Effect Of Acupuncture On Circulating Cytokines In Healthy Subjects
Mary Johansen, PharmD
Gui-Ju Yu, PhD
Timothy Madden, PharmD
Joseph S. Chiang, MD

ABSTRACT
Acupuncture therapy has been shown to produce global changes in immunological function including stimulation of circulating cytokine concentrations. We designed a single-blind crossover trial to further investigate the effect of LI 4 acupuncture vs sham acupuncture on circulating interferon-gamma (IFN-g) and interleukin-2 (IL-2) concentrations in a healthy population of 20 volunteer hospital employees. Each subject was randomized to receive an initial 20-minute acupuncture or sham acupuncture treatment, followed by the alternate treatment 2 weeks later. Peripheral blood cytokine concentrations were measured before and up to 48 hours after treatment. Vital signs were monitored in the first 10 subjects. Baseline plasma IFN-g and IL-2 concentrations were undetectable in all but 1 subject. Isolated increases in IFN-g and IL-2 concentrations were undetectable in 2 and 6 subjects, respectively, but no sustained increases were observed.

KEY WORDS
Acupuncture, Immune Function, Interferon, Cytokines, Interleukin-2, Sham Acupuncture

INTRODUCTION
Acupuncture therapy is thought to correct a wide variety of disease conditions. The general theory behind acupuncture therapy is based on the premise that there are patterns of energy flow through the body known as Qi that are essential for health. Disruptions of this energy flow, believed to be responsible for the onset of disease, are thought to be corrected by acupuncture stimulation.

The mechanisms by which acupuncture modulates physiological conditions, however, remain unknown. Numerous preclinical and clinical reports now describe the effects of acupuncture on cellular and humoral immunity, which may have far-reaching implications in the treatment and prevention of many diseases, including immune-mediated disorders. Modern anatomy and physiology relate acupuncture points to areas on the skin surface with lower electrical conduction compared to that of their immediate environment. Evidence also indicates that these points may correspond to peripheral cranial and spinal nerve endings.

The well-described analgesic effect of acupuncture has been shown to be mediated in part by stimulation of opioid-like peptides but both endorphin and non-endorphin systems may be involved. Further studies describe a myriad of specific immune parameter alterations induced by acupuncture. Included is modulation of globulins, enhancement of natural killer cell activity, increased phagocytic and fibrinolytic activity, increased plaque-forming cell concentration in spleen and bone marrow, and stimulation of leukocyte recovery following irradiation.

Other studies have demonstrated that acupuncture induces increases in certain T-lymphocyte subsets enhances E rosette forming rate, and may increase interleukin-2 (IL-2) receptor density on the surface of T-helper cells. Alterations in
cytokines have also been reported including stimulation of IL-2 and interferon-gamma (IFN-g) production, and suppression of IL-6 and IL-10 in patients with allergic asthma. To date, a large majority of published reports describing the effects of acupuncture on cellular and humoral immunity have originated outside the United States. Much of the literature specifically on cytokine stimulation consists of case reports or intervention studies. We therefore sought to perform a randomized single-blind controlled study investigating the effect of acupuncture on circulating cytokine concentrations and on immune function. In a healthy population of 20 subjects, we evaluated plasma concentration of IFN-g and IL-2 at several time points up to 48 hours following bilateral stimulation of the LI 4 acupuncture point.

METHODS
Subjects
Male or female subjects between the ages of 24 and 50 with no known systemic disease, immune dysfunction, or coagulation disorders were eligible. Exclusion factors included a history of infectious disease (cold, urinary tract infection, upper respiratory infection), prior use of prescription or over-the-counter medications within 4 weeks of study entry, skin infection at the proposed acupuncture site, and prior acupuncture therapy. This study was approved by the institutional review board (IRB) prior to initiation.

Hospital employees meeting eligibility criteria were randomly chosen and asked to volunteer for the study. Subjects had to confirm availability for each of 2 treatment sessions, 10-14 days apart, and for all blood sampling as defined by the protocol. An equal number of male and female subjects were asked to participate. A medical history was recorded and a baseline physical exam was performed prior to study entry. Informed consent was obtained from each subject indicating their awareness of the investigational nature of the study.

TREATMENT
Each subject received 1 acupuncture and 1 sham acupuncture treatment, administered 10-14 days apart. Subjects were randomized as to which treatment they received first, and were blinded to which treatment was received at each session. (The acupuncturist for the study was a Board-certified anesthesiologist and pain specialist with acupuncture training from China, Taiwan, and the United States.) Blood pressure, electrocardiogram, and oxygen saturation were monitored on the 1st 10 subjects to document the safety of the procedure.

