementation in vitro in this study may add to this resolution. However, the regulatory T cell and cytokines (IL-10 or TGF-β) were not included in our investigation. Additional work is necessary to elucidate the interaction of immune system activation with Mg supplementation. The underlying mechanisms for Mg supplementation in modulating Th1/Th2 immune responses in acute asthma deserve further investigation.

CONCLUSION

In summary, our study demonstrated that the serum Mg concentration in acute asthmatic patients was lower than that in the healthy participants. Mg supplementation was able to modulate the imbalance of the Th1/Th2 responses in the acute asthmatic CD4⁺ T lymphocytes. This provided preliminary in vitro evidence for Mg supplementation in regulating the immune system in acute asthma.

ACKNOWLEDGMENT

We thank Ke Yin, Yan Zhang, Xiao Peng, and Yan Shen for technical support at the core facilities of the Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, China.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

This work is supported by the National Natural Science Foundation of China (Rui-yun Liang, 81000015).

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