



## The $\alpha$ -adrenoceptor mediation of the immunomodulatory effects of electroacupuncture in DNP-KLH immunized mice

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### Abstract

Our previous study demonstrated that successive electroacupuncture (EA) at ST36 acupoint reduces IgE production in BALB/c mice immunized with 2,4-dinitrophenylated keyhole limpet protein (DNP-KLH) by suppression of the Th2 cell lineage development. Here, we report that pretreatment of phentolamine ( $\alpha$ -adrenoceptor antagonist, 10 mg/kg, i.p.) completely blocks the EA-induced suppression of antigen-specific and total IgE levels in serum and IL-4 production in anti-CD3 mAb-activated splenocytes in DNP-KLH immunized mice. The results suggest that  $\alpha$ -adrenoceptor play an important role in mediating the suppressive effects of EA on IgE production and Th2 cell response in DNP-KLH immunized mice.

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Acupuncture, which has been practiced for thousands of years in Eastern countries, is increasingly being used worldwide to treat various diseases with few side effects [12,21]. Electroacupuncture (EA) is a modified acupuncture technique that utilizes electrical stimulation. Acupuncture or EA stimulation is believed to facilitate the release of certain neurotransmitters, including opioids and catecholamines in the CNS, which elicits profound psychophysiological responses, especially analgesia [8–10].

Several lines of clinical evidence have indicated that EA treatment is effective for allergic disorders, such as asthma and chronic urticaria [3,4,11], of which the imbalance of Th1/Th2 cell response is the main cause. In general view, hyperproduction of IgE, for which Th2 cytokines are responsible, promotes the development of these allergic disorders [19,27]. Our previ-

ous study demonstrated that successive EA at ST36 acupoint reduces serum IgE levels in BALB/c mice immunized with 2,4-dinitrophenylated keyhole limpet protein (DNP-KLH), through the suppression of Th2 cytokines [24]. Interestingly, Kim et al. [16] reported that pretreatment of naloxone (opioid receptor antagonist) did not prevent EA-induced suppression of IL-4 mRNA levels from splenocytes in DNP-KLH immunized mice. Therefore, it is suggested that non-opioid mechanisms, such as catecholamines play a role in mediating the suppressive effects of EA on Th2 cell response in the immunized mice. In fact, it is well known that EA treatment regulates pain behaviors and autonomic nervous system functions via spinal  $\alpha$ -adrenoceptors or somato-sympathetic reflex in rats [15,17,25]. It also has been reported that acupuncture or EA treatment modulates immune responses in mice and humans by activation of autonomic nervous system [18,20]. Therefore, we hypothesized that  $\alpha$ -adrenergic signaling pathway mediates the immunomodulatory effects of EA. To explore this hypothesis, we examined whether phentolamine ( $\alpha$ -adrenoceptor antagonist) pretreatment reverses the suppressive effects of EA on IgE and IL-4 productions in DNP-KLH immunized mice.

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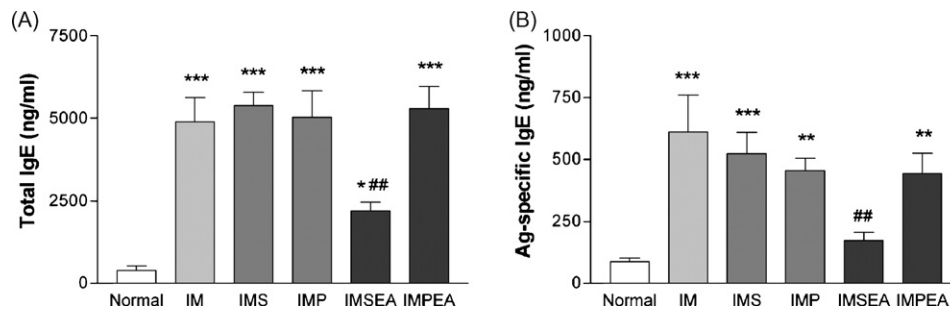


Fig. 1. Total IgE and antigen-specific IgE production from serum in DNP-KLH immunized mice. The amounts of total IgE (A) and antigen-specific IgE (B) were measured by ELISA. Data are presented as mean  $\pm$  S.E.M. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , vs. Normal group; ## $P < 0.01$ , IMSEA vs. IM or IMPEA group by Newman–Keuls multiple comparison test after one-way ANOVA. Normal = no treatments; IM = immunization; IMS = immunization + saline; IMP = immunization + phentolamine; IMSEA = immunization + saline + EA; IMPEA = immunization + phentolamine + EA.

