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The immune-enhancement effect by Falun Gong cultivation

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SUMMARY

Falun Gong (FG) is an advanced system of cultivation and practice, which is beneficial for both mind and body. In this study we investigated the effects of FG on the production of cytokines in FG practitioner (FGP). To study whether plasma cytokines levels were affected by FG, their levels were analyzed. The amount of interferon-γ (IFN-γ), interleukin (IL)-2, IL-4 and IL-6 (2.5-fold for IFN-γ, 1.2-fold for IL-2, 2.1-fold for IL-4 and 2.5-fold for IL-6, respectively) were significantly higher in the FGP group than normal group (P<0.05). Peripheral blood mononuclear cells obtained from normal healthy control and FGP were cultured for 24 h in the presence or absence of lipopolysaccharide. The amount of IFN-γ, IL-2, IL-4 and IL-6 in culture supernatant was quantified. However, there were no significant differences in the level of the same cytokines between the normal and FGP group. These data suggest that FG cultivation may contribute to immune-enhancement *in vivo*.

Key words: Falun Gong; Peripheral blood mononuclear cells; Plasma; Interferon-γ; Interleukin-2, 4, 6

Falun Gong is a high-level cultivation practice founded by Mr. Li Hongzhi (Li, 1994). It cultivates "Truthfulness-Benevolence-Forbearance," the highest characteristic of the universe. FG is an advanced system of cultivation and practice, which is beneficial for both mind and body. Since its introduction, it has been rapidly recognized worldwide for its effects on health improvement and stress relief, and more importantly, for its profound teaching that guides practitioners towards higher levels. FG has five sets of exercises including a meditation part. The movements are elegant, smooth, and easy to learn (Li, 1994).

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T-cells play a crucial role in immune functions as they act both as effectors (cytotoxic T-cells, Tc cells) and regulators (helper and suppressor T-cells, Th and Ts cells). Tc cells can kill virus-infected cells and cells that undergo malignant transformation. Their activation depends on antigen challenge and signals sent from activated Th cells. Th cells also mediate the link between antigen-presenting and the triggering of other cellular (natural and lymphokine-activated killer cells, macrophages, granulocytes) and humoral (B cell-produced antibodies) components of the immune response (Riddell *et al.*, 2002). Especially, Th cells are known to have two different subsets, Th1 and Th2. They are distinguished by cytokines they secret (Mosmann *et al.*, 1986; Del Prete *et al.*, 1991). Th1 lymphocytes produce interleukin (IL)-2, interferon (IFN)-γ and tumor necrosis factor, which promote cell-mediated immunity. Th2 lymphocytes produce IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, and GM-CSF, which promote humoral

antibody-mediated immune response (Mossman *et al*., 1989; Carter et al., 1996). As described above, various cytokines such as IFN-γ, IL-2, IL-4 and IL-6 are related to immune reaction, direct or indirect.

In the present study, we analyzed the production of IFN-γ, IL-2, IL-4 and IL-6 in the plasma between normal group and FGP group. We also measured change of the cytokines production from the lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMC).

MATERIALS AND METHODS

Reagents

Ficoll-Hypaque, LPS, avidin-peroxidase and 2'-azino-bis (3-ethylbenzithiazoline-6-sulfonic acid) tablets substrate were purchased from Sigma (St. Louis, MO, USA). RPMI 1640, ampicillin, streptomycin and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). Anti-human IL-2, 4, 6 and IFN-γ, biotinylated anti-human IL-2, 4, 6 and IFN-γ, and recombinant (r) human IL-2, 4, 6 and IFN-γ were purchased from R & D Systems (Minneapolis, MN, USA)

Sampling

Peripheral blood obtained from the 30 FGP (20 males and 10 females, mean age 48.3, range 41-62) and 30 healthy adults (20 males and 10 females, mean age 52.5 range 41-68) with no FG as a control group. The 26 persons out of 30 FGP answered the questionnaire and their cultivation periods were from 4 months to 6, 8 persons for 1 year, 12 persons for 2~3 years, and 6 persons for over 4 years, respectively. After the FG cultivation, clinical signs of each disease disappeared markedly (Table 1). Informed consent was obtained from subjects before performing this study. All samples were collected in a sterile glass tube and allowed to clot spontaneously for 15 min. Plasma was then collected by centrifugation and quickly frozen and stored in aliquots at −80°C until assay.

Scoring

Type of disease were rated as: 1= cured, 2= improved, 3= not change (Table 1).

