

Research article

Changes in cytokine production in healthy subjects practicing Guolin Qigong : a pilot study

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Abstract

Background: Guolin Qigong is a combination of meditation, controlled breathing and physical movement designed to control the vital energy (*qi*) of the body and consequently to improve spiritual, physical and mental health. Practice of Qigong has been reported to alter immunological function, but there have been few studies of its effects on cytokines, the key regulators of immunity.

Methods: Numbers of peripheral blood cytokine-secreting cells were determined by ELISPOT in 19 healthy volunteers aged 27 – 55, before they were taught the practice of Qigong and after 3, 7 and 14 weeks of daily practice. The effect of Qigong on blood cortisol was also examined.

Results: Numbers of IL4 and IL12-secreting cells remained stable. IL6 increased at 7 weeks and TNF α increased in unstimulated cultures at 3 and 7 weeks but decreased at these times in LPS and SAC-stimulated cultures. Of particular interest, IFN γ -secreting cells increased and IL10-secreting cells decreased in PHA-stimulated cultures, resulting in significant increases in the IFN γ :IL10 ratio. Cortisol, a known inhibitor of type I cytokine production, was reduced by practicing Qigong.

Conclusion: These preliminary studies in healthy subjects, although not necessarily representative of a randomized healthy population and not including a separate control group, have indicated that blood levels of the stress-related hormone cortisol may be lowered by short-term practice of Qigong and that there are concomitant changes in numbers of cytokine-secreting cells. Further studies of the effect of Qigong in patients with clinical diseases known to be associated with type 2 cytokine predominance are merited.

Background

Qigong is a combination of meditation, physical movements and controlled breathing. These exercises are designed to develop the skill of controlling *qi*, the vital energy of the body, and consequently to improve spiritual, physical and mental health. Adepts can reportedly use emitted *qi* to alter the performance of cells: "facilitating *qi*" increases cell growth while "inhibiting *qi*" suppresses growth of cultured tumour cells [1].

Guolin Qigong was introduced more than 50 years ago by Madam Guo, a long-term cancer survivor who developed a network of self-help groups throughout China and beyond. This form of Qigong, which is characterized by a slow walking exercise accompanied by arm movements coordinated with slight twisting movements of the waist, is said to be particularly helpful for cancer patients.

Among the organs apparently affected by qigong are brain, heart, kidney and the immune system, with bene-

fits being reported in senility, hypertension, stroke, asthma, blood chemistry and cancer [2–6]. (See also [http://www.goldenflower.org/reflib/articles/tc_as_treatment.shtml]).

The reported benefits of Qigong could be mediated by effects on immunological function and various aspects of immune function could perhaps be influenced by *qi* energy. However, there have been only a few peer-reviewed publications describing alterations in immune system activity as a result of practicing Qigong. Delayed cutaneous hypersensitivity responses were faster, stronger and more numerous in a Qigong trained group than a normal healthy group [7]. Patients with aplastic anaemia treated with Qigong showed significant improvement in CD4⁺:CD8⁺ T-cell ratio [8], which also increased in newly recruited Qigong trainees after 5 months of practice [9]. CD4:CD8 T-cell ratios and NK-cell activity tended to increase during a single 40-minute session of Qigong [10]. Various non-reviewed studies have described increased lymphocyte, neutrophil and natural killer (NK) cell functions in subjects practicing Qigong [see *Extension of Life: Documentation on the Effects of Chi Kong Qigong* (Edited by Zhang RM) Renming Tin Yue Press, China 1995; and [http://www.goldenflower.org/reflib/articles/tc_as_treatment.shtml]].

Cytokines are highly labile effector glycoproteins which are crucially involved in afferent and efferent limbs of immune responses as pro-inflammatory and anti-inflammatory regulators. Type 1 cytokines, such as interferon-gamma (IFN γ), tumour necrosis factor alpha (TNF α) and interleukin (IL) 12, promote cell-mediated responses, while type 2 cytokines, including IL4, IL6 and IL10, are anti-inflammatory and favour humoral responses [11,12]. Cell-mediated responses are important in protection against cancers, while overproduction of anti-inflammatory cytokines is potentially suppressive for anti-cancer immunity. The present pilot study was designed to investigate effects of Qigong on *in vitro* cytokine production in healthy volunteers and to determine whether further evaluation of its effects on the cytokine profiles of patients with cancer would be valuable. We measured production of the above 6 cytokines before Qigong training and for up to 14 weeks of performing the exercises. Significant increases in PHA-stimulated IFN γ :IL10 ratios were found, indicating a shift towards cytokines favoring cell-mediated immunity.