Eight-week old female BALB/c mice (OrientBio, Korea) were housed in an air-controlled, pathogen-free animal facility with a 12 h light:12 h dark cycle and were provided with food and water *ad libitum*. We regularly monitored the possibility of infection of animals by the measurements of body temperature and weight, and by the blood test for viral and mycoplasmal infections. All procedures were conducted in accordance with NIH guidelines and were approved by the Institutional Animal Care and Use Committee at Kyung Hee University.

Mice were randomly divided into six groups ( $n = 6$ –10/group): (1) Normal group (no treatments for 2 weeks, except for i.p. injection of saline on the 1st and 8th experimental day); (2) IM group (immunization on the 1st and 8th day); (3) IM + S group (immunization + daily i.p. injection of saline for 2 weeks); (4) IM + P group (immunization + daily i.p. injection of phentolamine for 2 weeks); (5) IM + S + EA group (immunization + daily i.p. injection of saline followed by ST36 EA for 2 weeks); (6) IM + P + EA group (immunization + daily i.p. injection of phentolamine followed by ST36 EA for 2 weeks).

EA stimulation was performed as previously described [24]. Briefly, a pair of stainless steel needles (0.2 mm in diameter and 3 cm long) was inserted (3 mm in depth) into the ST36, which is located at the anterior tibial muscle, 5 mm laterally and lower from the anterior tubercle of the tibia and at a point 5 mm distal from the first needle. This point (ST36) is known to modulate the immune response in mice [24,30]. Anode and cathode leads from an electrical stimulator were connected to the two acupuncture needles, and then train-pulses (1 Hz, 0.25 ms pulse width, 3–5 V) were applied for 20 min.

All mice, except for the Normal group, were immunized intraperitoneally with 4  $\mu$ g of DNP-KLH (Calbiochem, USA) and 4 mg of aluminum hydroxide (Sigma, USA) on the 1st and 8th day during the 2-week experimental period. Since the immunization with DNP-KLH causes a significant increase in IgE and IL-4 production on the 14th day after first immunization, EA stimulation was conducted daily for 2 weeks [24]. Phentolamine ( $\alpha$ -adrenoceptor antagonist, Tocris, USA) at a dose of 10 mg/kg [29] was dissolved in normal saline, and injected intraperitoneally (to the left and right abdominal side, alternatively) 10 min before EA stimulation. Normal saline (0.1 cc/mouse, i.p.) was served as control.

Serum and splenocytes from all groups of mice were obtained on the final experimental day. Serum levels of antigen-specific IgE and total IgE and the amounts of key Th1- and Th2-specific cytokines (i.e. IFN- $\gamma$  and IL-4) from anti-CD3 mAb-activated splenocytes were measured using ELISA method as described previously [24].

The one-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison test was used for the statistical analysis, since samples were normally distributed when assessed by the GraphPad Prism<sup>®</sup> windows 3.02. Data are presented as mean  $\pm$  S.E.M. In all cases,  $P < 0.05$  was considered significant.

Fig. 1 shows serum levels of antigen-specific and total IgE in mice. When compared with the Normal group, both the antigen-specific IgE and total IgE levels in the IM group were significantly increased. Saline or phentolamine injection alone did not affect this increase. EA treatment significantly reduced antigen-specific and total IgE secretions, in consistent with the results from our previous study [24] and phentolamine pretreatment completely blocked this EA effect.

The results of IL-4 and IFN- $\gamma$  levels from splenocytes are shown in Fig. 2. Similar to the previous reports [23,24], IL-4 secretion was markedly increased after DNP-KLH immunization while IFN- $\gamma$  was slightly suppressed. EA treatment significantly inhibited this IL-4 increase, which was completely reversed by phentolamine pretreatment. However, IFN- $\gamma$  secretion was not altered by EA treatment. Saline or phentolamine administration alone did not change IL-4 or IFN- $\gamma$  levels.

EA treatment can stimulate the release of specific neurotransmitters in the CNS, eliciting profound physiological effects and even activating self-healing mechanisms [10]. Numerous studies have demonstrated that EA stimulation increases the levels of endogenous opioids or catecholamines in the CNS, leading to potent analgesia. Pretreatment of antagonists against opioid receptors and  $\alpha$ -adrenoceptors blocks the analgesic effects of EA [8,9,15,17]. However, it is still unclear whether opioid or non-opioid mechanisms play an important role in the immunomodulatory effects of EA treatment.

