Original Article
Electroacupuncture improved immunocompetence of DCs and CIKs from peripheral blood in SAMP8 mice

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Abstract: Electroacupuncture (EA) can improve immunity in the elderly, but the effects of EA on the immunocompetence of dendritic cells (DCs) and Cytokine-induced killer cells (CIKs) remain unknown. SAMP8 mice were randomly divided into three groups: control group, sham EA group and EA group. Except for the control group, all the mice in the other groups (the sham EA group and the EA group) were pierced with needles at the Guanyuan (CV4) and Zusanli (ST36) acupoints, but only the mice in the EA group were treated with electrical stimulation. DCs and CIKs were isolated from peripheral blood and induced to be cultured. The cellular morphology and immune phenotype of DCs and CIKs were analyzed. The cytotoxicities, proliferative abilities and antitumor activities of CIKs and DC-CIKs were detected. The cellular cytotoxicity and inflammatory cytokines of DC-CIKs were evaluated via co-culture with DC-CIKs and SW480. These results showed that EA treatment significantly promoted the expressions of costimulatory molecules in DCs and the population of CD3+CD8+ and CD3+CD56+ cells in CIKs and DC-CIKs, and markedly increased the proliferative multiple of CIKs and DC-CIKs. EA also enhanced the antitumor ability of DC-CIKs on SW480 and that the effect was effector/target-ratio-dependent. And EA accelerated the production of IL-2, IL-12, IL-17 and TNF-α in DC-CIKs. Furthermore, acupuncture was observed to have similar and partial effects with EA, but these effects were weaker than EA. In conclusion, EA enhanced immunocompetence of DCs and CIKs in the aging mice by enhancing the function of DCs, promoting proliferation and cytotoxicity of CIKs and DC-CIKs, and promoting the release of inflammatory cytokines. These immunomodulatory functions of EA may be advantageous in enhancing the immunocompetence.

Keywords: Immunosenescence, electroacupuncture, dendritic cells, cytokine-induced killer cells, immunity

Introduction
Immunosenescence usually occurs during the progression of normal human aging and is characterized by quantitative and functional alterations in the immune system. And these changes are clearly observed in the adaptive immune system, particularly in T cells [1]. Thus, as the principal effector cells, the status of T lymphocytes is critical for the regulation of immune responses and protection against the autoimmune diseases and the risk of cancers. However, disorders of T lymphocytes negatively affected immune responses in the elderly [2]. In addition, it has been reported that aging promotes an increase in memory T cells and a decrease in naive T cells [3], which might be one of the reasons underlying low immunity.

Thus, in recent years, there has been great interests in the study of slowing down the aging of the body and enhancing the immunity of immune cells in the elderly.

Traditional Chinese Medicine (TCM) is widely used to delay aging by enhancing the function of the kidney and spleen, in which a deficiency of function of these organs is considered to be the main reason for aging [4]. Acupuncture, one type of TCM, is based on the ancient theory of oriental medicine. The clinical manipulation of acupuncture is conducted using thin needles inserted into specific body sites known as acupoints. Electroacupuncture (EA) is a modified technique of acupuncture that requires electrical stimulation. Clinical and experimental studies have shown that sequential EA stimulation
is effective in improving health and the treatment of various diseases [5-7]. Several other studies have shown that EA improves immunity by regulating immune cells and molecules [8-11]. Our previous studies also showed that EA at acupoints of Guanyuan (CV4) and Zusanli (ST36) induced an upregulation of T cell proliferation and IL-2/IL-2R, as well as CD8⁺CD28⁺ T cells, and decreased serum inflammatory factors IL-1β and IL-6 in a rat model of senile yang deficiency [12].

Cytokine-induced killer cells (CIKs), a type of immunocompetent T lymphocyte, are a group of effector CD8⁺ T cells possessing non-MHC-restricted cytolytic activities against tumor cells [13]. Like antigen-presenting cells (APCs), dendritic cells (DCs) play an important role in the activation of T cells via processing and presenting antigens to T cells in the immune system [14]. The expression of costimulatory molecules in the surface of DCs, including CD40, CD80, CD86 and HLA-DR, is necessary for the antigen-presenting capacity and activation of T cells. Cellular immunotherapy, which is based on DCs and CIKs, is a popular and novel method for the treatment of tumors in the clinic [15, 16]. The treatment based on the combination DCs and T cells promoted T cells proliferation and induced the antitumor effects of cytotoxic T lymphocytes (CTLs) [17]. The co-culture of DCs and CIKs elevated the expression levels of surface antigens and the antigen presenting capacity of DCs, promoting the proliferation and cytotoxicity of CIKs and rising the population of T cell subsets, including CD3⁺CD8⁺, CD3⁺CD56⁺ cells, which is positively related to the cytotoxicity ability of CIKs [18, 19]. Both the improvement of immunity by EA and immunocompetence of DC-CIKs have been well known; however, it is still unclear whether EA can improve immunocompetence by modulating the function of immune cells, especially DCs and CIKs at CV4 and ST36 in an aging model.