Methods

Subjects and evaluations

Nineteen healthy Chinese subjects aged 27 – 55 years (mean 43.9 \pm 7.8), of whom 11 were females, were recruited by advertisement for first-time training in Guolin

Qigong. One further volunteer was excluded on the grounds of ill-health. Each subject attended six 2-hour Qigong class in a two week period. They then practiced the exercises every day and kept diaries to record details. Ten subjects were still practicing Qigong at the end of the study period (14 weeks from starting training), while 9 ceased practice at 10 weeks. Blood pressure, pulse rate, blood cortisol level and production of IFN γ , IL4, IL6, IL10, IL12 and TNF α were measured before training and after 3, 7 and 14 weeks. One subject was excluded from the main study because of respiratory infection at 3 weeks, but was included in the 14 week comparison of subjects who completed the study with those who stopped, as the infection had cleared at that time.

ELISPOT assay

Numbers of cytokine-secreting cells (CK-SC) in unstimulated cultures or cultures stimulated with T-cell activators phytohaemagglutinin (PHA), Concanavalin A (Con A) or solid-phase anti-CD3, or monocyte activators *E. coli* lipopolysaccharide (LPS) or *S. aureus* Cowan I (SAC) were determined using ELISPOT assays [13,14]. Details of our adaptation of this method and its specificity and reproducibility have been reported [15–17]. Briefly, heparinized blood was processed for cytokine evaluations within one hour of collection. Peripheral blood mononuclear cells (PBM) were separated over Lymphoprep (Nycomed, Oslo, Norway) and added to 96-well Multiscreen plates (Millipore, Bedford, MA, USA) which had previously been coated with cytokine capture antibodies (PharMingen, San Diego, CA, USA) and blocked with 5% fetal calf serum (FCS) in medium RPMI 1640. Duplicate cultures of 10⁴ (for IL6 and TNF α) or 10⁵ (for IFN γ , IL4, IL10 and IL12) cells/well in RPMI + 5% FCS with or without stimulators were incubated for 18–22 hours at 37°C in 5% CO₂. Cells were then washed out and plates incubated sequentially with biotinylated detection anti-cytokine antibodies (PharMingen), streptavidin-alkaline phosphatase (Sigma, St. Louis, MO, USA) and 5-bromo-4-chloro-3-indolylphosphate-nitroblue tetrazolium (Calbiochem, La Jolla, CA, USA). Blue spots corresponding to each cytokine-SC were counted by microscopy with the aid of eyepiece graticules covering 1/8 or 1/72 of the culture well area. Results are expressed as CK-SC/10⁶ PBM.

Statistical analysis

Prism version 2.0 software (GraphPad, San Diego, CA, USA) was used to compare various parameters before and at 3, 7 and 14 weeks after starting training (paired t-test) and to compare results at 14 weeks for subjects who stopped performing Qigong at 10 weeks with those of subjects who continued beyond 14 weeks (unpaired t-test).