During acupuncture stimulation, needles (blunt-tipped stainless steel; 50 mm length, 0.2 mm diameter; Seirin, Tokyo, Japan; purchased from OMS, Oriental Medical Supply, Braintree, MA) were applied to the LI 4 acupuncture points bilaterally. This point is on the Large Intestine Principal Meridian, and is located on the hand in the center dorsal interosseous muscle between the 1st and 2nd metacarpal bones (Figure 1). Needles were maintained at this position for 20 minutes with intermittent manual rotation, and then removed. Manual rotation was performed by continuous alternating rotation of the needle, clockwise then counterclockwise, for 10 seconds every 5 minutes.

Sham treatments were performed exactly as LI 4 treatments, except needles were applied at nonacupuncture points approximately 1 inch from the LI 4 site bilaterally. It is thought that application of needles to nonacupuncture sites does not elicit the Qi stimulation induced at acupuncture points. We also considered the selection of sham acupuncture sites so that they were not in proximity of major vessels, bones, nerves, or internal organs.

During LI 4 treatments, the achievement of Qi was utilized as the endpoint for needle depth upon insertion. Propagation of this sensation along acupuncture channels is thought to carry the therapeutic signals to target areas. In order to maintain blinding, subjects were asked to voluntarily communicate what they felt during
needle insertion in order to assess achievement of De Qi. It can also be sensed by the operator as the needle being grabbed by the muscle.

Needle depth was expected to be approximately 0.5 cun for achievement of De Qi. If De Qi was not obtained at this depth as indicated by both the subject and the operator, the needle was adjusted until De Qi was achieved.

To determine plasma cytokine concentration, venous blood samples (5 mL) were collected from the antecubital space prior to acupuncture needle insertion, and at 0.5, 1, 2, 4, 8, 24, and 48 hours post needle removal. Samples were collected through a temporarily placed butterfly catheter into Vacutainer tubes containing heparin as an anti-coagulant, and plasma stored in aliquots of 0.5 mL at -80°C until analyzed. Plasma samples were analyzed for cytokine concentration by ELISA ("sandwich" enzyme immunoassay).

With 10 subjects, we sought to determine the proportion of patients with a sustained (in 2 consecutive samples) 4-fold or greater increase in plasma cytokine concentration relative to the lower limit of quantification of the assay. If sustained increases were observed, the percentage of patients with this degree of cytokine enhancement would be determined in both the acupuncture and sham acupuncture groups with 95% confidence intervals. If a nontrivial rise in any sample from the 1st set of 10 subjects was detected and defined as a cytokine concentration above the limit of quantification of the assay, 10 more subjects were entered onto the study.

RESULTS
Subject demographics are shown in Table 1. A variety of ethnic backgrounds were represented. Age and weight ranged from 23 to 50 years and 45 to 95 kg, respectively. An equal number of males and females were studied in each set of 10 subjects; none had received prior acupuncture therapy.

De Qi was achieved in all subjects during stimulation of the LI 4 acupuncture point. All subjects reported a feeling of aching, tingling, or numbness at the needle insertion site, which was exacerbated by manual rotation of the needles. During sham acupuncture treatment, 80% of subjects reported slight pain, pressure, or tingling upon needle insertion. The operator did not sense significant grabbing from the muscle during sham treatments. Blood pressure, heart rate, and oxygen saturation were unaffected by acupuncture and sham acupuncture procedures (Table 2).

Plasma IFN-g and IL-2 concentrations prior to acupuncture treatment were below the limit of quantification in all but 1 subject. One subject exhibited pretreatment IFN-y and IL-2 concentrations above 100 and 70 pg/mL, respectively, which remained stable in all subsequent post-treatment samples (data not shown). This observation suggested a preexisting stimulation of cytokine release which was unaffected by acupuncture treatment.

<table>
<thead>
<tr>
<th>Table 1. Demographic Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Men</td>
</tr>
<tr>
<td>Mean age, years (range)</td>
</tr>
<tr>
<td>Mean weight, kg (range)</td>
</tr>
<tr>
<td>Ethnic origin</td>
</tr>
<tr>
<td>African American</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>Hispanic</td>
</tr>
<tr>
<td>Middle Eastern</td>
</tr>
</tbody>
</table>

Figure 1. Large Intestine meridian showing location of the LI 4 acupuncture point. Reprinted from An Atlas of Acupuncture Points, with permission from Medical Acupuncture Publishers, Berkeley, CA, U.S.A, Copyright 1996, p 13.
Following LI 4 acupuncture treatment, no sustained increases in plasma cytokine concentrations were observed. Isolated increases in IFN-g and IL-2 were observed in 1 and 3 subjects, respectively, in each set of 10 subjects (Table 3). In 1 subject, an increase in IFN-g and IL-2 concentrations was observed concomitantly. No correlations could be made between subject age, race, or sex and the isolated increases in cytokine concentration. Cytokine concentrations were not detectable in any sample following sham treatment.

