The American Journal of Chinese Medicine, Vol. 35, No. 1, 35–51

Institute for Advanced Research in Asian Science and Medicine

Effect of Acupuncture Treatment on the Immune Function Impairment Found in Anxious Women

Lorena Arranz, Noelia Guayerbas, León Siboni and Mónica De la Fuente
Department of Physiology (*Animal Physiology II*), *Faculty of Biological Science*
Complutense University of Madrid, *Spain*

Abstract: It is presently accepted that emotional disturbances lead to immune system impairment, and that therefore their treatment could restore the immune response. Thus, the aim of the present work was to study the effect of an acupuncture treatment, designed specifically to relieve the emotional symptoms stemming from anxiety, on several functions (adherence, chemotaxis, phagocytosis, basal and stimulated superoxide anion levels, lymphocyte proliferation in response to phytohemagglutinin A (PHA) and natural killer (NK) activity) of leukocytes (neutrophils and lymphocytes) from anxious women. The acupuncture protocol consisted of manual needle stimulation of 19 acupoints, with each session lasting 30 min. It was performed on 34 female 30–60 year old patients, suffering from anxiety, as determined by the Beck Anxiety Inventory (BAI). Before and 72 hours after receiving the first acupuncture session, peripheral blood samples were drawn. In 12 patients, samples were also collected immediately after the first single acupuncture session and one month after the end of the whole acupuncture treatment, which consisted of 10 sessions during a year, until the complete remission of anxiety. Twenty healthy non-anxious women in the same age range were used as controls. The results showed that the most favorable effects of acupuncture on the immune functions appear 72 hours after the single session and persist one month after the end of the complete treatment. Impaired immune functions in anxious women (chemotaxis, phagocytosis, lymphoproliferation and NK activity) were significantly improved by acupuncture, and augmented immune parameters (superoxide anion levels and lymphoproliferation of the patient subgroup whose values had been too high) were significantly diminished. Acupuncture brought the above mentioned parameters to values closer to those of healthy controls, exerting a modulatory effect on the immune system.

Keywords: Anxiety; Acupuncture; Immune Functions; Women.

Correspondence to: Dr. Mónica De la Fuente, Department of Physiology (Animal Physiology II), Faculty of Biological Science, Complutense University of Madrid, José Antonio Nováis 2, 28040 Madrid, Spain. Tel: (+34) 91-394-4989, Fax: (+34) 91-394-4935, E-mail: mondelaf@bio.ucm.es

Introduction

Psychoneuroimmunology is a field of research that deals with the complex interactions between the central nervous system and the immune system, and how behavior and stress can modify these interactions (Glaser, 2005). Accordingly, several authors have shown the suppressive effect of chronic stress, depression and anxiety on immune functions (Herbert and Cohen, 1993; Koh, 1998). Though there are numerous studies on depression, the research dealing with anxiety is scarce (Koh, 1998). In this respect, it has been shown that untreated patients with anxiety exhibit significantly reduce lymphocyte proliferative response to PHA and interleukin-2 production (Koh and Lee, 1996). Nevertheless, anxiety may also be associated with increased immune function, which might be considered a transient phenomenon occurring prior to its down-regulation, indicating how the body's defenses cope with stress (Koh, 1993). However, the main reason for the diverse results on this subject is that some studies fail to distinguish between anxiety and depression. Adequate anxiety inventories with ability to differentiate between these two conditions, which show a high degree of overlap, are essential. Amongst them, the Beck Anxiety Inventory stands out, since it was specifically designed to differentiate between behavioral, emotional, and physiological symptoms in subjects with anxiety and those suffering depression. Aiming for that goal, the authors incorporated items that are specific for the physiological and cognitive symptoms of anxiety and independent of the symptoms of depression (Beck *et al*., 1988; Beck, 1993; Comeche *et al*., 1995).

In general, two types of treatment are available for anxiety: psychotherapy and pharmacotherapy. Psychotherapy, which includes cognitive behavioral therapy and collaborative care, has been shown to be insufficient in the majority of cases when used alone. Pharmacotherapy, alone or in combination with psychotherapy, is generally considered the standard treatment of anxiety. Anxiolytics, antidepressants or other agents, such as venlafaxine or monoamine oxidase inhibitors, are used depending on the specific diagnosis. However, all these drugs have side effects, limited efficacy, nonspecificity, risk of producing dependence, significant drug interaction and other problems, such as necessary dietary restrictions, which might be the leading causes of therapy change and patient nonadherence to the treatment (DeVane *et al*., 2005). Despite theses features, the treatment should be maintained for at least 12 months as anxiety tends to relapse. Even with long-term therapies, Yonkers *et al*. (2000) described that less than 50% of the patients with generalized anxiety disorder achieve full remission after 5 years, and of patients with full or partial remission, 27% and 39%, respectively, relapse within 3 years. In view of these facts, alternative therapy approaches are needed.

It is now accepted that both the neuroendocrine and the immune system are involved in homeostasis maintenance, showing a bidirectional communication mediated by shared chemical messengers and receptors, in order to maintain the health (Blalock, 1994; De la Fuente, 1999). As mentioned above, if one of these two systems suffers alterations, it will influence the other. Therefore, emotional disturbances lead to immune system impairment (Herbert and Cohen, 1993; De la Fuente *et al*., 1998). Likewise, therapeutic

interventions on one of the systems have an effect on the other. Thus, acupuncture provides the possibility to influence the immune system through its effects on the nervous system.

Acupuncture is an ancient therapeutic intervention widely practised in Eastern countries for thousands of years, owing to the favorable results obtained in the treatment of many diseases and pain relief (Richardson and Vincent, 1986; Ulett *et al*., 1998a). It seems to act stimulating sensory nerves (Yao *et al*., 1982; Chao *et al*., 1999), modulating hormonal factor release, including endogenous opioids (Kasahara *et al*., 1993; Kho *et al*., 1993; Jin *et al*., 1996; Joos *et al*., 2000), and the autonomic nervous system (Tam and Yin, 1975; Nishijo *et al*., 1997). Acupuncture may affect the pituitary gland via hypothalamus, causing β-endorphin release into circulation (Pomeranz, 1976; Kendall, 1989). It is unlikely that opioids in the periphery are analgesically active (He, 1987), but they probably have effects on non-analgesic systems, including the immune system (Mittleman and Gaynor, 2000). In addition to β-endorphin, there are other hormones and neuropeptides that have been found to be released during the acupuncture treatment (Bucinskaite *et al*., 1996). Thus, several authors have detected changes in ACTH (Xie, 1981), leu- and met-enkephalin, dynorphin, serotonin and biogenic amines (He, 1987), among others (Mittleman and Gaynor, 2000). Rather few, but interesting reports are available on acupuncture and its possible benefits in altered states of the nervous system, such as anxiety (Sher, 1998; Ullet *et al*., 1998b). In fact, it is well-known that anxiety is related to endogenous opioid system deficits (Sher, 1998).