Thus, this study aimed to investigate the relationship between EA and immunocompetence in the aging model of animal and to examine the potential mechanisms of EA. The senescence accelerated prone mouse P8 (SAMP8) is the only mammalian model used to evaluate accelerated senescence and is widely used as an aging model in the study of geriatrics [20, 21]. These mice are characterized by learning and memory impairment, as well as pathological changes in the cerebral cortex and hippocampus in the central nervous system [22, 23]. SAMP8 mice were used as an aging model to investigate the effects of EA on the immunocompetence of immune cells in the elderly in this study.

Materials and methods

Animals

SAMP8 mice (9 months of age, weighing 25-28 g, male) were obtained from the first affiliated hospital of Tianjin Medical University (Tianjin, China). All mice were maintained under pathogen-free conditions using air conditioners and a 12 h light/dark cycle (20-25°C, 45-55% relative humidity). All mice had free access to food and water during the experiments. All experimental procedures were approved by the Animal Ethics Committee of Hubei University of Chinese Medicine (Wuhan, Hubei) in accordance with the Guide for the Care and Use of Laboratory Animals of the State Scientific and Technological Commission of China.

Experimental design and electroacupuncture treatment

A total of 30 SAMP8 mice were randomly arranged into the three groups: Control, EA and Sham EA (n=10). It was reported that the electroacupuncture at CV4 and ST36, which were commonly used as acupoints to study acupuncture effects on immunity, showed a better regulating effect on senile immunologic function than conventional acupuncture [12, 24]. The CV4 and ST36 points were selected as reported by Gao et al [25]. CV4 point was located at the point under 1/7 of the connecting line between the lower edge of the xiphoid of the sternum and the upper edge of the pubic bone and ST36 point was located at 2 mm lateral to the anterior tubercle of the tibia, near the mouse’s knee joint of the hind limb [26, 27]. A pair of needles (diameter, 0.35 mm; length, 25 mm), were inserted into the CV4 (oblique needling forward lower abdomen, 1.5 mm) and ST36 (perpendicular needling, 3.0 mm) acupoints of mice in the EA and sham EA group. Then the needles in mice in the EA group and not in the sham EA group, were connected to an electrical acupuncture apparatus (HANS LH202H, Huawei, Beijing, China) where the...
parameters of electrical stimulation had been set at 2 Hz frequency and 1 mA intensity. The mice were treated with electrical stimulation for 15 min and each treatment with EA stimulation was performed at 9:00-11:00 am once a day over four consecutive weeks. Mice of the sham EA group underwent the same manipulation as the EA group but without the electrical current during treatment.

**Cell culture and trypan blue staining**

Mononuclear cells were isolated from peripheral blood using lymphocyte separation medium. The adherent cells were collected to culture DCs, and the suspension cells were transferred for inducing CIKs. All cytokines of cell culture were purchased from R&D Systems. The adherent cells were cultured in RPMI-1640 medium containing RhIL-4 (1000 U/ml), RhGM-CSF (1000 U/ml), TNF-α (10 ng/ml) and 10% fetal bovine serum (FBS, Gibco). DCs reached maturity on the 9th day due to mature morphology. The suspension cells were cultured in RPMI-1640 supplemented with IFN-γ (50 ng/ml) and 10% FBS. After 24 h, IL-2 (500 U/ml) and CD3 (50 ng/ml) were added. Half of the culture medium was replaced every 3 days. After being cultured for 9 days, DCs and CIKs were co-cultured at a ratio of 1:3 to generate DC-CIKs. Half of the culture medium was replaced every 2 days. Human colorectal cancer SW480 cells were cultured in complete medium composed of Dulbecco’s Modified Eagles Medium (DMEM) supplemented with 10% FBS, and penicillin and streptomycin (100 U/ml each). All cells were maintained in 5% CO₂ at 37°C. In order to evaluate the cell viabilities of DCs and CIKs, trypan blue staining was performed with 0.4% (w/v) trypan blue solution (Beyotime biotechnology Co. Ltd., Shanghai, China). Cell suspension solution was mixed with 0.4% trypan blue solution at a ratio of 9:1 and then live cells and dead cells were counted. Cell viability equals to number of living cells/total number of cells ×100%.

