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Assessment of immunological parameters following a qigong training program

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Summary

Background: Qigong is a type of Chinese psychosomatic exercise that integrates meditation, slow physical movements, and breathing, and to which numerous physical as well as mental benefits have been classically ascribed. The aim of the present study was to analyze the effects of a qigong program on various immunological parameters.

Material/Methods: 29 naive subjects participated in the study, of whom 16 were allocated to the experimental group and the rest to the control group. The experimental subjects underwent a qigong training program, conducted by a qualified instructor, consisting of half an hour of daily practice for one month. The day before the experiment commenced and the day after it finished, blood samples were drawn from all subjects for the quantification of immunological parameters (leukocytes, immunoglobulins, and complement). As statistical analysis, analysis of covariance (ANCOVA) was carried out.

Results: Statistically significant differences were found between the control and experimental groups, with the experimental group showing lower numbers of total leukocytes and eosinophils, number and percentage of monocytes, as well as complement C3 concentration. In addition, a similar result with a trend towards significance was observed in the number of eosinophils.

Conclusions: These findings demonstrate that after one month of practicing qigong, significant immunological changes occurred between the experimental and control groups, with a consistently lower and broadly significant profile of these measures within the qigong practitioner group.

key words: **qigong • immune system • meditation • physical exercise • stress**

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BACKGROUND

Qigong is a Chinese psychosomatic discipline that is part of traditional Chinese medicine, together with acupuncture [1] and other, less known, practices such as moxibustion [2,3] and herb therapy [4]. Qigong is commonly referred to as Chinese meditation [5], qigong meditation [6], or moving meditation [7], as it often implies movement. However, qigong practice has some particular features [8,9] which differentiate it from some of the other meditation forms such as yoga or transcendental meditation. In this sense, qigong generally entails a more dynamic approach than the other meditative traditions and its movements are said to stimulate acupuncture meridians [10,11]. Consequently, it has often been regarded as having a number of physical as well as psychological benefits [12–16].

The neuroendocrine and immune systems are two key systems whereby psychosomatic interactions have been found to be of relevance. In fact, the neuroendocrine system is considered to be the main link between the psychological state and the immune system [17]. Thus, a mutual regulation exists between the neuroendocrine and immune systems; the neuroendocrine system influences immune function through hormonal and neural pathways, while the immune system affects neuroendocrine function by means of cytokines [18]. This precise communication between both systems can be seen by the existence of hormone receptors on immune cells and the physical contact, by means of innervation, between the nervous system and immune system lymphoid organs [19,20].

Qigong, like Tai Chi, a specific method of qigong, has a significant physical component from which some of its benefits are supposedly directly derived. In the last two decades, physical exercise has attracted great interest due to its effects on immune function. The effects of exercise on blood leukocyte subpopulations may vary considerably, depending on variables such as the acute or chronic nature of the exercise [21] and the duration and intensity of each session [22,23]. Duration, for instance, can be a critical factor, as it can turn exercise into a physiologically stressful situation, capable of compromising glycemia, thus inducing cortisol liberation which, in turn, affects the immune system. In this respect, administration of corticosteroids to humans has been shown to have a diminishing effect on the counts of most immune cells [24]. In particular, chronic stress, whether physical or otherwise, has been associated with reduced immunity, while acute stress has often been said to be related to activation of acute-phase immune responses [25]. Therefore, it has generally been suggested that short moderate exercise improves immune function, whereas intense exercise of long duration suppresses it [26,27].

The meditative aspect of qigong and, therefore, its link with the psychological state of the individual, is also a serious element of qigong practice. The psychological component is so relevant to qigong training that deviation from correct practice has been reported to induce mental disorders [28–32]. Meditation can affect the psychological state, and this, in turn, influences the neuroendocrine system, whereby influence on immune cells can be exerted. In fact, various methods of meditation have been found to affect immune function [33–38].

Given the degree of interconnection between psychological factors and the functioning of the neuroendocrine and immune systems, the behavioral modulation of immune function through physical exercise or meditation can represent a potentially beneficial approach to health. In this regard, physical exercise can be effective in augmenting resistance to infectious disease and wound healing [39], as well as in combating tumor cell proliferation [40]. Likewise, meditation has also been reported to be useful in controlling growth of malignant tumors [36,41]. Therefore, it seems surprising that, despite the abundant evidence indicating a possible protective effect of physical exercise [24,42] and meditation [43,44], little attention has been put on this integral behavioral approach as a potential strategy in cancer control and health in general.

