Neuroimmunomodulatory effects of acupuncture in mice

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The purpose of this study was to assess the effect of acupuncture on the immunological response. The induction of anti-sheep red blood cells (SRBC) plaque-forming cells (PFC) was used as a measurement of the immune response to treatment. In normal non-immunized mice, enhancement of PFC was seen after a single acupuncture treatment when spleen cells from stimulated mice were cultured with SRBC in vitro. After 3 acupuncture treatments, spleen cells from mice did not show PFC enhancement after treatment with anti-Thy-1.2 antibody and complement, nor after the removal of non-adherent cells. Serum obtained from mice 1 h after acupuncture stimulation enhanced the PFC of normal spleen cells in vitro, but the enhancement was abolished by the addition of propranolol. These results suggest that acupuncture, by activation of the autonomic nervous system, modulates the immune response.

Recent reports have suggested that the immune reaction is regulated by the central nervous system [4, 8]. This is interesting to note, as experimental studies have also shown that acupuncture stimulation affects the activity of several systems within the brain and induces the release of endogenous opioids [11].

Opiates and endogenous opioids have been shown to have immuno-compromising effects [2, 3, 7]. Further, other studies have shown that erythrocytes, monocytes, granulocytes and lymphocytes all have specific opiate binding sites [1, 14]. These findings, and the fact that acupuncture has been tried in the treatment of patients with immuno-deficiency diseases, induced us to study the effects of acupuncture on the immune responses [17].

Acupuncture treatment was carried out for periods of 30 min. Six points were chosen (Jingmen G25; Zhaohai K6; Dazhu B11, bilaterally). Thirty male mice, 6–10 weeks old, were assigned to 3 groups with 10 mice in each, for each of the treatment categories.

Group 1 (acupuncture): the needles were inserted between 0.3 and 0.9 cm and rotated. This caused a local muscle contraction, recognized as de Qi, and was repeated for 10 sec every 5 min.

Group 2 (superficial acupuncture): the needles were inserted intradermally and left for 30 min without eliciting any response.

Group 3 (controls): ten mice, untreated, served as controls. After the trials the mice were killed by decapitation.

Spleens were removed aseptically, sliced in tissue culture media (RPMI 1640, Sigma) solution, and passed through a wire mesh. The cells obtained were washed 3 times in phosphate-buffered saline (pH 7.4) and then hemolyzed with 0.75 % Tris-ammonium chloride solution (pH 7.65). The cells were washed again 3 times and then suspended in tissue culture media (RPMI 1640, Sigma) in a concentration of 1 × 10⁷ cells/ml [9].

Measurement of anti-sheep red blood cells (SRBC) IgM plaque-forming cells (PFC) in vivo was performed by determining the number of anti-SRBC PFC spleen cells on the 5th day after sensitization with 5 × 10⁷ SRBC [6].

The β-adrenergic blocking drug propranolol (Inderal), the α-adrenergic blocking agent phentolamine (Regitine) and hexamethonium (Methobromine) were dissolved in physiological saline and administered orally to the mice. The local anaesthetic lidocaine (Xylocain) was injected subcutaneously. All drugs were given in amounts of 0.1 ml/10 g body weight.

Regulatory cells: spleen or thymic cells from CBF1 mice were treated with mitomycin C (25 μg/ml, 37°C, 30 min), washed 3 times with RPMI 1640, and cultured at

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TABLE I
EFFECTS OF SUPERFICIAL ACUPUNCTURE OR ACUPUNCTURE ON ANTI-SHEEP RED BLOOD CELLS (SRBC) IgM PLAQUE-FORMING CELLS (PFC) PRODUCTION IN IMMUNIZED MICE

Treatment was given once a day from day 1 to day 3 after immunization with SRBC. Interaction of phentolamine, propranolol, hexamethonium, and lidocaine with the different modes of treatment. All drugs were administered 90 min before treatment. Saline was administered s.c. daily before each mode of treatment. Values (anti-SRBC PFC, PFC/10^6, spleen cells) are given as mean ± S.D.