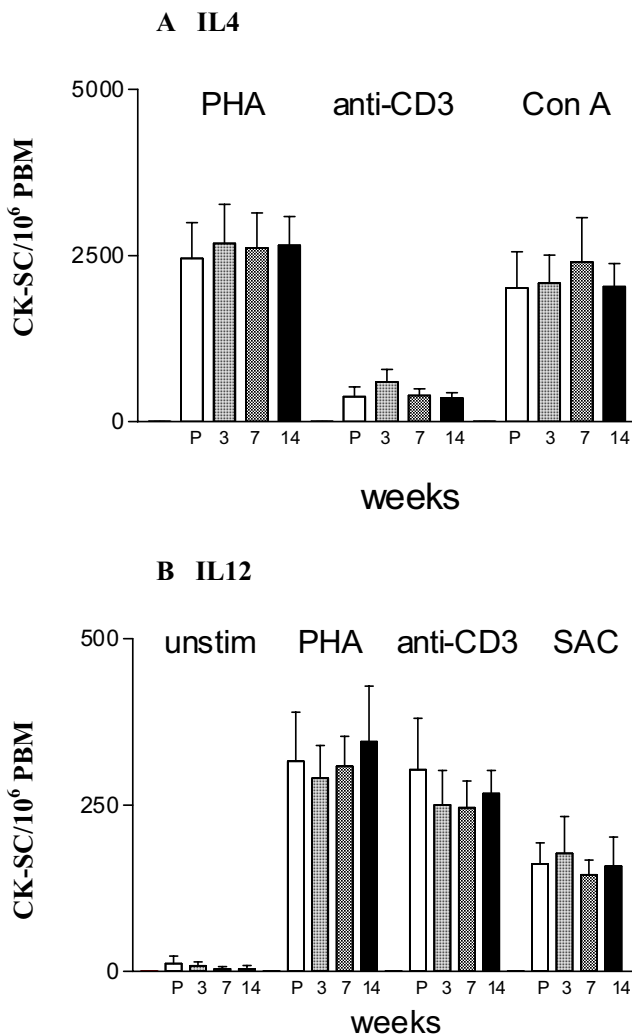


Figure 1
Numbers of IL4 (A) and IL12 (B) cytokine-secreting cells (CK-SC) per 10⁶ peripheral blood mononuclear cells (PBM) measured by ELISPOT assay in 19 healthy volunteers before starting Qigong training (P) and after 3, 7 and 14 weeks of practice. There were no significant changes during the study period.

Results

No significant changes in systolic or diastolic blood pressure occurred over the study period, but pulse rate decreased from 76.3 ± 11.4 (mean \pm SD) before training to 69.8 ± 9.4 at 3 weeks ($p < 0.05$). However, values at 7 and 14 weeks (71.4 ± 9.5 and 74.3 ± 13.3 respectively) were not significantly different from baseline. Plasma cortisol levels at baseline were 394 ± 115 nmol/l and at 3, 7 and 14 weeks were 328 ± 126 ($p < 0.05$), 376 ± 109 (not significant) and 318 ± 110 ($p < 0.05$) respectively.

Numbers of PHA, anti-CD3 and Con A-stimulated IL4-SC (Fig. 1A) and unstimulated and PHA, anti-CD3 and

SAC stimulated IL12-SC (Fig. 1B) remained stable before and after Qigong training. No IL4 (nor IFN γ)-SC were seen in unstimulated cultures, as observed previously [15–17].

IL6-SC were significantly increased after 7 weeks of exercising in unstimulated, PHA-stimulated and LPS-, but not SAC-, stimulated cultures and returned to baseline levels at week 14 (Fig. 2A). TNF α -SC were significantly increased at 3 and 7 weeks in unstimulated cultures and returned to near baseline at 14 weeks. There was no significant increase in TNF α -SC stimulated by T-cell or monocyte activators and in fact LPS and SAC-stimulated TNF α -SC were significantly reduced at 14 weeks compared to pretraining (Fig. 2B).

Reduction in IL6 and TNF α -SC between week 7 and week 14 could have been because approximately half of the subjects stopped exercising at 10 weeks. This was not so for IL6, since cytokine production was similar in those who continued and those who stopped performing Qigong (Fig. 3A). In contrast, stopping Qigong was associated with a significantly lower level of unstimulated and PHA-stimulated TNF α -SC than in those who continued (Fig. 3B).

IFN γ -SC were significantly increased in PHA-stimulated cultures at 3, 7 and 14 weeks. The more powerful IFN γ inducers Con A and anti-CD3 stimulated levels of cytokine production that were less influenced by practicing Qigong, only Con A-induced IFN γ -SC at 3 weeks being significantly higher than before training (Fig. 4A). IL10 production in unstimulated cultures increased significantly after 7 weeks of exercises but fell back to pretraining levels at 14 weeks. PHA, LPS and SAC-induced IL10-SC were similar to pretraining levels at weeks 3 and 7 and lower at week 14, with PHA-induced IL10-SC being significantly lower than before training (Fig. 4B). The ratio of IFN γ :IL10-SC in PHA-stimulated cultures was significantly higher at 3, 7 and 14 weeks than before training (Fig. 4C).

IFN γ -SC, IL10-SC and the ratio of PHA-stimulated IFN γ :IL10-SC at 14 weeks were not significantly different in subjects who stopped performing Qigong exercises at 10 weeks compared with those who continued beyond 14 weeks (Fig. 5).