Since qigong has an important psychological as well as physical component due to its meditative and body movement elements, it would be expected to find some sort of changes within the immune system after engaging subjects in the daily practice of this Chinese moving meditation exercise. Notwithstanding, qigong has scarcely been investigated with regard to its effects on the immune system. Most of the research undertaken has been published in Chinese; thus the results have not been easily accessible to the western world. Therefore, this study was designed with the purpose of investigating the effects of a particular and widely practiced qigong method on immunological parameters in subjects who volunteered to follow a program with the duration of one month.

MATERIAL AND METHODS

Participants

29 subjects, aged 18–21 years, of whom 14 were male and 15 female, all psychology students at the University of Malaga, were selected from among a larger group of volunteers. Only healthy subjects not taking any type of drug and with regular life habits were chosen to be part of the experimental or control groups. None of them practiced sports regularly or had any previous experience with qigong, yoga, or other such methods. All were asked to follow their normal life-styles while the experiment was being conducted. Subjects were screened to exclude those with any pathological conditions and/or those who had received pharmacological treatment in the three months prior to the experiment. 16 subjects were randomly allocated to the experimental group and 13 to the control group, balancing the number of males and females in each case. Female subjects were all within the first 7 days of their menstrual cycle. One experimental subject (male) decided to abandon the experiment within the first few days of onset, and a further two (one male and one female) were excluded from the sample for non-attendance at the qigong sessions on more than two occasions.

Intervention

The form of qigong taught is known as the "eight pieces of brocade" (*Ba Duan Jin* in Chinese pinyin transliteration). It is a simple qigong method that contains eight distinct movements and integrates them with breathing and a relaxed state of the mind. The whole physical sequence contains eight discrete movements which are repeated 8 times

each, making a total of 64 physical movements to complete the entire set. Throughout the practice, natural, relaxed, and rhythmic breathing is required. This method of qigong reportedly dates back hundreds of years, and a number of physical and psychological benefits has traditionally been attributed to it. More recently, two reports published in important international journals focused on this qigong style and its health-promoting features [45,46].

The experimental subjects gathered every day in the afternoon, Monday to Friday, in a room adjoining our laboratory where the practice sessions were conducted. This psychosomatic training took place and was taught under the guidance of a qualified qigong instructor of this discipline. Each session lasted for 30 minutes and the subjects were encouraged, but not required, to keep practicing on their own on the weekends. The subjects followed this qigong training for a period of one month. The training included 20 group sessions conducted by the instructor and some additional sessions carried out individually over the weekends. The amount of individual training varied from one subject to another, but typically consisted of one extra session each weekend of half the duration of the regular ones. During the experiment, control subjects were not required to do anything in particular and were just asked to follow their daily life habits.

Blood sampling

The day before the study commenced, blood samples were taken from all subjects, in both the control and experimental group, at 9: 30 in the morning, and again one month later, at the end of the study, when qigong training was concluded for the experimental subjects. The immunological parameters investigated included the number of leukocytes (total leukocytes, monocytes, neutrophils, eosinophils, basophils, lymphocytes, T lymphocytes, and T helper lymphocytes), the percentages of leukocytes (monocytes, neutrophils, eosinophils, basophils, lymphocytes, and T helper lymphocytes), as well as the concentrations of immunoglobulins (IgA, IgG, and IgM) and complement (C3 and C4). The procedure was as follows:

- Total blood count (total leukocytes, monocytes, neutrophils, eosinophils, basophils, and lymphocytes): four or five milliliters of blood were introduced into an EDTA tube and analyzed by an impedance and absorbance method in a Pentra 120 ABX analyzer.
- Serum immunoglobulins and complement: four or five milliliters of blood were introduced into a Vacutainer tube and centrifuged at 3500 rpm. Immunoglobulins and complement were then determined by nephelometry in a Immage Immunochemistry System (Beckman Coulter).
- Lymphocytes subsets: T lymphocytes and T helper lymphocytes were determined by flow cytometry in a FACScan (Becton Dickinson). A complete blood lysis method was employed to stain cells with the conjugated antibodies/fluorescent dyes. 50 µl of EDTA-blood was mixed with 10 µl of the different monoclonal antibodies (anti-CD3 and anti -CD4) and incubated for 15 minutes at room temperature. Lysis took place for six to eight minutes with cold lysis buffer at 4°C. Before the supernatant was pipetted off, it was centrifuged at 1200 rpm for 5 minutes. Washing was then carried out with 3 ml of phosphate buffered saline (PBS), and, finally, 500 µl of PBS were added. The different cell subtypes were analyzed by forward and side scatter to determine size and granularity, respectively. Finally, the appropriate cell population was gated, analyzed, and counted according to three types of fluorescence: phycoerythrin, fluorescein, and Per-CP.