**Cell proliferative capacity and cytotoxicity detected by CCK-8 assay**

The CCK8 (cell counting kit 8) assay was used to evaluate the cell proliferative capacities and cytotoxic activities of CIKs or DC-CIKs. In order to detect the cell proliferative capacities, single-cell suspension of CIKs and DC-CIKs were placed into 96-well plates after CIKs were cultured for 9 days, 1×10⁴ cells for each well; and cells were continuously cultured for 3, 5, 7 days. Three compound holes were set for each group. At the end of experiments, a 10 µl of CCK8 solution (5 mg/ml in PBS) was then added to each well, and the plates were incubated at 37°C for 4 h. The optical density (OD) was measured at 450 nm. The proliferative multiple was calculated according to the OD values. The proliferative multiple equals to the ratio of the OD value of cells at the end of the experiment to the value of cell at the beginning of the experiment.

During the experiment to measure the cytotoxic activities of DC-CIKs, SW480 cells were used as target cells and DC-CIKs were used as effector cells. The concentration of SW480 cells was adjusted to 1×10⁵ cells/ml, and the concentration of DC-CIKs was adjusted to gradient concentration. The ratios of concentration of DC-CIKs to the concentration of SW480 cells were 5, 10, 20 and 30. In order to ensure the effector/target ratios of 30:1, 20:1, 10:1 or 5:1, each 100 µl of single-cell suspension of SW480 cells and DC-CIKs were placed into 96-well plates as DC-CIK-SW480 group. Additionally 100 µl of single-cell suspension of SW480 cells and medium were placed as SW480 group; and 100 µl of single-cell suspension of different concentration of DC-CIKs and medium were placed as DC-CIKs group. Each group was set three compound holes. Then mixed cells were incubated for 24 h. At the end of the experiment, a 10 µl of CCK8 solution (5 mg/ml in PBS) was then added to each well, and the plates were incubated at 37°C for 4 h. The optical density (OD) was measured at 450 nm. The killing rate of DC-CIKs was calculated according to the OD values. The formula used as following: killing rate=[1-(OD<sub>DC-CIK-SW480</sub>−OD<sub>DC-CIK</sub>)/OD<sub>SW480</sub>]×100%.

**The immune phenotype of DCs, CIKs and DC-CIKs detected by flow cytometry**

A flow cytometric analysis was performed to detect immune phenotype of DCs, CIKs and DC-CIKs. DCs and DC-CIKs were induced to be cultured for 9 days. Then DCs and CIKs were co-cultured to generate DC-CIKs at a ratio of 1:3 for 3 days. At the end of the experiment, cells were collected and washed twice with ice-cold
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Results

DC and CIK cells were isolated and cultured

The adhered cells and the suspension cells were separated 2 h after the culture of isolated peripheral blood monocytes, aiming to induce the differentiation and formation of DC precursors and CIK by different cytokines. The mor-

Figure 1. Cell morphology and cell viability of DCs and CIKs. Cell morphology of DCs (A) and CIKs (B) on the 9th day after induction by different cytokines was observed and photoed using an inverted microscope with a magnification of 200 (upper) or 400 (under) times. The cell viability of DCs and CIKs (C) was measured by trypan blue staining. The data was expressed as the mean ± SD for three independent experiments.

phosphate-buffered saline (PBS), adjusted to a cell density of 1×10^6 cells/ml, and then incubated with FITC anti-mouse CD40 (cat NO. 561845) and PE anti-mouse CD80 (cat NO. 553769) antibodies or FITC anti-mouse CD86 (cat NO. 561962) and PE HL-ADR antibody (cat NO. sc-33718 PE) for 20 min. All antibodies were purchased from Becton Dickinson and Company (BD Pharmingen) or Santa Cruz Biotechnology, Inc. After fixation with 4% paraformaldehyde, the expression of markers was evaluated by flow cytometry.