PBMC isolation and culture

PBMC (5 FGP and 2 normal control) from heparinized venous blood were isolated by Ficoll-gradient centrifugation, washed three times in phosphate-buffered saline (PBS) solution and resuspended in RPMI 1640 medium (Gibco) supplemented with 2 mM L-glutamin, 100 U / ml penicillin G, 100 μg / ml streptomycin, and 10% FBS inactivated for 30 min at 56°C. PBMC were adjusted to a concentration of 2×10^6 / ml in 30 ml falcon tube, and 100 μl aliquots of cell suspension were placed in a four-well cell

Table 1. Effect of FG on symptom changes of various diseases

Type of disease	Total Cases	Disease curing status					
		Cured		Improved		No change	
		Case	%	Case	%	Case	%
[a]**Digestive system** Gastritis, ulcers, Liver and gallbladder diseases, etc.	15	12	80	3	20	0	0
[a]**Musculoskeletal System** Lumbago, spinal and joint disease etc.	15	13	86.7	2	13.3	0	0
[a]**Cardiovascular System** Coronary artery disease, congestive heart failure, hypertension etc.	12	11	91.6	1	8.4	0	0
[a]**Nervous System** Anxiety, depression etc.	12	10	83.3	2	16.7	0	0
[a]**Ear and Nose System** Empyema, otitis media rhinitis etc.	8	6	75	0	0	2	25
[a]**Respiratory System** Bronchitis, asthma etc.	7	7	100	0	0	0	0

Twenty-six out of 30 FGP answered the questionnaire.
[a]$P<0.05$ (Pearson Chi-Square tests).

culture plate. PBMC were cultured for 24 h in 95% humidified air containing 5% CO_2 (37°C), in the presence or the absence of LPS, and the supernatants were collected by centrifugation and stored at −20°C.

ELISA of cytokines

Sandwich enzyme-linked immunosorbent assay (ELISA) for IFN-γ, IL-2, IL-4 and IL-6 and was carried out in duplicate in 96-well ELISA plates (Nunc, Denmark) coated with each of 100 μl aliquots of anti-human IFN-γ, IL-2, IL-4 and IL-6 monoclonal antibodies (R&D Systems, Minneapolis, MN, USA) at 1.0 μg / ml in PBS at pH 7.4 and was incubated overnight at 4°C. The plates were washed in PBS containing 0.05% Teeen-20 (Sigma, St. Lousis, MO, USA) and blocked with PBS containing 1% BSA, 5% sucrose and 0.05% NaN_3 for 1 h. After additional washes, sample or IFN-γ, IL-2, IL-4 and IL-6 standards were added and incubated at 37°C for 2 h. After a 2 h incubation at 37°C, the wells were washed and then each of 0.2 mg / ml of biotinylated anti-human IFN-γ, IL-2, IL-4 and IL-6 were added and again incubated at 37°C for 2 h. After washing the wells, avidin-peroxidase was added and plates were incubated for 20 min at 37°C. Wells were again washed and ABTS substrate (Sigma) was added. Color development was measured at 450 nm using an automated microplate ELISA reader. A standard curve was run on each assay plate using recombinant IFN-γ, IL-2, IL-4 and IL-6 (R&D Systems) in serial dilutions.

Statistical analysis

To compare clinical characteristics between the normal group and FGP groups, cross-tables were produced using Pearson Chi-Square test to determine statistical significance. Levels of cytokines among the clinical groups were compared using the two-tailed Student's-*t* test; a value of $P<0.05$ was accepted as statistically significant. Values of cytokines are given in the text as mean±standard deviation (SD).

RESULTS

To study which plasma cytokine levels are changed by FG cultivation, their levels were analyzed by ELISA method.

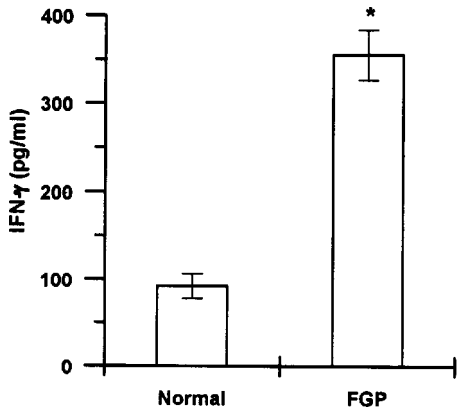

Fig. 1. Effect of FG on plasma IFN-γ level. Data are shown as mean±SD.

IFN-γ levels in plasma

As can be seen in Fig. 1, FGP group showed IFN-γ levels comparable to that of normal group. The IFN-γ plasma level in FGP group was higher than that in normal group (355.6±28.9 pg / ml in FGP group vs 91.7±14.5 pg / ml in normal group). There were significant differences between FGP group and normal group by two-tailed Students- *t* test at $P<0.05$.