Statistical analyses

A between-group analysis of covariance (ANCOVA) was performed on several dependent variables: the numbers of total leukocytes, monocytes, neutrophils, eosinophils, basophils, lymphocytes, T lymphocytes, and T helper lymphocytes; the percentages of lymphocytes, T helper lymphocytes, monocytes, neutrophils, eosinophils, and basophils; as well as the concentrations of IgG, IgA, IgM, and the complements C3 and C4. The qigong training was considered as an independent variable with two levels (absence or control group, and presence or experimental group) and the respective pretest scores of each dependent variable as covariants. Thus, the differences between groups were estimated with the differences in pretest scores removed. A value of $p<0.05$ was considered to be significant, while $p<0.1$ was considered a trend towards significance.

RESULTS

Following one month of qigong training (Table 1), effects of experimental treatment, after adjustment for covariants, were found in total leukocytes, eosinophils monocytes, and complement C3. The adjusted means, *F* statistics, and *p*-values are presented in Table 1. Specifically, it was found that the experimental group had lower values than the control group in the total number of leukocytes ($p=0.03$), the number of eosinophils ($p=0.04$), the number ($p=0.02$) and the percentage ($p=0.05$) of monocytes, as well as the complement C3 levels ($p=0.004$). In addition, a trend towards significance was observed in the number of neutrophils ($p=0.07$). No significant changes were observed in any of the lymphocyte measures (total lymphocytes, T lymphocytes, T helper lymphocytes), in the concentrations of complement C4, or the immunoglobulins (IgA, IGM, and IgG).

DISCUSSION

Our results show that after one month of training in this form of qigong, significant immunological changes occurred between the experimental and control groups. These results are in accordance with other studies, that found qigong practice [47–51] to be capable of inducing significant modifications in immune cells. However, unlike the above-mentioned studies, our findings show lower values in the immune measures of the experimental group compared with the control group. We found lower concentrations of complement and a lower number of leukocytes in the qigong group. The lower leukocyte counts consisted, specifically, of a reduction of phagocytic cells. This also differs from most of the cited studies, which reported increases in T lymphocytes [47,48] and in some cytokines [50]. Although we did not measure cytokines, we did measure T cells and found no differences between the experimental and control groups in these parameters. It is probable that the different methods of qigong followed in the aforementioned reports and the various durations of the programs, some of which lasted up to 5 months, may account

Table 1. Adjusted means of each dependent variable for control and experimental groups, F statistics and p-value.

Variable	Control group	Experimental group	*F*	*P*
Leucocytes ($\times 10^3$ cells/µl)	6.73	5.66	4.9	0.03*
Monocytes ($\times 10^3$ cells/µl)	0.60	0.45	6.25	0.02*
Neutrophils ($\times 10^3$ cells/µl)	3.63	2.85	3.55	0.07
Eosinophils ($\times 10^3$ cells/µl)	0.29	0.19	4.38	0.04*
Basophils ($\times 10^3$ cells/µl)	0.04	0.05	0.29	0.59
Lymphocytes ($\times 10^3$ cells/µl)	2.19	2.00	1.9	0.18
T Lymphocytes (cells/µl)	1753.77	1687.91	0.28	0.60
T Helper Lymphocytes (cells/µl)	1027.19	1031.12	0.01	0.95
Monocytes (%)	9.05	7.84	4.35	0.05*
Neutrophils (%)	52.06	52.01	0.01	0.99
Eosinophils (%)	4.43	3.69	1.57	0.22
Basophils (%)	0.78	0.87	0.35	0.56
Lymphocytes (%)	33.69	35.60	0.62	0.43
T Helper Lymphocytes (%)	58.49	60.29	2.21	0.15
IgA (mg/dl)	196.90	200.03	0.24	0.63
IgG (mg/dl)	1084.97	1093.78	0.90	0.76
IgM (mg/dl)	151.12	162.59	2.39	0.14
C3 (mg/dl)	109.74	98.89	10.13	0.004*
C4 (mg/dl)	18.02	16.51	7.71	0.020

* p<0.05, Exp-post vs. Control-post; # p<0.005, Exp-post vs. Control post;
** p<0.02 Exp-post vs. Control-post; ## p<0.01, Exp-post vs. Control post;
*** p<0.05 Control-post vs. Control-pre; ### p<0.01, Exp-post vs. Exp-pre

for some of the discrepancies between the results of our research and those of the other authors.