In recent years, it has been reported that acupuncture may also have favorable effects on the treatment of diseases that involve the immune system, i.e., allergic processes in both mice (Kasahara *et al*., 1993) and human models (Joos *et al*., 1997; 2000), inflammatory processes in humans (Berman *et al*., 1999; Ernst, 2000) or different traumatisms in rats (Sun *et al*., 2000). Acupuncture tends to normalize leukocyte (granulocyte and lymphocyte), circulating concentrations when they are disturbed (Sin *et al*., 1983). Furthermore, several studies have concluded that the phagocytic activity of macrophages (Sin, 1983), monocytes and neutrophils (Zhou *et al.*, 1988; Sliwinski and Kulej, 1989), as well as neutrophil migration (Sin *et al.*, 1983), are improved after acupuncture treatment. In addition, acupuncture regulates lymphocyte proliferation (Sin *et al*., 1983; Bianchi *et al*., 1991; Joos *et al.*, 1997) and stimulates NK activity (Yu *et al*., 1998; Du *et al*., 1998).

In view of the above, the aim of the present work was to study, in adult anxious women, whether several immune functions, which have been described to be impaired in anxiety (Koh and Lee, 1996) and are good health markers (Wayne *et al.*, 1990), can be restored to the levels of healthy women after an acupuncture treatment designed to relieve the symptoms derived from such emotional situations.

Materials and Methods

Subjects

The acupuncture treatment was performed on 34 female patients, age from 30 to 60 years, who suffered high levels of anxiety, as determined by the Beck Anxiety Inventory (Beck

et al., 1988; Beck, 1993; Comeche *et al*., 1995). A group of 20 healthy women in the same age range, to avoid gender and age related differences, was used as the control.

The subjects were patients of Dr. Siboni (Acupuncturist Physician by the Faculty of Medicine of the Complutense University of Madrid), who attended his consulting room to treat their anxiety symptoms. The control group was formed by women who responded to e-mail and press advertisements, and posters. All subjects were Spanish and recruited from the population of Madrid. The inclusion criteria were: (a) female; (b) 30–60 years old; (c) exhibit high levels or absence of anxiety as shown by the Beck Anxiety Inventory. None of the participants received any kind of psychopharmacological treatment or other psychotherapy. Exclusion criteria consisted of malnourishment (imbalance between the organism needs and the nutrient intake, either hyper- or hyponutrition), pregnancy, severe allergies, immunodeficiency or autoimmune diseases, neoplasia, rheumatic fever, diabetes, seizures, endocrine disorders, anemia, radiation therapy, chemotherapy, intake of adrenal corticosteroids or estrogen replacement therapy, smoking, consuming alcohol or drugs, or having performed endurance training shortly before admission. Women who had recently been diagnosed as patients of infectious diseases, had suffered any surgical treatment, consumed immune-modulating drugs, showed pathologic laboratory findings, were unable to complete the evaluation protocol or to speak, read or write in Spanish, were also excluded.

All participants received information on the purpose of the study and gave their written consent for their blood samples to be used for academic research. All procedures were carried out according to the Declaration of Helsinki. The clinical interview, conducted by Dr. Siboni, was followed by the performance of the psychological questionnaire (Spanish version of the Beck Anxiety Inventory). Blood samples were collected afterwards.

Beck Anxiety Inventory (*BAI*)

The BAI is a self-applied instrument to assess anxiety (emotional state in which the individual experiments a permanent feeling of distress and despair, unknowing the reasons consciously), specially in its physical aspects (Beck *et al*., 1988; Beck, 1993; Comeche *et al*., 1995). It consists of 21 items, including a broad range of symptoms associated with clinical manifestations of anxiety, such as nervousness, inhability to relax, headache and tachycardia. Each item consists of four statements representing varying degrees of severity. Subjects were asked to select the statement that best reflected their experience during the past week of the test. The time of administration was 5 min. A sum score was calculated, with higher scores indicating higher levels of anxiety symptoms (0–9 normal or lack of anxiety; 30–63 high anxiety). The BAI differentiates between anxiety and depression, and also between anxious and healthy subjects, but not between anxiety disorders. It has a test-retest reliability of $r = 0.67–0.93$ and its internal consistency is Cronbach's $\alpha = 0.90–0.94$ (Beck *et al*., 1988; Beck, 1993; Comeche *et al*., 1995).

Treatment

The manual acupuncture protocol to treat anxiety consisted of needle stimulation of 19 acupoints: SI3, HT3, 5, PC6, LI4, 11, TH5, CV3, 4, 6, 15, GB34, 43, ST36, SP6, LV2, UB60, KD6, GV20. Chinese needles (0.26 mm diameter, Huan Qiu, Suzhou, China), which were inserted to 10 mm, depending on the muscular mass, and Corean needles (0.18 mm diameter, Huan Qiu, Suzhou, China), inserted to a depth of 4 mm, were used. Each of them was chosen on the basis of the anatomical location of the acupoint. Both were selected because they were easy to manipulate, were better accepted by patients and resulted in less traumatic injury. Each session lasted 30 min. Before the start of the treatment course and 72 hours after the first acupuncture session, peripheral blood samples were drawn from all patients by vein puncture at 9 a.m., in order to control the effect of circadian variations on the immune parameters, into tubes containing citrate. In 12 patients, samples were collected under the same conditions, before, immediately and 72 hours after the first single acupuncture session, and one month after finishing the whole acupuncture treatment, which consisted of 10 sessions in one year, as anxiety disappearance was determined by the BAI. All acupuncture points were used in each session. The same data were collected before and after patient treatment. Samples from the controls were drawn only once.

Assay of Neutrophil and Lymphocyte Adherent Capacity

For adherent capacity measurement, the method described by De la Fuente *et al*. (1997) was used. This method resembles, *in vitro*, the cellular adherence to endothelium *in vivo* (De la Fuente *et al.,* 1997). Adherence Index (A.I.) percentage for neutrophils and lymphocytes was calculated according to the equation:

$$\text{A.I.} = \frac{\text{Cells/ml}_{\text{initial}} - \text{Cells/ml}_{\text{effluent}}}{\text{Cells/ml}_{\text{initial}}} \times 100.$$

Separation of Human Blood Neutrophils and Lymphocytes

Peripheral blood neutrophils and lymphocytes were obtained following a method previously described (De la Fuente and Víctor, 2000). Collected cells, containing 98% neutrophils or lymphocytes, were diluted to the corresponding final concentrations for each assay. Cell viability was checked by the trypan blue exclusion test before and after each assay, and it was higher than 95% in all cases.