Yu et al. [30] showed that EA treatment upregulated NK cell activity with increased IFN- $\gamma$  and  $\beta$ -endorphin in non-immunized mice, and naloxone pretreatment attenuated these EA effects. Meanwhile, Kasahara et al. [13] reported that

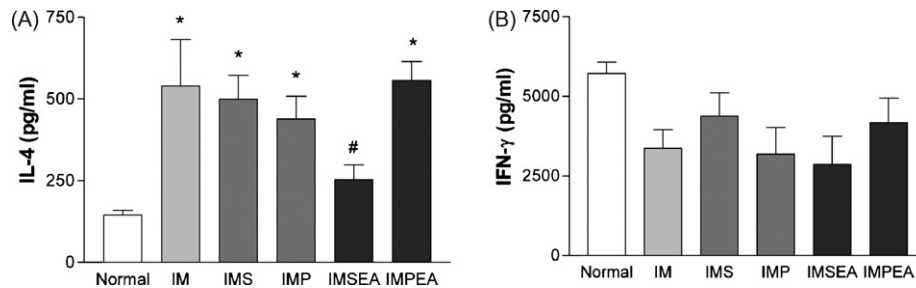


Fig. 2. IL-4 and IFN- $\gamma$  secretion from splenocytes in DNP-KLH immunized mice. The amounts of IL-4 (A) and IFN- $\gamma$  (B) were determined by ELISA. Data are presented as mean  $\pm$  S.E.M. \*  $P < 0.05$ , vs. Normal group; #  $P < 0.05$ , IMSEA vs. IM or IMPEA group by Newman–Keuls multiple comparison test after one-way ANOVA. Normal = no treatments; IM = immunization; IMS = immunization + saline; IMP = immunization + phentolamine; IMSEA = immunization + saline + EA; IMPEA = immunization + phentolamine + EA.

intracisternal pretreatment of naloxone blocked the suppressive effects of EA on 2,4,6-trinitrochlorobenzene (TNCB)-induced delayed type hypersensitivity in mice at 0 and 3 h after TNCB challenge, but not at 24 h after TNCB challenge, indicating that both the central opioid and non-opioid mechanisms are involved in the EA effects. In contrast, Kim et al. [16] showed that naloxone pretreatment did not prevent EA-induced suppression of IL-4 mRNA expression from splenocytes in DNP-KLH immunized mice. In this study, we re-evaluated the effects of naloxone pretreatment on EA-induced suppression of IL-4 production in DNP-KLH immunized mice. In line with the previous study [16], naloxone pretreatment did not significantly block the suppressive effects of EA on IL-4 production (data not shown). Since each study adopted different methods in terms of immunization, EA stimulation and cytokines assay, it may not be surprising that discrepant results were obtained. Despite the contradictory results, it can be suggested that non-opioid mechanisms, such as catecholamines play a major role in mediating the suppressive effects of EA on Th2 cell response under our experimental condition (Th2 dominant condition).

It is widely accepted that EA treatment affects the neuronal activity of the hypothalamus [6,7], which is a center for endocrine-immune modulation. Furthermore, EA treatment can regulate pain behavior and various autonomic nervous system functions, such as blood pressure regulation and gastric motility, through activation of spinal  $\alpha$ -adrenergic receptors or somato-sympathetic reflex in rats [5,15,17,22,25]. In response to activation of the hypothalamus–pituitary–adrenal axis or sympathetic stimulation, noradrenaline is released and influences cellular activities of immunocompetent cells through  $\alpha$ - and/or  $\beta$ -adrenoceptors, resulting in modulation of various immune responses [14,28]. The sympathetic nervous system can inhibit antibody production caused by a protein antigen, keyhole limpet hemocyanin in rats [1]. In this report, we demonstrate that phentolamine pretreatment completely reverse the suppressive effects of ST36 EA treatment on serum levels of antigen-specific and total IgE production in DNP-KLH immunized mice. The data also show that EA-induced suppression of IL-4 levels from splenocytes is completely blocked by phentolamine. These results suggest that  $\alpha$ -adrenergic signaling pathway, rather than opioid system, play an important role in mediating the suppressive

effects of EA on IgE production and Th2 cell response in DNP-KLH immunized mice.

The immunomodulatory effects of catecholamines are mediated by  $\alpha$ - and  $\beta$ -adrenoceptors, which are known to be coupled to various G-proteins that modulate adenylate-cyclase and cAMP levels. Noradrenaline predominantly activates  $\alpha$ - and  $\beta_1$ -adrenoceptors and is a weak stimulator of  $\beta_2$ -adrenoceptors, whereas adrenaline is a strong stimulator of  $\beta$ -adrenoceptors [2,26]. However, the present study could not examine the possible involvement of  $\beta$ -adrenoceptor in EA-induced immunomodulation, since propranolol ( $\beta$ -adrenoceptor antagonist) itself had effects under our experimental condition (unpublished observation). Therefore, we cannot rule out the possibility that  $\beta$ -adrenergic pathway is also involved in the immunomodulatory effects of EA. In addition, other neurotransmitters, such as 5-HT and acetylcholine, which were known to be involved in various kinds of EA effects [8,9], may play a role in the suppressive effects of EA on IgE and IL-4 production in the immunized mice. In ongoing studies, those possibilities are to be investigated.

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