DISCUSSION
The growing acceptance and use of acupuncture therapy in Western medical practice has led to increased interest in understanding the mechanisms postulated to support its claimed benefits. Recent evidence suggests the effects of acupuncture may be mediated through multiple pathways, involving both the nervous and immune systems. Acupuncture stimulates endogenous opioid peptides and may modulate other neurotransmitters involved in pain perception at various levels of the central and peripheral nervous system, including serotonin.7,26

Table 2. Physiologic Data*

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>LI 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>132/80</td>
<td>132/80</td>
<td>131/73</td>
<td>128/79</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>131/85</td>
<td>131/85</td>
<td>155/84</td>
<td>154/91</td>
<td>97</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>86/52</td>
<td>86/52</td>
<td>94/62</td>
<td>93/60</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>123/99</td>
<td>123/99</td>
<td>123/80</td>
<td>124/63</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>5</td>
<td>107/61</td>
<td>107/61</td>
<td>137/100</td>
<td>138/75</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>134/86</td>
<td>134/86</td>
<td>113/60</td>
<td>113/48</td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td>7</td>
<td>111/67</td>
<td>111/67</td>
<td>113/38</td>
<td>119/48</td>
<td>96</td>
<td>98</td>
</tr>
<tr>
<td>8</td>
<td>127/55</td>
<td>127/55</td>
<td>129/70</td>
<td>111/59</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>9</td>
<td>121/57</td>
<td>121/57</td>
<td>103/57</td>
<td>97/62</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>10</td>
<td>156/32</td>
<td>156/32</td>
<td>142/52</td>
<td>122/50</td>
<td>99</td>
<td>100</td>
</tr>
</tbody>
</table>

*1st 10 subjects only

Table 3. Plasma IFN-g and IL-2 Concentrations in Subjects Following LI 4 Acupuncture*

<table>
<thead>
<tr>
<th>Subject</th>
<th>Timepoint, Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IFN-g, pg/mL</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&lt;15.6</td>
</tr>
<tr>
<td>7</td>
<td>&lt;15.6</td>
</tr>
<tr>
<td>9</td>
<td>&lt;15.6</td>
</tr>
<tr>
<td>13</td>
<td>&lt;0.39</td>
</tr>
</tbody>
</table>
In recent years, endogenous opioid peptides have been closely associated with cellular and humoral immunity. The suggestion that acupuncture's influence may be partially expressed through immune mechanisms is reinforced by the understanding of the coupling of the pain and immune systems via common signaling molecules. β-endorphin and met-enkephalin seem to be mediators between the central nervous system and the immune system, demonstrating abilities to enhance natural killer cell activity and increase in vitro T-lymphocyte rosette formation from human T-lymphocytes. Other reports demonstrating opioid receptor-mediated effects on immunity, and reversal of these effects at least in part by naloxone, also support the hypothesis that acupuncture may influence the immune system as well as the antinociceptive system.

Among recent studies reporting stimulation of cytokine release, one study performed in Taiwan demonstrated a more than 10-fold increase in plasma interferon concentration after piqure of the LI 4 acupuncture point in a population of healthy volunteers. In that study, serum interferon concentration was determined immediately before LI 4 acupuncture was initiated, and at 24, 48, and 96 hours after withdrawal of the needles. Mean plasma interferon concentration rose from < 23 IU/mL at baseline to 323.2 IU/mL and 167.6 IU/mL at 24 and 48 hours after needle removal, respectively. Of 32 subjects, 24 demonstrated a response. It was reported in that study that the interferon stimulated was of the gamma type. Our study resulted in only a few isolated increases in IFN-γ and IL-2 plasma concentrations, and none were sustained in consecutive samples. Other clinical studies have also demonstrated a lack of effect of acupuncture on immunoglobulin levels and leukocyte counts in patients following upper abdominal surgery.

Numerous confounding variables may contribute to isolated increases in plasma cytokine concentrations as observed in our study. Acute infectious or viral exposures, hormonal variations, or changes in diet or exercise can all contribute to isolated bursts of cytokine release. Varying results regarding cytokine stimulation by acupuncture may be due to several other factors. The influence of steril practice utilized in previous studies indicating cytokine stimulation has been considered, but the need for skin and hand disinfection for acupuncture procedures is in itself controversial and is not likely a significant variable in these studies. The varying techniques employed by different operators may influence results. Many reports on immune stimulation with acupuncture have utilized electroacupuncture techniques employing electrical stimulation of acupoints, which may impart additional effects. Other schools of thought suggest that acupuncture induces homeostatic effects on immunity which may be more obvious when abnormalities exist first. Therefore, baseline immunological status of the organism may contribute to varying results on immune effects attributed to this intervention.