The levels of inflammatory cytokines detected by ELISA

The DC-CIKs and SW480 cells were co-cultured at a ratio of 30:1 in 24-well plates for 24 h, 5×10^5 cells of SW480 in each well. Then interleukin-2 (IL-2), IL-12, IL-17 and tumor necrosis factor-α (TNF-α) production were quantified with mouse IL-2, IL-12, IL-17 and TNF-α ELISA kit (Bio-Swamp), following the manufacturer's recommended assay procedures. The optical densities (OD) were measured at 450 nm using a microplate reader. The cytokine concentrations were determined using a standard curve established with the appropriate recombinant cytokine, and are expressed in pg/ml.

Statistical analysis

All data are presented as the means ± SD. The SPSS 19.0 statistical software was used for data analysis. Differences between multi-groups were determined using one-way ANOVA and the mean values among groups were evaluated using Student's t-test. Results with P < 0.05, P < 0.01 and P < 0.001 were considered significant.
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The morphology of the adherent cells was obviously dendritic on the 5th day. The displayed a large number of dendrites and pseudopodia, characteristics of matured DC cells, on the 9th day after induction by RhIL-4, RhGM-CSF and TNF-α (Figure 1A). The suspension cells grew gradually in form of aggregate clusters at the beginning of the 3rd day after induction by IFN-γ, IL-2 and CD3 antibody, and they displayed a small circle and other irregular shapes which are the characteristics of CIK cells after being induced by cytokines on the 9th day after induction (Figure 1B). The results of trypan blue staining showed that the percentage of live cells of both DCs and CIKs on the 9th day were above 95% (Figure 1C), indicating cell viabilities of both isolated DCs and CIKs were strong enough to progress the following experiments.

EA promoted the immune phenotype of DCs, CIKs and DC-CIKs

To investigate the effect of EA on the function of DCs, CIKs and DC-CIKs, the expressions of some cell surface specific molecules were detected by flow cytometry. Results indicated that the percentages of positive DCs which expressed costimulatory molecules of DCs, such as CD40, CD80, CD86 and HLA-DR, significantly increased in the EA group on the 9th day compared to both the control group and the sham EA group (all of $P < 0.05$) (Figure 2A). The percentages of CD3+CD8+ and CD3+CD56+ cells in the EA group were also significantly higher than that in the control group and sham EA group on the 9th day ($P < 0.05$) (Figure 2B). Interestingly, compared to the control group, the percentages of simple-positive DCs, such as CD80 and CD86, and double-positive CIKs, such as CD3+CD56+ in the sham EA group obviously rose ($P < 0.05$). This data indicated that EA could enhance the ability of antigen presentation of DCs and killing ability of CIKs through promoting the expression of surface specific molecules of DCs and the formation of CD3+CD8+ and CD3+CD56+ cells of CIKs. Undeniably, acupuncture without electrical stimulation also exerted these effects to a certain extent.

In addition, when DCs and CIKs were co-cultured at a ratio of 1:3 for 3 days, as shown in Figure 2C and 2D, the percentages of CD3+CD8+ and CD3+CD56+ cells in three groups were higher in DC-CIKs than those in CIKs ($P < 0.05$). Compared to the control group and the sham EA group, the percentages of these two double-positive CIKs in DC-CIKs in the EA group were increased ($P < 0.05$). And there were also significant differences for the percentages of these cells between the control group and sham EA group. The killing ability of CIKs and DC-CIKs was dependent on the immune phenotypes of CD3+CD8+ and CD3+CD56+. Thus, this data verified that the killing ability of DC-CIKs was stronger than CIKs and that EA could strengthen their killing ability by increasing the formation of those two kinds of immune phenotype cells.

EA enhanced the cell proliferative capacities of CIKs and DC-CIKs

In order to evaluate the effects of EA on the cell proliferative capacities of CIKs and co-cultured DC-CIKs, CCK-8 assays were performed when these cells were seeded into 96-well plates and...
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continuously cultured in vitro for 3, 5 and 7 days. As shown in Figure 3A and 3B, the OD values of CIKs and DC-CIKs in the EA group were significantly higher than those in the control group and sham EA group ($P < 0.05$) for 3, 5 and 7 days. And proliferative curve showed that cell proliferative multiples of CIKs and DC-CIKs in the EA group were remarkably higher than those in the control group (Figure 3C, 3D). These data suggested that EA could enhance the cell proliferative capacities of CIKs and DC-CIKs. The proliferative multiple of DC-CIKs was also higher than that of CIKs in the EA group ($P < 0.05$) (Figure 3E), which indicated that the proliferative ability of DC-CIKs was stronger than that of CIKs.