As we have indicated, after one month of practicing *Ba Duan Jin* qigong, our experimental subjects showed lower leukocyte counts than the control subjects, particularly in the number of monocytes and granulocytes (including eosinophils and, to a certain extent, neutrophils), as well as lower concentrations of the C3 complement fraction. It is remarkable that the experimental subjects were found to have lower values than the control subjects in innate immune response components, whereas adaptive immune response cells (e.g. lymphocyte subpopulations) remained unaffected. These data are, to our knowledge, the first to be published in the western world with respect to changes of this nature in these aspects of the immune system in subjects undergoing qigong training. Our results differ from current literature about meditation, either in the immune parameters where significance was observed, the direction of the change, or both [34,37,52], and the same discrepancy applies to physical exercise [24]. We must, however, take into consideration that these studies have important methodological differences, not only among them, but also with respect to the present research. In this respect, in some of the reports meditation was studied using subjects exposed to considerable physical stress, often from a clinical population. This considerable difference in methodology may account for some of the differences in results.

Since our findings reveal lower values in the experimental group with regard to monocytes, some granulocytes, and complement components, it may initially appear as a sign of a detrimental effect of qigong on the immune system. Nevertheless, cell counts, the main variable we measured, cannot be considered an indicator of immune functionality and, therefore, these results by themselves do not necessarily suggest a negative effect induced by qigong on the immune system. At any rate, it is important to emphasize that our results are extremely consistent throughout all the significances observed. Given this consistency, it would be interesting to conduct further research in future studies regarding the functionality of these leukocyte subsets by assessing their cytotoxicity after following a practice program in this qigong system. In this sense, a recent study reported enhanced cellular function of neutrophils and NK cells after qigong training [53].

In view of these noteworthy and specific cellular and biochemical changes within the immune system, it is interesting to speculate the extent to which these changes in the various parameters measured could be of clinical interest. In fact, the consistently lower concentrations of some complement

components and innate immune response cells we found in the qigong group may be relevant from a clinical point of view. To this day, complement proteins do not seem to have been studied in relation to any psycho-physiological training methods such as qigong, meditation or yoga. Nonetheless, a small number of papers has explored the complement system with respect to physical training [54–56], and one of these investigations also found significant differences [54]. Interestingly, a lower level of both fractions of complement, C3 and C4, was reported in runners compared with sedentary people. While this is similar to what we found, it is difficult to establish a possible medical implication of this finding. The human complement system is known to have a protective role against microbial agents [57]; however, C3 and C4 factors are also known to be markers of inflammation [58], and complement activation has been said to be associated with a number of pathological conditions, such as atherosclerotic processes [59].

Likewise, monocytosis and reduced monocyte phagocytic activity have been found to be related to anxiety [60] and physical stress [19]. While it is a well-known fact that monocyte inactivation augments the possibility of infectious processes [61], monocytes are also considered to play a role in the pathogenesis of various diseases, such as Crohn's disease [62,63] and rheumatoid arthritis [64]. Increased eosinophil levels have also been found to be related to psychological stress and, specifically, academic stress [65]. In this sense, although eosinophils play an important role in eliminating helminthic parasites [66,67], they also have a marked proinflammatory potential and, consequently, have been associated pathologically with a number of diseases, particularly allergic processes such as asthma and allergic rhinitis [68]. Similarly, increases in neutrophils have been associated with stress in patients with various pathological conditions [69,70], especially with mental [71] and physical [56,72] stress in healthy subjects.

Since the pathogenesis of various diseases has been characterized by an increased immune response, treatments which can control immune bursts may be beneficial. Therefore, our study appears to suggest that qigong practice may constitute an effective psycho-physiological method for immune modulation, which may be of potential clinical relevance. Besides, while it is still premature to conclude any immunomodulatory role for qigong, other types of meditation have been reported to reduce the impact of stress on the immune system [34]. In addition, since it is well established that stress can upregulate the number of various leukocyte subpopulations, and considering the lower profile of the qigong group in this respect, it would be reasonable to wonder whether any psychological factor played a role in these results. Some additional data (unpublished) from our laboratory actually support the notion of a significant psychological influence induced by qigong, given that the experimental group exhibited significantly lower anxiety scores than the controls, as measured by STAI. Thus, in this context these results reveal an anti-anxiety effect of qigong. Furthermore, it is possible that the influence of qigong on the immune system may be partially mediated by its psychological effect.

The link between psychological factors and neuro-immuno-endocrinological function suggests that approaches concentrating on enhanced psychological function will gain increasing acceptance as potentially effective therapies in the near future. As a matter of fact, while some years ago the concept of neuroimmunomodulation was considered a strange approach to health, of dubious scientific validity, today it is regarded as the fastest growing area within biomedical science research [73].

Conclusions

Our results show that after practicing qigong for a period of one month, the experimental subjects exhibited lower values than controls in innate immune response cells and proteins, including monocytes, granulocytes, and complement. Therefore, these results demonstrate that qigong training can exert a significant effect on various immunological parameters following a short period of practice and suggest that qigong may represent an effective psychosomatic training for immune modulation. Further studies would be necessary to elucidate the whole scope of the influence of qigong on the immune system and the possible clinical implications that this might have.

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