Assay of Chemotaxis

Chemotaxis was evaluated according to the technique described by De al Fuente *et al*. (1997). The Chemotaxis Index (C.I.) was determined by counting the number of neutrophils and lymphocytes in 20 microscope fields of the lower filter face using an immersion objective (100×).

Assay of Phagocytosis

Phagocytosis assay of inert particles (latex beads) (Sigma, St. Louis, USA) was carried out following the method described by De la Fuente *et al*. (1997). The number of particles ingested by 100 neutrophils was expressed as Phagocytic Index (P.I.) and the number of ingesting neutrophils per 100 neutrophils as Phagocytic Efficiency (E.F.), both determined by counting in an optical microscope (100×).

Superoxide Anion Level Measurement

Superoxide anion was evaluated by the method for mouse macrophages (Alvarado *et al*., 2005), using human neutrophils. The results were expressed as nmol/10^6 neutrophils.

Assay of Lymphoproliferation

A previously described method was used (De la Fuente and Víctor, 2000). The results were expressed as ^{3}H-thymidine uptake (counts per min, cpm).

Assay of Cytotoxicity

For target cells cytolysis measurement, an enzymatic colorimetric assay was used (Cytotox 96 TM Promega, Boeringher Ingelheim, Biberach, Germany), which is based on lactate dehydrogenase (LDH) determination using tetrazolium salts (Ferrández *et al*., 1999). To determine the lysis percentage of target cells, the following equation was used:

$$\% \text{ lysis} = \frac{\text{E} - \text{ES} - \text{TS}}{\text{M} - \text{TS}} \times 100$$

where E is the mean of absorbances in the presence of effector and target cells; ES, the mean of absorbances of effector cells incubated alone; TS, the mean of absorbances in target cells; and M is the mean of maximum absorbances after incubating target cells with lysis solution.

Statistical Analysis

Data are given as mean ± SEM of the values from the number of experiments shown in the figure legends. Normality of the samples was assesed by the Kolmogorov-Smirnov test. The differences between the patient and the control group were studied by the student's *t*-test for unrelated samples, and differences inside the patient group, due to the treatment, were determined by the student's *t*-test for related samples, $P < 0.05$ was considered the minimum significant level in all cases.

Results

Effects of a Single Acupuncture Session (72 Hours After It) on the Immune Function Changes in Anxious Women

Neutrophil Functions: There were no differences in neutrophil adherence capacity (Table 1) between women suffering anxiety and the control group, whereas the adherence index was significantly lower ($p < 0.001$) 72 hours after the acupuncture session. In regard to neutrophil chemotaxis function (Fig. 1A), anxious women showed a significantly lower index ($p < 0.001$) than the controls, which was increased ($p < 0.01$) 72 hours after one single acupuncture session and appeared to be similar to the control value at that time. Phagocytic index and efficiency (Fig. 1B and 1C, respectively) were lower in patients ($p < 0.01$ and $p < 0.001$, respectively) as compared to healthy subjects, and appeared to be increased ($p < 0.01$ and $p < 0.001$, respectively) by acupuncture, reaching the control value in the case of the phagocytic index, 72 hours after the session. Superoxide anion levels in non-stimulated and stimulated samples (Fig. 1D) were both increased in patients ($p < 0.01$ and $p < 0.05$, respectively) as compared to the control group, and decreased ($p < 0.05$) to the healthy values 72 hours after acupuncture.

Lymphocyte Functions and NK Activity: There was no difference in lymphocyte adherence (Table 1) between patients and healthy women, whereas the acupuncture treatment decreased ($p < 0.001$) this capacity. As regards to the lymphocyte chemotaxis function (Fig. 2A), it was significantly lower ($p < 0.001$) in anxious women in comparison with the control group, and appeared to be restored to the control value 72 hours after the acupuncture session. Lymphoproliferation in response to PHA results are shown in Fig. 2B. There were two statistically different subgroups in the patient group, one showing an increased (A; $p < 0.001$) and the other a diminished (B; $p < 0.001$) lymphocyte T proliferative response to mitogens with respect to the control group. 72 hours after the session, this function appeared to be modulated to the control value by acupuncture, decreased in A ($p < 0.05$) and increased in B ($p < 0.001$). As regard to the NK activity (Fig. 2C), women suffering anxiety showed a decreased function ($p < 0.001$) with respect to the control group, which was stimulated ($p < 0.01$) 72 hours after the acupuncture session.

Study of the Effects of a Single Acupuncture Session (Immediately After It) on the Immune Function Changes in Anxious Women and the Persistence of Effects One Month After Finishing the Complete Acupuncture Treatment

Neutrophil Functions: With regard to neutrophil adherence (Table 2), no difference between patients and controls was observed. Adherence index was decreased ($p < 0.05$) immediately after the acupuncture session, and remained so one month after finishing the whole acupuncture treatment ($p < 0.001$). Neutrophil chemotaxis capacity (Fig. 3A), significantly lower in patients ($p < 0.001$) as compared to healthy subjects, was increased by acupuncture ($p < 0.05$) one month after the end of the whole treatment, and appeared

Table 1. Adherence Index of Human Peripheral Blood Neutrophils and Lymphocytes (%) from Women Suffering Anxiety, Before and 72 Hours After Receiving One Single Acupuncture Session (Patients), and From Healthy Women of the Same Age Range (Controls)

	Anxious	72 Hours After Acupuncture	Controls
Neutrophil Adherence Index (%)	50 ± 2	26 ± 3*** $^{\bullet\bullet\bullet}$	51 ± 2
Lymphocyte Adherence Index (%)	54 ± 3	35 ± 5*** $^{\bullet\bullet\bullet}$	56 ± 2

Values represent the mean ± SEM of 34 patients and 20 control subjects, each value being the mean of duplicate assays. $^{***}p < 0.001$ with respect to the value in the control group. $^{\bullet\bullet\bullet}p < 0.001$ with respect to the value in those patients before the acupuncture treatment.