IL-2 levels in plasma

As can be seen in Fig. 2, FGP group showed IL-2 levels comparable to that of normal group. The average IL-2 plasma level in FGP group was higher than that in normal group (220±44 pg / ml vs 134.5±24.7 pg / ml). But there were no significant differences between FGP group and normal group.

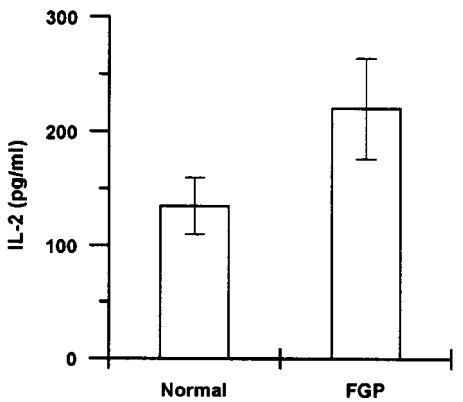

Fig. 2. Effect of FG on plasma IL-2 level. Data are shown as mean±SD.

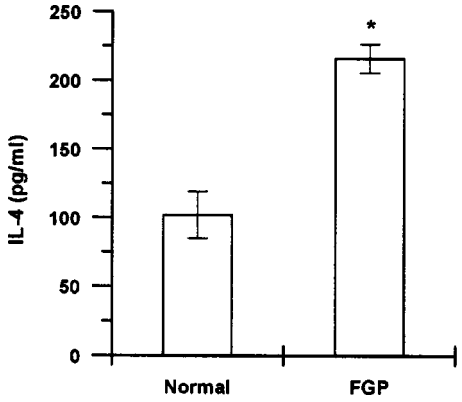

Fig. 3. Effect of FG on plasma IL-4 level. Data are shown as mean±SD.
*Significant differences from normal group and FGP group by two-tailed Student's-*t* test at $P<0.05$.

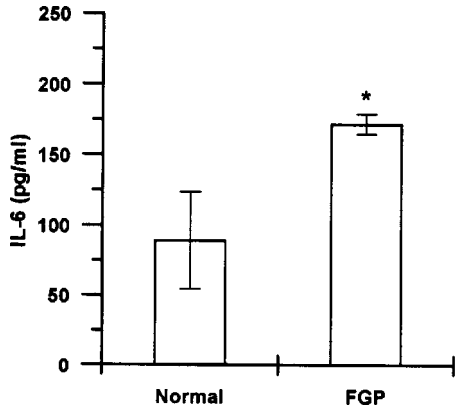

Fig. 4. Effect of FG on plasma IL-6 level. Data are shown as mean±SD.
*Significant differences from normal group and FGP group by two-tailed Student's-*t* test at $P<0.05$.

IL-4 levels in plasma

Higher levels of plasma IL-4 than normal group (101.7±17.2 pg/ml) were measured in the FGP group (216±10.5 pg/ml) (Fig. 3). There were significant differences between FGP group and normal group by two-tailed Student's-*t* test at $P<0.05$.

IL-6 levels in plasma

Higher levels of plasma IL-6 than normal group (88.8±34.6 pg/ml) were measured in the FGP group (171.7±7 pg/ml) (Fig. 4). There were significant differences between FGP group and normal group by two-tailed Student's-*t* test at $P<0.05$.

IL-4/IFN-γ cytokine ratio in plasma

By comparing the Th1/Th2 cytokine ratio, we calculated the IL-4/IFN-γ cytokine ratio. The cytokine ratio was significantly lower in FGP group than normal group (1.1±0.01 in normal group vs 0.6±0.011 in FGP) (Fig. 5, $P<0.05$). This result showed that Th1 cytokine, IFN-γ levels was higher than Th2 cytokine, IL-4 levels in FGP group.

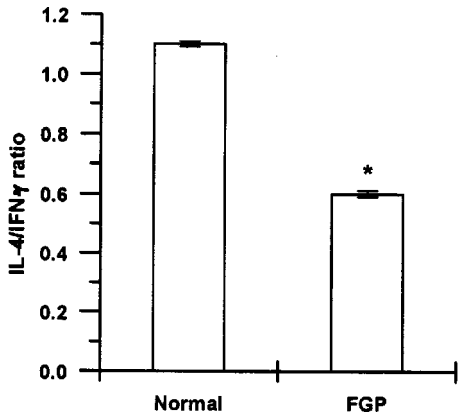

Fig. 5. Effect of FG on IL-4/IFN-γ cytokine ratio in plasma. Data are shown as mean±SD.
*Significant differences from normal group and FGP group by two-tailed Student's-*t* test at $P<0.05$.