Discussion

Qigong has been practiced for over 3,000 years in China and evidence of its beneficial effects in maintaining and improving health has been presented, though largely in journals inaccessible to the West. Wider application of Qigong could possibly contribute to a healthier population less dependent on modern medicines, the cost of

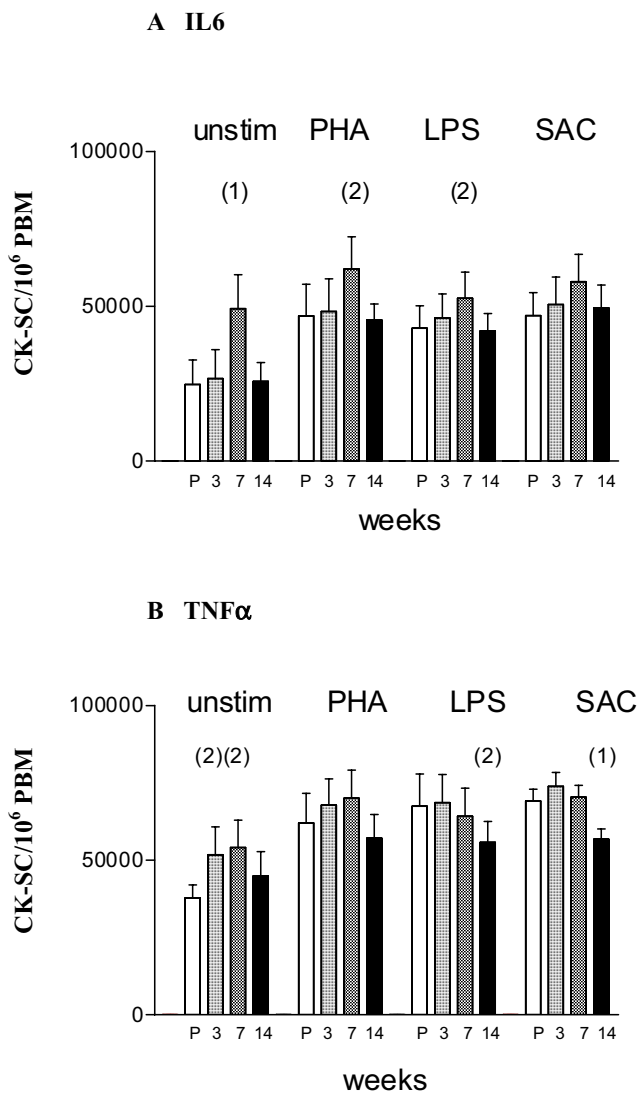


Figure 2
 Numbers of IL6-SC/10⁶ PBM were increased after 7 weeks of Qigong practice compared with pretraining (A). Numbers of unstimulated TNFα-SC/10⁶ PBM were increased at 3 and 7 weeks, but LPS and SAC-stimulated TNFα-SC/10⁶ PBM were decreased at 14 weeks compared to pretraining (B). (1) p < 0.01, (2) p < 0.05 compared with P, paired t-test.

which is spiraling. If it were possible to identify the precise mechanisms by which Qigong achieves these supposed benefits, more focused application of the exercises and of *qi* energy could possibly result in generally acceptable therapies to supplement current practices.

The present study attempted to determine whether the key regulator molecules of the immune system, cytokines, are influenced by Qigong. I chose to evaluate cytokine production using ELISPOT assays because these measure actual secreted cytokine rather than intracyto-