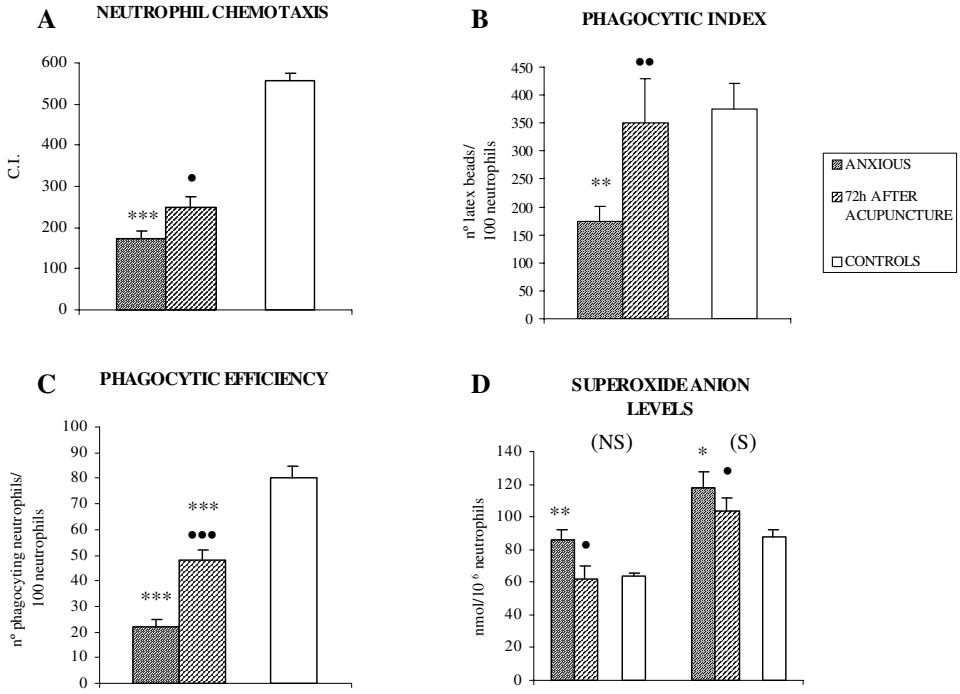

Figure 1. Chemotaxis index (A) (C.I.), phagocytic index (B) (nº latex beads/100 neutrophils), phagocytic efficiency (C) (nº phagocyting neutrophils/100 neutrophils) and superoxide anion levels (D) (nmol/10^6 neutrophils) in non-stimulated (NS) and stimulated (S) samples of human peripheral blood neutrophils from women suffering anxiety, before and 72 hours after receiving one single acupuncture session (patients), and from healthy women of the same age range (controls). Each column represents the mean ± SEM of 34 patients and 20 control subjects, each value being the mean of duplicate assays. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ with respect to the value in the control group. $^{\bullet}p < 0.05$, $^{\bullet\bullet}p < 0.01$, $^{\bullet\bullet\bullet}p < 0.001$ with respect to the value in those patients before the acupuncture treatment.

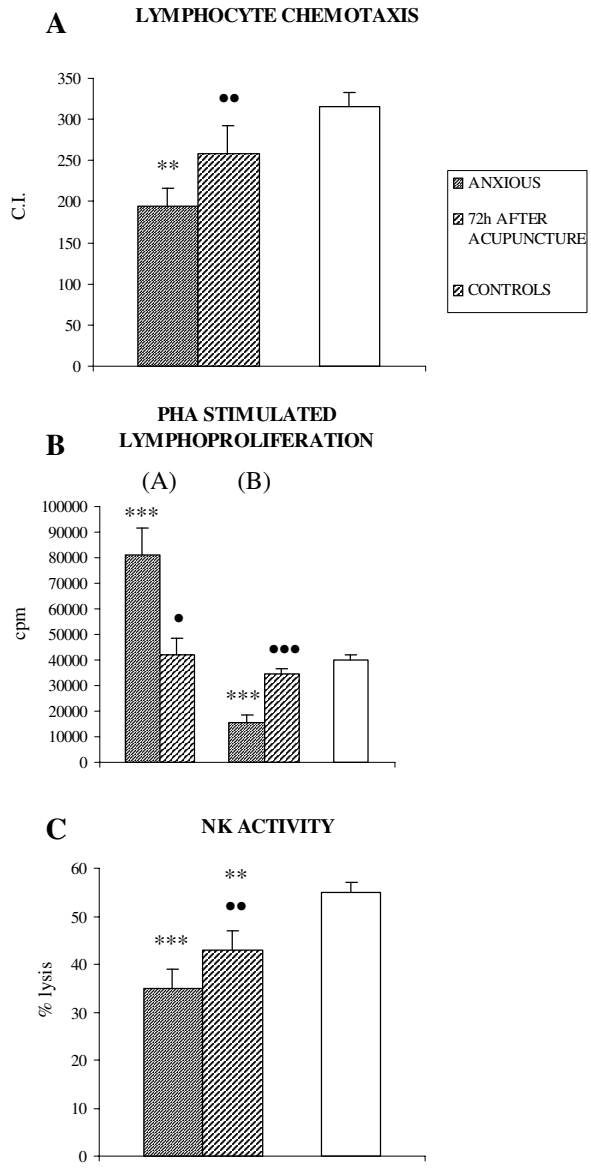

Figure 2. Chemotaxis index (A) (C.I.), PHA stimulated proliferation (B) (cpm) and NK activity (C) (% lysis) of human peripheral blood lymphocytes from women suffering anxiety, before and 72 hours after receiving one single acupuncture session (patients), and from healthy women of the same age range (controls). Each column represents the mean ± SEM of 34 patients (except in 2B; A subgroup n = 10 and B subgroup n = 24) and 20 control subjects, each value being the mean of duplicate assays. $^{**}p < 0.01$, $^{***}p < 0.001$ with respect to the value in the control group. $^{\bullet}p < 0.05$, $^{\bullet\bullet}p < 0.01$, $^{\bullet\bullet\bullet}p < 0.001$ with respect to the value in those patients before the acupuncture treatment.

Table 2. Adherence Index of Human Peripheral Blood Neutrophils and Lymphocytes (%) from Women Suffering Anxiety, Before, Immediately and 72 Hours After Receiving One Single Acupuncture Session, and One Month After Finishing the Whole Acupuncture Treatment (Patients), and from Healthy Women of the Same Age Range (Controls)

	A	IAA	72 Hours	1MAA	C
Neutrophil Adhrence Index (%)	51 ± 8	25 ± 3$^{***\,\bullet}$	25 ± 7$^{***\,\bullet\bullet}$	17 ± 5$^{***\,\bullet\bullet\bullet}$	51 ± 2
Lymphocyte Adhrence Index (%)	58 ± 4	32 ± 8$^{***\,\bullet\bullet}$	28 ± 5$^{***\,\bullet\bullet}$	30 ± 4$^{***\,\bullet\bullet}$	56 ± 2

Values represent the mean ± SEM of 12 patients and 20 controls, each value being the mean of duplicate assays. $^{***}p < 0.001$ with respect to the value in the control group. $^{\bullet}p < 0.05$, $^{\bullet\bullet}p < 0.01$, $^{\bullet\bullet\bullet}p < 0.001$ with respect to the value in those patients before the acupuncture treatment. A: Anxious women before the acupuncture treatment, IAA: immediately after one single acupuncture session, 72 hours: 72 hours after one single acupuncture session, 1MAA: one month after finishing the whole acupuncture treatment, C: controls.