**EA enhanced antitumor ability of DC-CIKs on human colorectal cancer cells**

Then cytotoxicity assay was performed to evaluate the effect of EA on the antitumor ability of DC-CIKs against human colorectal cancer SW480 cells. When DC-CIKs were co-cultured with SW480 cells in any condition of different effector/target ratios for 24 h, compared with both the control group and sham EA group, the OD values in the EA group significantly decreased ($P < 0.05$), whereas OD values didn’t obviously change between the sham EA group and control group until effector/target ratios was up to 20:1 (Figure 4A-D). As shown in Figure 4E, the killing rates of DC-CIKs in three groups continuously increased following the increasing of effector/target ratios, indicating that the killing effect of DC-CIKs was effector/target-ratio-dependent. The killing rates of DC-CIKs in the EA group, however, were significantly higher than those in both the control group and sham EA group ($P < 0.05$), while the killing rates in the sham EA group were higher than those in the control group, suggesting that acupuncture without electricity also enhanced the antitumor ability of DC-CIKs to some extent. These data illustrated that EA markedly enhanced the antitumor ability of DC-CIKs on human colorectal cancer cells and that the effect was effector/target-ratio-dependent.

**EA accelerated the release of inflammatory cytokines by DC-CIK cells**

According to the results obtained from cytotoxicity assay of DC-CIKs in different effector/target ratios, the killing rate of DC-CIKs was maximized when the effector/target ratio was 30:1. Thus, we continued to design the experiments to evaluate the inflammatory response in SW480 cells when co-cultured with DC-CIKs at an effector/target ratio of 30:1. As shown in Figure 5, the levels of IL-2, IL-12, IL-17 and TNF-α markedly increased in DC-CIK-SW480 cells compared to DC-CIKs in the three groups ($P < 0.05$), indicating that the release of inflamm-
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Mandatory cytokines were induced after the co-culture of DC-CIKs and SW480. Compared to the control group, the levels of IL-2, IL-12, IL-17 and TNF-α, released by DC-CIKs or both DC-CIK-SW480 cells, predominantly upgraded in the EA group. The data demonstrated that EA accelerated the release of inflammatory cytokines by DC-CIKs against SW480. Additionally, it was noted that significant changes also existed for the levels of those inflammatory cytokines induced by DC-CIKs or DC-CIK-SW480 cells between sham EA group and control group (P < 0.05), indicating acupuncture without electricity also exerted the function to promote the release of inflammatory cytokines. Even so, there were also significant changes for the levels of those cytokines between the EA group and sham EA group. Therefore it could be considered that the acceleration effect of EA on the release of inflammatory cytokines was superior to the effect of acupuncture without electricity.

Discussion

Human aging over time is accompanied by immunosenescence mediated by the immune system. During immunosenescence the decrease and dysfunction in immune cell subsets obviously affect the effects on the inflammatory...
conditions of human bodies, autoimmune diseases, the risk of cancers and response to medical treatment [28, 29]. Therefore, Immune cells, which refer to a series of specific cells participating in the immune response, play a critical role in defense of the immune system. Natural kill (NK) cells, T helper (Th) 1 cells, Th2 cells, T lymphocytes and other cells are important immune cells. In addition, dendritic cells (DCs) are known to be the most potent APC, while cytokine-induced killer cells (CIKs) possess antitumor activity against a variety of tumors [13, 14]. Due to low immunity caused by quantitative and functional alterations of immune cells with age, the elderly are prone to some diseases, e.g., cancers and susceptibility to infection [28, 29]. Thus it is a novel perspective to reduce the prevalence of diseases, particularly tumors, in the elderly by enhancing the immunity. It is unclear whether EA can improve immunocompetence and kill tumor cells by regulating the function of immune cells in an aging model. Therefore, this study explored the effects of EA on immunocompetence in the aging model, and further examined the potential antitumor effects of EA through cell experiments.