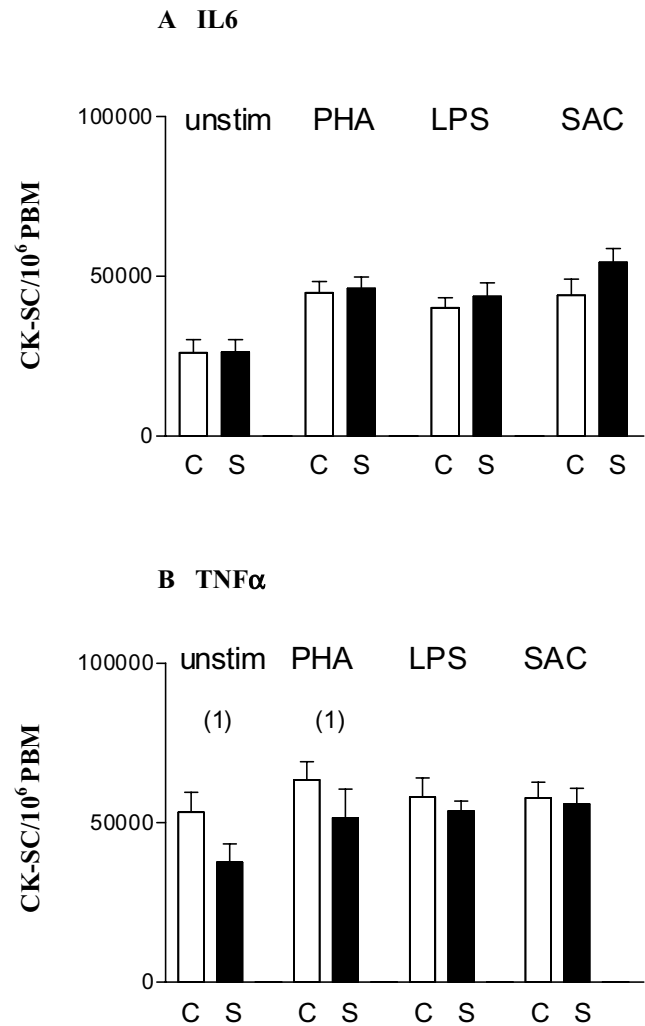


Figure 3
 Numbers of IL6-SC/10⁶ PBM (A) and TNFα-SC/10⁶ PBM (B) at 14 weeks in subjects who continued (C) Qigong practice beyond 14 weeks and those who stopped (S) at 10 weeks. (1) p < 0.05, unpaired t-test.

plasmic protein (as measured by flow cytometry) or message (by reverse transcriptase polymerase chain reaction) and the problem of cytokine neutralization by concomitantly secreted antagonists or soluble receptors, which affects measurement of cytokines in culture supernatants, is avoided.

The ELISPOT assays established in our laboratory are sensitive, specific (no ELISPOTs develop when unmatched pairs of anti-cytokine antibodies are used for capture and detection) and reproducible, with intra-assay coefficients of variation (CV) of 8.8 ± 5.8% for combined data for IFNγ, TNFα, IL4, IL6, IL10 and IL12 and all stimuli [15]. Inter-assay variation is 13.2 ± 4.9%, reflecting considerable individual day-to-day variation,