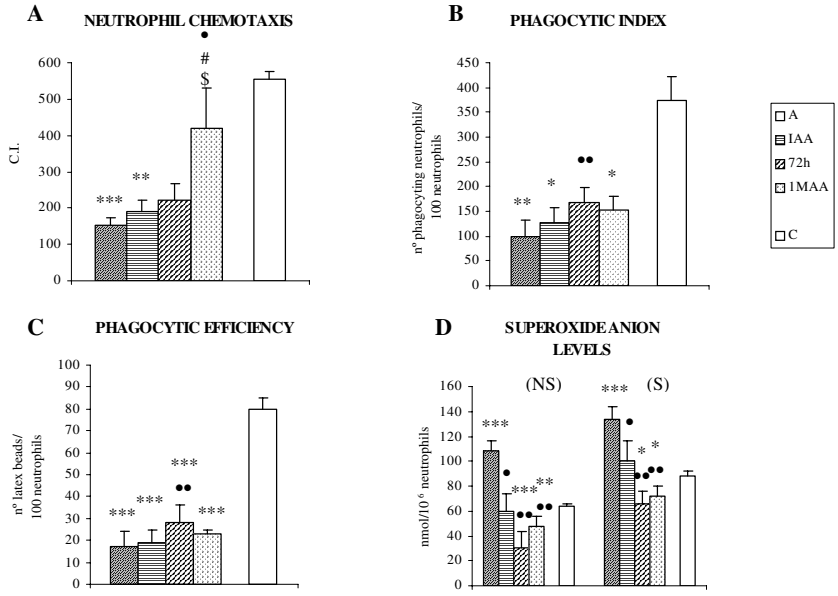

Figure 3. Chemotaxis index (A) (C.I.), phagocytic index (B) (nº latex beads/100 neutrophils), phagocytic efficiency (C) (nº phagocyting neutrophils/100 neutrophils) and superoxide anion levels (D) (nmol/10^6 neutrophils) in non-stimulated (NS) and stimulated (S) samples of human peripheral blood neutrophils from women suffering anxiety, before, immediately and 72 hours after receiving one single acupuncture session, and one month after finishing the whole acupuncture treatment (patients), and from healthy women of the same age range (controls). Each column represents the mean ± SEM of 12 patients and 20 control subjects, each value being the mean of duplicate assays. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ with respect to the value in the control group. $^{\bullet}p < 0.05$, $^{\bullet\bullet}p < 0.01$ with respect to the value in those patients before the acupuncture treatment. $^{\#}p < 0.05$ with respect to the value in those patients immediately after receiving one single acupuncture session. $^{\$}p < 0.05$ with respect to the value in those patients 72 hours after receiving one single acupuncture session. A: anxious women before the acupuncture treatment, IAA: immediately after one single acupuncture session, 72 h: 72 hours after one single acupuncture session, 1MAA: one month after finishing the whole acupuncture treatment, C: controls.

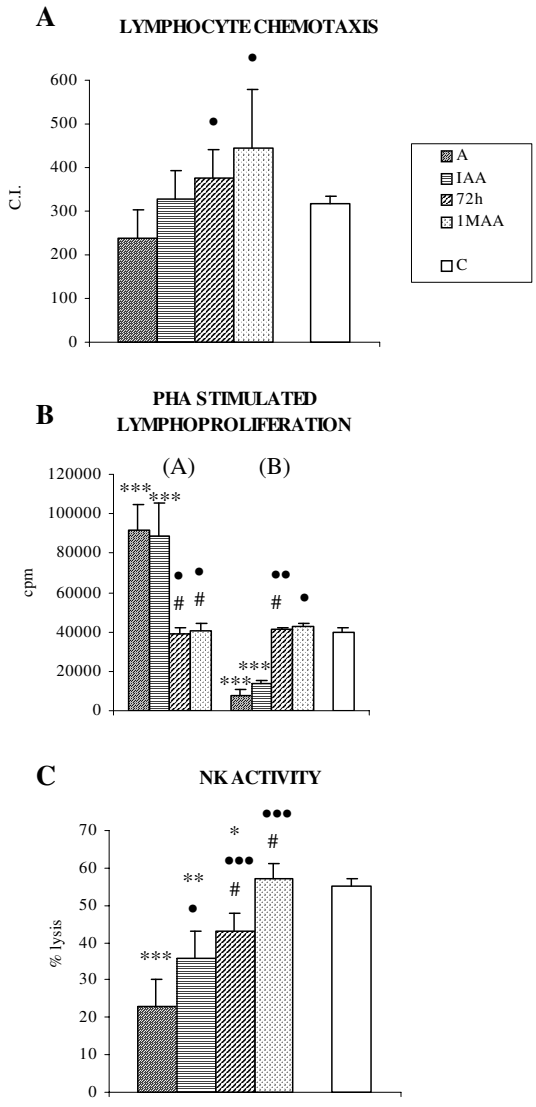

Figure 4. Chemotaxis index (A) (C.I.), PHA stimulated proliferation (B) (cpm) and NK activity (C) (% lysis) of human peripheral blood lymphocytes from women suffering anxiety, before, immediately and 72 hours after receiving one single acupuncture session, and one month after finishing the whole acupuncture treatment (patients), and from healthy women of the same age range (controls). Each column represents the mean ± SEM of 12 patients (except in 2B; A subgroup n = 5 and B subgroup n = 7) and 20 control subjects, each value being the mean of duplicate assays. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ with respect to the value in the control group. $^{\bullet}p < 0.05$, $^{\bullet\bullet}p < 0.01$, $^{\bullet\bullet\bullet}p < 0.001$ with respect to the value in those patients before the acupuncture treatment. $^{\#}p < 0.05$ with respect to the value in those patients immediately after receiving one single acupuncture session. A: anxious women before the acupuncture treatment, IAA: immediately after one single acupuncture session, 72 h: 72 hours after one single acupuncture session, 1MAA: one month after finishing the whole acupuncture treatment, C: controls.

to be similar to the control value 72 hours after one single session as well as one month after the treatment. As regards to the ingestion of latex beads, both phagocytic index (Fig. 3B) and efficiency (Fig. 3C) were lower ($p < 0.01$ and $p < 0.001$, respectively) in anxious women as compared to the control group. These functions were significantly increased ($p < 0.01$) 72 hours after one single acupuncture session, reaching the control value in case of the phagocytic index. As regards to superoxide anion levels (Fig. 3D), in the presence and absence of stimulation, the patient group showed higher values ($p < 0.001$) than those of the control group, which were decreased ($p < 0.05$) immediately after one acupuncture session. These functions were significantly lower ($p < 0.001$ and $p < 0.05$, respectively) than the control value 72 hours after the session, and remained so one month after the end of the treatment ($p < 0.01$ and $p < 0.05$, respectively).