<table>
<thead>
<tr>
<th>Mode of treatment</th>
<th>No treatment (control)</th>
<th>Superficial acupuncture</th>
<th>Acupuncture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>2083 ± 368</td>
<td>2168 ± 328</td>
<td>4165 ± 550*</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>3304 ± 508</td>
<td>3345 ± 436</td>
<td>5739 ± 705*</td>
</tr>
<tr>
<td>Propranolol</td>
<td>2688 ± 339</td>
<td>2656 ± 337</td>
<td>2619 ± 425</td>
</tr>
<tr>
<td>Hexamethonium</td>
<td>2076 ± 362</td>
<td>2105 ± 296</td>
<td>2533 ± 347</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>2003 ± 270</td>
<td>2081 ± 223</td>
<td>2926 ± 590</td>
</tr>
</tbody>
</table>

*P < 0.05 comparing no treatment (controls) with the different modes of acupuncture.

5 x 10^6 cells/well with 5 x 10^6 normal spleen cells and 5 x 10^6 SRBC (9).

RPMI 1640 was supplemented with 25 mM HEPES, 300 µg/ml of L-glutamine, 100 U/ml of penicillin, 100 µg/ml of streptomycin, 5 x 10^{-5} M 2-mercaptoethanol, and 10% fetal bovine serum for the culture of mice spleen cells [16].

Spleen cells, adjusted with RPMI 1640, were placed in Petri dishes that had been treated with 10% fetal bovine serum, and incubated for 1 h at 37°C [9]. The fraction of cells non-adherent to the dishes was removed. The procedure was repeated 3 times.

Anti-Thy-1.2 antibody was diluted to 1/5000 with RPMI 1640 and was used to adjust the concentration of spleen cells to 1 x 10^7 cells/ml. The suspension was left at 4°C for 30 min. The cells were then resuspended in RPMI 1640 which contained guinea pig dried complement, incubated at 37°C for a further 30 min, then washed 3 times with RPMI 1640 [9]. Control cells were treated with complement only.

The values presented represent mean ± S.D. Significances of differences between groups were tested by analysis of variance. Intergroup differences of categorical variables were tested for significance with Fisher’s exact test. Statistical significance was defined as P < 0.05.

Acupuncture stimulation resulted in an increase in the PFC production. Pre-administration (90 min before stimulation) of phentolamine 5 mg/kg p.o. further enhanced the increase of PFC production. However, the increase induced by acupuncture was blocked by pre-treatment with propranolol 5 mg/kg p.o., hexamethonium 5 mg/kg p.o. and lidocaine 5 mg/kg s.c. (Table I). Mice, normal or immunized with SRBC, were treated with acupuncture for 3 days. Spleen cells from these mice were then cultured with SRBC in vitro. As shown in Table II, the spleen cells from both treated groups showed an increase in PFC due to acupuncture stimulation. This increase appeared 3 h after an acupuncture treatment and lasted for at least 24 h.

The effect of anti-Thy-1.2 antibody on the helper activity was enhanced by acupuncture and the enhancement was reduced by the addition of anti-Thy-1.2 antibody and complement, but not by complement alone (Table III). The helper activity was seen in spleen cells that were non-adherent to the dishes, but not in adherent cells or thymic cells. The number of anti-SRBC PFC was increased by treatment with serum taken from mice 1 h after acupuncture stimulation (Table IV). This serum-influenced activity was abolished by the addition of propranolol 10^{-7} g/ml, but not by phentolamine 10^{-7} g/ml (Table V).

The present study shows that acupuncture treatment may result in enhancement of the PFC activity. Three hours after treatment, there was enhanced PFC activity. This enhanced PFC activity was not blocked by in vitro treatment with mitomycin C, but was abolished by the addition of anti-Thy-1.2 antibody and complement, and by the removal of non-adherent cells. Serum obtained from mice 1 h after acupuncture treatment showed enhanced PFC in normal mice spleen cells, but this effect was reversed by the addition of the β-adrenergic blocking agent propranolol.
TABLE III
CHARACTERIZATION OF HELPER ACTIVITY ON PFC ENHANCED BY SUPERFICIAL ACUPUNCTURE OR ACUPUNCTURE

Spleen and thymic cells were used 3 h after treatment. These cells were treated with mitomycin and were used as regulatory cells of in vitro anti-SRBC PFC production. Normal spleen cells were used as effector cells. Values (PFC/culture) are given as mean ± S.D.