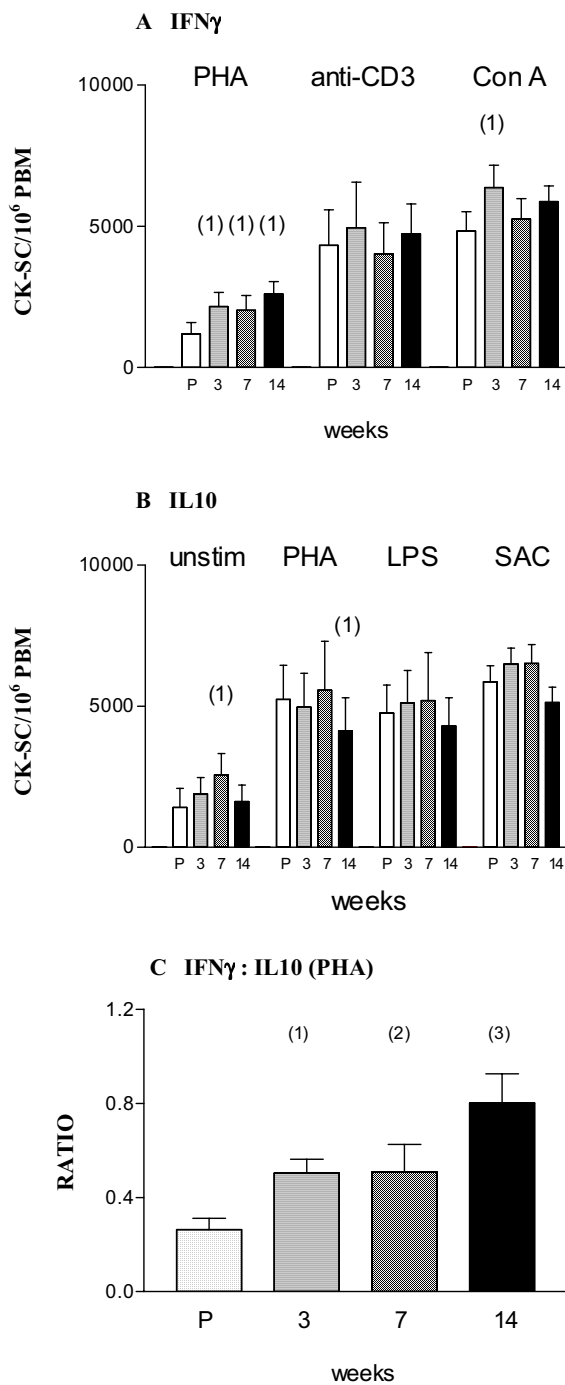


Figure 4 Increased numbers of PHA-induced IFN γ -SC/10⁶ PBM at 3 – 14 weeks of Qigong practice and of Con A-induced IFN γ -SC/10⁶ PBM at 3 weeks compared with pretraining (A). Increased numbers of unstimulated IL10-SC/10⁶ PBM at 7 weeks and decreased numbers of PHA-stimulated IL10-SC/10⁶ PBM at 14 weeks (B). The ratio of PHA-induced IFN γ : IL10 increased with duration of Qigong practice (C). (1) p < 0.01, (2) p < 0.05, (3) p < 0.001 compared with P, paired t-test.

but when groups of subjects are evaluated at different time points, mean values vary by only $5.3 \pm 3.8\%$ [15]. This is acceptable for studying effects of interventions in groups of subjects.

All recruited volunteers were normotensive before training and practicing Guolin Qigong for at least 10 weeks did not alter blood pressure greatly. Pulse rates were lower than baseline at 3 weeks but not at 7 or 14 weeks. Interestingly, reduction in cortisol levels was biphasic, with a return to pretraining levels at 7 weeks despite significant reductions at 3 and 14 weeks. There were no significant differences between cortisol levels in those who stopped exercising at 10 weeks and those who continued. It is possible that practicing Qigong improved the stress-coping skills of our volunteers and that this ability was retained even after exercising was discontinued. This possibility requires further study.

Previous studies have shown that *in vitro* production of IFN γ , IL12 and TNF α is suppressed by cortisol and that these cytokines are negatively correlated with plasma cortisol levels [18]. Administration of cortisone acetate decreases the IFN γ :IL10 ratio by >70% [19]. Improved coping with stress might therefore be expected to lower cortisol and favour type 1 over type 2 cytokine production. Our subjects had significantly increased IFN γ and reduced IL10-SC in PHA-stimulated cultures after practicing Qigong for as little as 3 weeks, with even greater increases at 14 weeks. This effect was still seen at 14 weeks in subjects who stopped exercising at 10 weeks.

However, polarization towards type 1 responses was not found with all *in vitro* stimuli. Increases in IFN γ production after Qigong training were less apparent when Con A or solid-phase anti-CD3 were used. IL10 production was increased rather than decreased at 7 weeks in unstimulated cultures and there was only a trend towards reduced LPS or SAC-induced IL10 at 14 weeks. Furthermore there were no noticeable effects of Qigong on IL12, the key inducer of IFN γ and type 1 cytokines [20], nor on IL4, the central player in type 2 responses [21].

Unstimulated and stimulated IL6-SC were significantly increased at 7 weeks, suggesting that a transient inflammatory event may have occurred at this time. Cortisol levels had risen from the reduced levels seen at 3 weeks and were to fall again at 14 weeks, which might indicate an increase in stress level of unknown causation at this time. However, DeRijk *et al* [22] found that physiological increased levels of cortisol induced by strenuous exercise had no effect on LPS-induced IL6, and pharmacologically increased levels were suppressive.