Lymphocyte Functions and NK Activity: Regarding to the adherence (Table 2), no differences were observed between the patients and the controls. This function was decreased immediately after one acupuncture session ($p < 0.01$) and remained so after one month after the end of the whole acupuncture treatment ($p < 0.01$). With respect to the lymphocyte chemotaxis (Fig. 4A), no significant differences between anxious and healthy women were found. The chemotaxis index of lymphocytes was increased in patients 72 hours after the first acupuncture session ($p < 0.05$) and remained so one month after the end of the whole treatment ($p < 0.05$). Figure 4B shows the results of lymphoproliferation capacity in response to PHA. This function appeared to be modulated by acupuncture, decreased ($p < 0.05$) for A subgroup, in which had been high before the treatment ($p < 0.001$), and increased ($p < 0.01$) for B subgroup, in which it had initially been low ($p < 0.001$), being similar to the control value 72 hours after one acupuncture session. This effect appeared to be maintained one month after finishing the whole treatment ($p < 0.05$). With regard to NK activity (Fig. 4C), anxious women showed a diminished function ($p < 0.001$) as compared to their healthy counterparts, which was increased immediately after the first acupuncture session ($p < 0.05$), appearing similar to the control value at one month after the end of the whole treatment ($p < 0.001$).

Discussion

Immune modulation by acupuncture has received very little attention. In the present work, we have studied the effect of acupuncture treatment on several immune functions in women suffering anxiety. Bossy (1990) indicated that the response to one single acupuncture session frequently shows a 12–24 hours delay and remains effective for 5–7 days. Therefore, we analyzed the related parameters at several times, i.e., immediately and 72 hours after the first single acupuncture session, and one month after the end of a complete treatment consisting of ten sessions during one year. To our knowledge, this is the first study in regard to the particular characteristics.

The age and gender of the control and patient groups were similar in order to avoid any differences in the studied parameters caused by those factors. It must also be considered that, although the acupuncture session itself may influence both the subjective state of the

individuals and certain measured data, several placebo-controlled studies on the effects of acupuncture on immunity have shown significant improvements in patients treated with true acupuncture with respect to those who received sham acupuncture (Karst *et al*., 2003; Kou *et al*., 2005). Thus, the control group used in the present work was formed by healthy women who were not treated at all. In addition, a large number of strict exclusion criteria were used in order to seek homogeneity in both the control and the anxiety sample and exclude other possible factors affecting the immunity of the subjects.

The results showed that the impaired immune functions in anxious women (chemotaxis, phagocytosis, lymphoproliferation and NK activity) were significantly improved by acupuncture, whereas the increased immune parameters (superoxide anion levels and lymphoproliferation of the patient subgroup showing abnormally high values) were significantly diminished. Acupuncture brought all these immune parameters to or close to the healthy non-anxious control values, exerting not only a merely stimulatory action but a modulatory effect on the immune system. Indeed, as Bossy (1990) pointed out, a relatively small effect was shown immediately after one single session, while the effect was very marked at 72 hours after the session. Benefits were maintained one month after the whole acupuncture treatment.

More concretely, acupuncture treatment of anxiety decreases immune cell adherence, facilitating the migration of cells through the endothelial layer towards the infection focus. In fact, chemotaxis of immune cells to the source of infection, which was impaired in anxious women, appears to be stimulated by acupuncture. Accordingly, other authors have also suggested the possible role of acupuncture in regulating leukocyte migration (Sliwinski and Kulej, 1989).

With regard to phagocytic capacity, the functional decline observed in women suffering from anxiety, seems to be improved by acupuncture. In this respect, numerous studies confirm macrophage, monocyte and neutrophil phagocytic activity stimulation after acupuncture treatment in animals (Sin, 1983) and humans (Rogers and Bozzy, 1981; Sin *et al*., 1983; Zhou et *al*., 1988).

Besides, superoxide anion levels, measure of the generation and release of reactive oxygen species (ROS), which were increased in anxious women as compared to their healthy counterparts, are brought down by acupuncture. However, other authors have not found any immediate change in the neutrophil respiratory burst after one acupuncture session (Karst *et al*., 2002), while its increase has been described 48 hours thereafter (Karst *et al*., 2003). Nevertheless, Zhao *et al*. (2002) found an inhibition of rat peritoneal macrophage activity increase after surgical trauma by acupuncture. In this context, it must be considered that ROS produced by phagocytes are important in host defense, but the excessive levels in anxiety disorders, could be deleterious, leading to an oxidative stress state in immune cells (Víctor *et al*., 2004), which will alter membrane fluidity and cell membrane-related functions, such as chemotaxis and phagocytosis (Víctor *et al*., 2003). Additionally, although there is evidence for a positive correlation between low levels of superoxide anion and bactericidal activity (Boxer, 1995), there is no evidence that superoxide overproduction has such an effect on bactericidal activity (Sirota *et al*., 2003). In fact, Wolach *et al*. (2000) have shown a high superoxide generation by neutrophils with no parallel effect on their

bactericidal activity. Thus, acupuncture could exert its immunomodulatory action, at least in part, by decreasing the oxidative stress condition of immune cells.

With respect to the lymphoproliferative response, the two possible different profiles found in response to anxious emotional situations, either increased or diminished function, appear to be restored by acupuncture. Several authors have found stimulation of lymphocyte proliferation in response to acupuncture (Bianchi *et al.*, 1991; Joos *et al*., 1997). Our results demonstrate that acupuncture exerts not only a merely stimulatory effect but also an immunomodulatory action, depending on the physiological need.

As regards to NK activity, the suppression of this function in anxious situations is normalized by acupuncture, which is congruent with that reported by Yu *et al.* (1998).

In view of the above, it appears that the acupuncture treatment specifically designed to relieve the emotional symptoms derived from anxiety effectively improved the immune function suppression occurring in this disorder, which is evident at least as regards to the functions studied in the present work, even as early as 72 hours after one single session.

In this context, it must be considered that the general impairment of the immune responsiveness in anxious women might lead them to suffer diverse diseases in the long-run, if not treated. Frequent infectious illnesses and other immune system-related diseases could be prevented, at least in part, by the acupuncture treatment of anxiety, improving the quality of life of these patients. In addition, acupuncture lacks the adverse side effects of the traditional pharmacotherapy of anxiety, and this could result in an improvement of patient adherence to the treatment, resulting in increased effectiveness. However, further research studying the possible relapse over the years would be of great interest.