<table>
<thead>
<tr>
<th></th>
<th>No treatment (controls)</th>
<th>Superficial acupuncture</th>
<th>Acupuncture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen cells</td>
<td>320 ± 474</td>
<td>2414 ± 386</td>
<td>4797 ± 356*</td>
</tr>
<tr>
<td>Treated with complement</td>
<td>2337 ± 415</td>
<td>4707 ± 594*</td>
<td></td>
</tr>
<tr>
<td>Treated with anti-Thy-1.2 and complement</td>
<td>2280 ± 403</td>
<td>2877 ± 261</td>
<td></td>
</tr>
<tr>
<td>Adherent cells</td>
<td>2320 ± 474</td>
<td>2230 ± 338</td>
<td></td>
</tr>
<tr>
<td>Non-adherent cells</td>
<td>2377 ± 435</td>
<td>4152 ± 437*</td>
<td></td>
</tr>
<tr>
<td>Thymic cells</td>
<td>2377 ± 435</td>
<td>2520 ± 360</td>
<td>2427 ± 427</td>
</tr>
</tbody>
</table>

*P < 0.05 comparing no treatment (controls) with the different modes of acupuncture or within the treatment groups.

The present and previous results indicate that the enhancement of PFC by acupuncture may be due to the activation of non-specific helper T lymphocytes by β-action of endogenous epinephrine, released through stimulation of the sympathetic nervous system [9]. This result is supported by the fact that an i.p. injection of painful substance raises the level of endogenous epinephrine in the spleen [10]. Furthermore, the autonomic nervous system may be activated by the local stimulus of externally administered catecholamines [9, 10], and increased antibody production is caused via the β-action of epinephrine from the sympathetic nervous system [9]. It is likely that part of the activation of the autonomic nervous system is mediated through sensory neuropeptides released during acupuncture [13]. This is interesting to note as lymphocytes possess receptors for these mediators and are affected by such mediators not only in proliferation [15] but also in rosette formation [12], antibody formation [5], cytotoxicity [18] and the intracellular level of cyclic nucleotides [19].

In summary, it has been reported that the peripheral and central nervous system may exert an effect on the immunological response [2, 3]. Acupuncture treatment results in changes in the activity of the central and peripheral nervous system [11, 13]. The results of the present study indicate that acupuncture enhances the immune reaction through the activation of non-specific T lymphocytes through the autonomic nervous system.

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TABLE IV
EFFECT OF SERUM FROM MICE GIVEN SUPERFICIAL ACUPUNCTURE OR ACUPUNCTURE ON ANTI-SRBC PFC OF SPLEEN CELLS FROM NORMAL MICE IN VITRO

Sera were added at 0.3 ml to culture plate before beginning culture. Values (PFC/culture) are given as mean ± S.D. NS between groups.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>No treatment</th>
<th>Superficial acupuncture</th>
<th>Acupuncture</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1077 ± 82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1070 ± 91</td>
<td>1041 ± 74</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1095 ± 97</td>
<td>1175 ± 110</td>
<td></td>
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<tr>
<td>60</td>
<td>1142 ± 87</td>
<td>1761 ± 97</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>1145 ± 135</td>
<td>1401 ± 183</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>1083 ± 106</td>
<td>1094 ± 194</td>
<td></td>
</tr>
</tbody>
</table>

TABLE V
EFFECTS OF PROPRANOLOL AND PHENTOLAMINE IN VITRO ON THE ENHANCEMENT OF PFC BY SERUM FROM MICE TREATED WITH SUPERFICIAL ACUPUNCTURE OR ACUPUNCTURE

Sera were obtained from mice 60 min after superficial acupuncture or acupuncture treatment. Phentolamine and propranolol were used at dose 10⁻¹ g/ml. Values (PFC/culture) are given as mean ± S.D. NS between groups.

<table>
<thead>
<tr>
<th></th>
<th>No treatment</th>
<th>Superficial acupuncture</th>
<th>Acupuncture</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug</td>
<td>1750 ± 477</td>
<td>1851 ± 470</td>
<td>2506 ± 381</td>
</tr>
<tr>
<td>Propranolol</td>
<td>1830 ± 307</td>
<td>2010 ± 383</td>
<td>1279 ± 183</td>
</tr>
<tr>
<td>Phentholamine</td>
<td>1728 ± 428</td>
<td>1793 ± 193</td>
<td>2408 ± 332</td>
</tr>
</tbody>
</table>