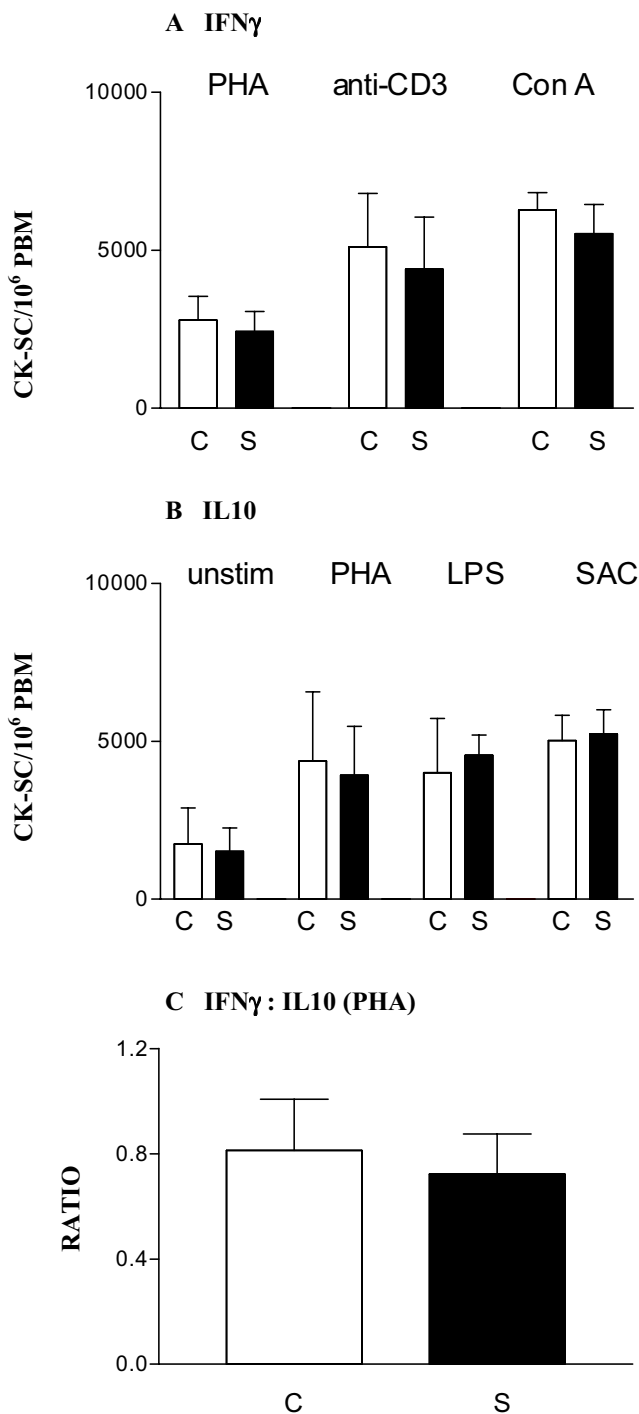


Figure 5
IFN γ production (A), IL10 production (B) and IFN γ :IL10 ratio (C) at 14 weeks after training in subjects who continued (C) Qigong practice beyond 14 weeks and those who stopped (S) at 10 weeks.

TNF α -SC increased over baseline in unstimulated cultures at 3 and 7 weeks and fell back to normal at 14 weeks in subjects who stopped performing exercises at 10 weeks. There was a similar but not significant trend in PHA-stimulated cultures, whereas in cultures stimulated with monocyte activators TNF α levels were suppressed at 14 weeks even in subjects who stopped exercising at 10 weeks. Unlike IL6, TNF α is sensitive to suppression by physiologically-induced increases in cortisol [22] but our results suggest that if the changes in TNF α seen in this study were due to fluctuating cortisol levels then TNF α induction by T-cell activators and *in vivo* stimuli may be affected by cortisol in a different way to that induced by monocyte activators.

Conclusions

A balance between type 1 and type 2 cytokines is required for maintenance of health, a preponderance of the former being associated with certain organ-specific autoimmune diseases and of the latter with allergy, systemic autoimmunity and suppressed responses to viruses and cancer [23]. Preliminary evidence from a small, non-randomized, uncontrolled study has been provided here that Guolin Qigong might alter cytokine production in healthy subjects in the direction of type 1 responses. There is no evidence at present to indicate whether this would be beneficial, leading to stronger protection against, for example, cancer and asthma; harmful, leading to later complications such as autoimmune disease; or without effect, due to the relatively minor changes induced. Further studies of the effects of Qigong, and of the various components of Qigong, on clinical, immunological and physiological parameters in various disease situations are, however, clearly merited.

Competing interests

None declared

Acknowledgements

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