There are some shortcomings of the present study that need to be mentioned, which also point out directions for future research. This study has focused on anxious women, thus limiting its relevance for extrapolation to male patients. Nevertheless, the available evidence shows that women appear to be more anxious and exhibit more psychosomatic complaints than men (Brezinka *et al*., 1998), thus the immunological benefits found in response to acupuncture could be particularly noteworthy because the population used in this study may be quite unhealthy. However, our results are still preliminary and need to be confirmed using a larger sample, exploring gender differences. Further placebo-controlled studies to investigate the proportion of effects due to true acupuncture would also be interesting.

References

Alvarado, C., P. Álvarez, L. Jimenez and M. De la Fuente. Improvement of leukocyte functions in young prematurely aging mice after a 5-week ingestion of a diet supplemented with biscuits enriched in antioxidants. *Antioxid. Redox Signal*. 7: 1203–1210, 2005.

Beck, A.T. *Beck Anxiety Inventory*. Psychological Corporation, San Antonio, 1993 (Spanish version).

Beck, A.T., N. Epstein, G. Brown and R.A. Steer. An inventory for measuring anxiety: psychometric properties. *J. Consult. Clin. Psychol*. 56: 893–897, 1988.

Berman, B.M., B.B. Singh, L. Lao, P. Langenberg, H. Li, V. Hadhazy, J. Bareta and M. Hochberg. A randomized trial of acupuncture as an adjunctive therapy in osteoarthritis of the knee. *Rheumatology* 38: 346–354, 1999.

Bianchi, M., E. Jotti, P. Sacerdote and A.E. Panerai. Traditional acupuncture increases the content of beta-endorphin in immune cells and influences mitogen induced proliferation. *Am. J. Chin. Med*. 19: 101–104, 1991.

Blalock, J.E. The syntax of immune-neuroendocrine communication. *Immunol. Today* 15: 504–511, 1994.

Bossy, J. Immune system, defense mechanisms and acupuncture: fundamental and practical aspects. *Am. J. Acupunct*. 18: 219–232, 1990.

Boxer, L.A. Neutrophil disorders: qualitative abnormalities of the neutrophil. In: W.J. Williams, E. Beutler, A.J. Erslev and M.A. Lichtman (eds.) *Hematology*, 5th ed. McGraw-Hill, New York, 1995.

Brezinka, V., E. Dusseldorp and S. Maes. Gender differences in psychosocial profile at entry into cardiac rehabilitation. *J. Cardpulm. Rehabil*. 18: 445–449, 1998.

Bucinskaite, V., E. Theodorsson, K. Crumpton, C. Stenfors, A. Ekblom and T. Lundeberg. Effects of repeated sensory stimulation (electro-acupuncture) and physical exercise (running) on open-field behaviour and concentrations of neuropeptides in the hippocampus in WKY and SHR rats. *Eur. J. Neurosci*. 8: 382–387, 1996.

Chao, D.M., L.L. Shen, S. Tjen-a-Looi, K.F. Pitsillides, P. Li and J.C. Longhurst. Naloxone reverses inhibitory effect of electroacupuncture on sympathetic cardiovascular reflex responses. *Am. J. Physiol*. 276: H2127–H2131, 1999.

Comeche, M.I., M.I. Díaz and M.A. Vallejo. *Cuestionarios, Inventarios y Escalas, Ansiedad, Depresión y Habilidades Sociales*. Fundación Universidad-Empresa, Madrid, 1995.

De la Fuente, M. Modulation of immune functions by neuropeptides. *Curr. Trends Immunol.* 2: 111–122, 1999.

De la Fuente, M., M. Carrasco and A. Hernanz. Modulation of human neutrophil function *in vitro* by gastrin. *J. Endocrinol*. 153: 475–483, 1997.

De la Fuente, M., M.D. Ferrández, M.S. Burgos, A. Soler, A. Prieto and J. Miquel. Immune function in aged women is improved by ingestion of vitamins C and E. *Can. J. Physiol. Pharmacol.* 76: 373–380, 1998.

De la Fuente, M. and V.M. Víctor. Antioxidants as modulators of immune function. *Immunol. Cell Biol*. 78: 49–54, 2000.

DeVane, C.L., E. Chiao, M. Franklin and E.J. Kruep. Anxiety disorders in the 21st century: status, challenges, opportunities, and comorbidity with depression. *Am. J. Manage. Care* 11: S344–S353, 2005.

Du, L.N., G.C. Wu and X.D. Cao. Modulation of orphanin FQ or electroacupuncture (EA) on immune function of traumatic rats. *Acupunct. Electrother. Res*. 23: 1–8, 1998.

Ernst, E. Complementary and alternative medicine in rheumatology. *Baillieres Best Pract. Res. Clin. Rheumatol.* 14: 731–749, 2000.

Ferrández, M.D., R. Correa, M. Del Río and M. De la Fuente. Effects *in vitro* of several antioxidants on the natural killer function on aging mice. *Exp. Gerontol*. 34: 675–685, 1999.

Glaser, R. Stress-associated immune dysregulation and its importance for human health: a personal history of psychoneuroimmunology. *Brain Behav. Immun*. 19: 3–11, 2005.

He, L.F. Involvement of endogenous opioid peptides in acupuncture analgesia. *Pain* 31: 99–121, 1987.

Herbert, T.B. and S. Cohen. Depression and immunity: a metaanalytic review. *Psychol. Bull*. 113: 474–486, 1993.

Jin, H.O., L. Zhou, K.Y. Lee, T.M. Chang and W.Y. Chey. Inhibition of acid secretion by electrical acupuncture is mediated via β-endorphin and somatostatin. *Am. J. Physiol*. 271: G524–G530, 1996.

Joos, S., C. Schott, H. Zou, V. Daniel and E. Martin. Acupuncture-immunological effects in treatment of allergic asthma. *Allergologie* 20: 63–68, 1997.

Joos, S., C. Schott, H. Zou, V. Daniel and E. Martin. Immunomodulatory effects of acupuncture in the treatment of allergic asthma: a randomized controlled study. *J. Altern. Complement. Med.* 6: 519–525, 2000.

Karst, M., D. Scheinichen, T. Rueckert, T. Wagner, B. Wiese and M. Fink. Acupuncture has no immediate treatment effect on the neutrophil respiratory burst: a randomized single-blinded two-period crossover study. *Brain Behav. Immun*. 16: 813–816, 2002.

Karst, M., D. Scheinichen, T. Rueckert, T. Wagner, B. Wiese, S. Piepenbrock and M. Fink. Effect of acupuncture on the neutrophil respiratory burst: a placebo-controlled single-blinded study. *Complement. Ther. Med*. 11: 4–10, 2003.