Cytokine production on the LPS-stimulated PBMC

As shown Table 2, Spontaneous cytokines (IFN-γ, IL-2, IL-4 and IL-6) production in the culture supernatants of non-stimulated PBMC from FGP group was similar to that in the culture supernatants from normal group. The cytokines production in the culture supernatants of LPS-stimulated PBMC from normal group or FGP group was no significant differences each other (Table 2). Cell toxicity by LPS was not observed (data not shown).

DISCUSSION

We found that plasma IFN-γ, IL-2, IL-4 and IL-6 levels were significantly increased in the FGP group than non-FGP group. Production of the cytokines from LPS-stimulated PBMC was not observed significant differences.

Th cells are thought to be important in the development of various diseases. While Th cells of the Th1 type predominantly produce IL-2 and IFN-γ, and are involved in cell-mediated immune responses, Th cells of Th2 type produce large quantities of IL-4 and IL-6, which promote the development of

Table 2. Effect of FG on the cytokine production from PBMC

(ng / ml)	LPS (ng / ml)	IFN-γ	IL-2	IL-4	IL-6
FGP	-	0.073±0.008	0.07±0.001	0.01±0.003	0.04±0.001
	+	0.323±0.16	0.153±0.05	0.08±0.001	0.64±0.09
Normal	-	0.07±0.003	0.08±0.007	0.02±0.005	0.05±0.009
	+	0.497±0.21	0.176±0.08	0.087±0.004	0.67±0.1

PBMC suspensions (2×10^5 cells) were stimulated with LPS (10 ng / ml) for 24 h. The cells were separated from the released cytokines by centrifugation at 400×g for 5 min at 4°C. Cytokine levels in culture supernatants were measured using ELISA.

inflammation (Romagnani. 1991; Parronchi *et al*., 1991; Prete *et al*., 1991; Zurawski *et al*., 1994). An alteration of cytokine levels could shift the Th1 / Th2 balance toward Th2 dominance, the results being augmented eosinophil recruitment and inflammation (Alenius *et al*., 2001). Other researchers also reported that Th2 cytokine level was higher than Th1 cytokine levels in various diseases including cerebral infarction, allergy and asthma (Kim *et al*., 2000; Jeong *et al*., 2002). IL-2, the cytokine also known as T-cell growth factor, has multiple immunoregulatory functions and biological properties. IL-2, together with other factors and in conjunction with antigens, mitogens, or anti-immunoglobulin antibodies, controls B cell proliferation and differentiation into antibody-producing plasma cells (Jelinek *et al*., 1987). Natural killer and lymphokine-activated killer cells, monocytes and macrophages all have the ability to respond to IL-2 with increased activity or proliferation (Kuziel *et al*., 1991; Minami *et al*., 1992). Shinkai *et al.* (1995) reported that production and responsiveness to IL-2 most notably decreased in the aging process. IFN-γ is also an important cytokine in the host defense against infection by viral and microbial pathogens (Samuel, 2001). IFN-γ induces a variety of physiologically significant responses that contribute to immunity. Recently, other researchers reported that IFN-γ and IFN-related genes were also significantly increased in FGP (Samuel, 2001). In this study, plasma IL-2 and IFN-γ levels in the FGP group were higher than in non-FGP group. These results suggest that FG might have a beneficial effect in the immune-enhancement.

Proinflammatory cytokines involved in hemostatic and immunological imbalance leading to enlargement of various tissue damages. But IL-4 has been also called the prototypic immunoregulatory cytokine. Like many cytokines, it can affect a variety of target cells in multiple ways. IL-4 has an important role in regulating antibody production, hematopoiesis and inflammation, and the development of effector T-cell responses (Brown *et al.*, 1997). IL-6 is also a multifunctional regulator of immune and inflammatory processes that has a range of biologic activities, including important roles in the development of plasma cells and stimulation of the production of acute phase response protein by hepatocytes (Gauldie *et al*., 1987). In this study, the level of IL-4 and IL-6 in FGP group was very higher compared with the normal group. Therefore, we can speculate that IL-4 and IL-6 increased by FG may contribute to immune-enhancement response. But further study is necessary to clarify the role of the IL-4 and IL-6 increased by FG cultivation.

FG has been documented that spiritual practice and mind / body approaches may enhance the effectiveness of immune systems and further are clinically effective in treating a variety of diseases (Coker, 1999; Jones, 2001; Shang, 2001).

In conclusion, our results demonstrate that FG cultivation enhances the cytokines production in plasma. Therefore, we can speculate that a turn for the better of various diseases by FG may be responsible for the enhancement of immune function.

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