Acupoint selection is a variable of primary importance. Although no standard guidelines exist for the selection or combination of acupoints to induce effects on immunity, published reports are indicative of those that may give the most promising results. Stimulation of the LI 4 acupoint has enhanced recovery of total leukocytes, lymphocytes, and neutrophils in irradiated rats. Other animal and human studies have demonstrated the effectiveness of combining this point with SP 6 and ST 36. The use of this combined approach has yielded increases in circulating white blood cells and immunoglobulins and significantly increased IL-2 concentrations in patients with rheumatoid arthritis.

In patients with a variety of cancers, LI 4 acupuncture combined with ST 36, PC 6 or Nei Guam, and Renmai 4 (CV 4 or Guan Yuan) increased the percentage of some T-lymphocytes subsets (CD4+/CD8+ ratio alteration), increased circulating β-
endorphins, and decreased the concentration of soluble IL-2 receptor in peripheral blood.\textsuperscript{11,38}

A recent study showing suppression of IL-6 and IL-10 concomitantly with increases in IL-8 in asthma patients utilized an acupuncture regimen comprising 15 points across 8 meridians, some of which were varied in accordance with specific diagnoses.\textsuperscript{25}

**CONCLUSION**

The lack of cytokine stimulation observed in this study following single-point acupuncture suggests that further evaluation is needed to define whether acupuncture modulates cytokine release. The role played by endorphins in this mechanism is perhaps another subject worthy of study. It is clear that stimulation of the endogenous release or production of immune mediators by acupuncture may have far-reaching implications in the treatment of disease. Use of this technique to stimulate the immune system could play a role in cancer treatment, as well as in reversing the often therapy-limiting effect of disease or treatment-related immunosuppression. Stimulation of the immune system using this technique may also provide important advantages over other forms of immune stimulation. Not only is acupuncture a cost-efficient procedure, but in contrast to several other exogenously administered immune-modulating agents, acupuncture does not pose a risk of increasing infectious susceptibility or inducing antigenic responses, and is not known to produce severe side effects, such as hypotension and capillary leak syndrome, known to occur with exogenously administered IL-2.\textsuperscript{39,40} Continued evaluation of immune modulation by acupuncture should perhaps be performed in well-defined patient populations with various acupoint combinations, treatment regimens, and techniques in order to further elucidate its potential role in disease treatment.

**REFERENCES**

33. Kho HG, Van Egmond J, Eijk RJR, Kapteyns WWMJ. Lack of influence of acupuncture and trancutaneous stimulation...


AUTHORS’ INFORMATION

Mary J. Johansen, PharmD, is Assistant Professor of Cancer Medicine, Dept of Experimental Therapeutics, and Manager, Pharmaceutical Development Center, at UTMD Anderson Cancer Center in Houston, Texas.

Mary Johansen, PharmD*  
Department of Experimental Therapeutics  
UTMD Anderson Cancer Center  
1515 Holcombe Blvd, Unit 601  
Houston, TX 77030  
Phone: 713-745-3043  
Fax: 713-745-2908  
E-mail: mjohanse@mdanderson.org

Gui-Ju Yu, PhD, is a Senior Research Assistant, Pharmaceutical Development Center at UTMD Anderson Cancer Center in Houston, Texas. Dr Yu earned her PhD in Physics at the Soviet Academy of Science, Institute of Physics, in Russia.

Gui-Ju Yu, PhD  
Pharmaceutical Development Center  
UTMD Anderson Cancer Center  
8000 El Rio St  
Houston, TX 77054  
Phone: 713-745-3220  
Fax: 713-745-2908  
E-mail: gyu@mdanderson.org

Timothy Madden, PharmD, is Director, Pharmaceutical Research, Division of Pharmacy, and Co-Director, Pharmaceutical Development Center at UTMD Anderson Cancer Center in Houston, Texas.

Timothy Madden, PharmD  
UTMD Anderson Cancer Center  
1515 Holcombe Blvd, Unit 90
Dr Joseph Chiang is a Board-certified Anesthesiologist and Pain Specialist, and Professor, Dept of Anesthesiology, UTMD Anderson Cancer Center in Houston, Texas. Dr Chiang's efforts contributed to acupuncture therapy being approved as a formal therapeutic modality offered to ill patients in M.D. Anderson Cancer Center.

Joseph S. Chiang, MD
UTMD Anderson Cancer Center
1515 Holcombe Blvd, Unit 42
Houston, TX 77030
Phone: 713-794-5363
Fax: 713-794-4590
E-mail: jchiang@mdanderson.org