Kasahara, T., M. Amemiya, Y. Wu, and K. Oguchi. Involvement of central opioidergic and nonopioidergic neuroendocrine systems in the suppressive effect of acupuncture on delayed type hypersensitivity in mice. *Int. J. Immunopharmacol*. 15: 501–508, 1993.

Kendall, D.E. A scientific model of acupuncture, part I. *Am. J. Acupunct*. 17: 251–268, 1989.

Koh, K.B. The relationship between stress and natural killer-cell activity in medical college students. *Jpn. J. Psychosom. Med*. 3: 3–10, 1993.

Koh, K.B. Emotion and immunity. *J. Psychosom. Res*. 45: 107–115, 1998.

Kho, H.G., P.W. Kloppenborg and J. Van Egmond. Effects of acupuncture and transcutaneous stimulation analgesia on plasma hormone levels during and after major abdominal surgery. *Eur. J. Anesthesiol*. 10: 197–208, 1993.

Koh, K.B. and B.K. Lee. Reduced lymphocyte proliferation and interleukin-2 production in anxiety disorders. *Psychosom. Med*. 58: 80, 1996.

Kou, W., J.D. Bell, I. Gareus, G. Pacheco-López, M.U. Goebel, G. Spahn, M. Stratmann, O.E. Janssen, M. Schedlowski and G.J. Dobos. Repeated acupuncture treatment affects leukocyte circulation in healthy young male subjects: a randomized single-blind two-period crossover study. *Brain Behav. Immun.* 19: 318–324, 2005.

Mittleman, E. and J.S. Gaynor. A brief overview of the analgesic and immunologic effects of acupuncture in domestic animals. *J. Am. Vet. Med. Assoc*. 217: 1201–1205, 2000.

Nishijo, K., H. Mori, K. Yosikawa and K. Yazawa. Decreased heart rate by acupuncture stimulation in humans via facilitation of cardial vagal activity and suppression of cardiac sympathetic nerve. *Neurosci. Lett*. 227: 165–168, 1997.

Pomeranz, B. Naloxone blockade of acupuncture analgesia: endorphin implicated. *Life Sci*. 19: 1757–1763, 1976.

Richardson, P.H. and C.A. Vincent. Acupuncture for the treatment of pain: a review of evaluative research. *Pain* 24: 15–40, 1986.

Rogers, P.A.M. and J. Bozzy. Activation of defense systems of the body in animals and man by acupuncture and moxibustion. *Acupunct. Res. Q* 5: 47–54, 1981.

Sher, L. The role of endogenous opioid system in the pathogenesis of anxiety disorders. *Med. Hypotheses* 50: 473–474, 1998.

Sin, Y.N. Effect of electric acupuncture and moxibustion on phagocytic activity of the reticulo-endothelial-system of mice. *Am. J. Acupunct*. 11: 134–137, 1983.

Sin, Y.M., A.R. Sedgewick and M.B. Mackay. Effect of electric acupuncture on acute inflammation. *Am. J. Acupunct*. 11: 359–362, 1983.

Sirota, P., R. Gavrieli and B. Wolach. Overproduction of neutrophil radical oxygen species correlates with negative symptoms in schizophrenic patients: parallel studies on neutrophil chemotaxis, superoxide production and bactericidal activity. *Psychiatry Res*. 121: 123–132, 2003.

Sliwinski, J and M. Kulej. Acupuncture induced immunoregulatory influence on the clinical state of patients suffering from chronic spastic bronchitis and undergoing long-term treatment with corticosteroids. *Acupunct. Electrother. Res*. 14: 227–234, 1989.

Sun, T., L.N. Du, G.C. Wu and X.D. Cao. Effect of intrathecal morphine and electroacupuncture on cellular immune function of rats and increment of mu-opioid receptor mRNA expression in PAG following intrathecal morphine. *Acupunct. Electrother. Res*. 25: 1–8, 2000.

Tam, K.C. and H.H. Yin. The effect of acupuncture on essential hypertension. *Am. J. Chin. Med*. 3: 369–375, 1975.

Ulett, G.A., S.P. Han and J.S. Han. Traditional and evidence-based acupuncture: history, mechanisms, and present status. *South Med. J*. 91: 1115–1120, 1998a.

Ulett, G.A., S.P. Han and J.S. Han. Electroacupuncture: mechanisms and clinical application. *Biol. Psychiatry* 44: 129–138, 1998b.

Víctor, V.M., M. Rocha and M. De la Fuente. Regulation of macrophage function by the antioxidant N-acetylcysteine in mouse-oxidative stress by endotoxin. *Int. Immunopharmacol*. 3: 97–106, 2003.

Víctor, V.M., M. Rocha and M. De la Fuente. Immune cells: free radicals and antioxidants in sepsis. *Int. Immunopharmacol*. 4: 327–347, 2004.

Wayne, S.J., R.L. Rhyne, P.J. Garry and J.S. Goodwin. Cell-mediated immunity as a predictor of morbidity and mortality in subjects over 60. *J. Gerontol.* 45: 45–98, 1990.

Wolach, B., R. Gavrieli and A. Pomeranz. Effect of granulocyte and granulocyte macrophage colony stimulating factors (G-CSF and GM-CSF) on neonatal neutrophil functions. *Pediatr. Res.* 48: 369–373, 2000.

Xie, Q.W. Endocrinological basis of acupuncture. *Am. J. Chin. Med*. 9: 298–304, 1981.

Yao, T., S. Andersson and P. Thoren. Long-lasting cardiovascular depression induced by acupuncture-like stimulation of the sciatic nerve in unanaesthetized spontaneously hypertensive rats. *Brain Res*. 240: 77–85, 1982.

Yonkers, K.A., I.R. Dyck, M. Warshaw and M.B. Keller. Factors predicting the clinical course of generalised anxiety disorder. *Br. J. Psychiatry* 176: 544–549, 2000.

Yu, Y., T. Kasahara, T. Sato, K. Asano, G.D. Yu, J.Q. Fang, S.Y. Guo, M. Sahara and T. Hisamitsu. Role of endogenous interferon-γ on the enhancement of splenic NK cell activity by electroacupuncture stimulation in mice. *J. Neuroimmunol*. 90: 176–186, 1998.

Zhao, H., L.N Du, J.W. Jiang, G.C. Wu and X.D. Cao. Neuroimmunal regulation of electroacupuncture (EA) on the traumatic rats. *Acupunct. Electrother. Res*. 27: 15–27, 2002.

Zhou, R.X., F.L. Huang, S.R. Jiang and J.C. Jiang. The effect of acupuncture on the phagocytic activity of human leukocytes. *J. Tradit. Chin. Med*. 8: 83–84, 1988.