Acupuncture is one therapeutic approach used to treat multiple illnesses, such as pain relief, and is accepted worldwide, not only in the East [30]. The effect of acupuncture or EA on different immune cells was different under different conditions. Several studies have shown that EA increased the activities of NK cells in the spleen and there were no effects on the population of NK cells in the spleen in normal mice and rats [31, 32]. EA can effectively alleviate tissue damage and reduce inflammatory reaction in ulcerative colitis by reducing Th17 cells and increasing Treg cells to achieve dynamic balance of Treg/Th17 cells [33]. EA also effectively upregulates peripheral blood CD3+ , CD4+ and NK cells levels in stress-training rats [11]. Our results showed that EA and acupuncture promoted maturity of DCs and enhanced the cytotoxicity of CIKs and DC-CIKs by increasing the expression of whole or partial costimulatory molecules in the surface of DCs, the population of CD3+CD8+ and CD3+CD56+ cells in CIKs and DC-CIKs in SAMP8 mice. But the significant differences between the EA group and sham EA group suggested that the regulatory effect of EA on the functions of DCs, CIKs and DC-CIKs were superior to acupuncture without electrical stimulation. Interestingly, compared to the control group, EA significantly increased the proliferative multiple of CIKs and DC-CIKs, and there was no significant increase in the proliferative multiple of the two types of cells in sham EA group. Our research confirmed that EA can improve immune function of the aging organism, hence resisting various diseases. Tang et al. also reported that EA elevated the population of T cell subsets (CD3+, CD4+ and CD4+CD8+) and IgG in senile yang deficiency [34]. After moxibustion treatment, the aging scores of 223 cases were all substantially reduced, along with a strengthened cellular immune function and an increase of total T-lymphocyte count [35]. These studies showed that EA or acupuncture was effective in the regulation of the population and activities of immune cells and that the effect of EA was better.

Additionally, EA also improves immune function by balancing T helper 1 (Th1) and Th2 cytokines in splenic T cells of traumatized rats [5]. In recent studies, DC-CIKs, which are a result of co-cultured DCs and CIKs, exerted efficient antitumor activity [36]. Thus the effects of EA on the antitumor activities of DC-CIKs were evaluated by detecting the cell viabilities of DC-CIK-SW480 cells and calculating killing rates of DC-CIKs using human colorectal cancer SW480 cells as target cells. These results showed that EA significantly decreased the cell viability of DC-CIK-SW480 cells; and that EA increased the killing rates of DC-CIKs following the increase of effector/target ratio. Similar tendency was observed in the sham EA group. Thus, EA enhanced the antitumor activity of DC-CIKs from SAMP8 mice, while acupuncture exerted a relative weaker function than EA. A report showed that acupuncture at ST36, Hegu (LI4) and Sanyinjiao (SP6) increased immune function and inhibited tumor growth in Walker-256 liver cancer, gastric cancer and hypodermic tumor rats [6]. Combined with our studies, EA at CV4 and ST36 has been shown to activate immunity and increase the cytotoxic activity of DC-CIK on human colorectal cancer cells. DC-CIK adoptive immunotherapy is developing rapidly as a new antitumor therapy [37]. Lin et al found that DC-CIK adoptive immunotherapy reduced the severity of adverse effects and prolonged the survival of colorectal cancer.
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patients [38]. This study showed that EA can enhance the antitumor activities of DC-CIK against SW480 cells in the aging model. Therefore, our findings provide new basic research data for the antitumor effects EA, and supported EA as an adjunct to DC-CIK adoptive immunotherapy. However, the antitumor effects and mechanism of EA need further study.

Immunosenescence, is accompanied by chronic inflammatory conditions, which is also known as inflammation, featured by an increase in inflammatory cytokines (e.g., interleukin (IL)-6, IL-1β and tumor necrosis factor (TNF)-α [39]. Th1 cells and Th17 cells are key immune cells to induce the inflammatory responses by releasing specific cytokines. IL-2 and IL-12 are representative cytokines in Th1 cells produced by activated T cells, while cytokine IL-17 is a typical cytokine in Th17 cells [40].

In our study, the levels of IL-2, IL-12, IL-17 and TNF-α released by DC-CIKs or DC-CIK-SW480 in the EA group significantly increased compared to the control group, and the levels were higher in DC-CIK-SW480 cells than that in DC-CIKs in the EA group. These findings illustrated that the inflammatory response was strongly induced when the SW480 was co-cultured with DC-CIKs, whereas EA stimulation enhanced this effect. TNF-α induced by EA treatment played an essential role in the cytotoxic activity, which is consistent with the results of killing activity of DC-CIKs. In our study, EA induced IL-2, IL-12 and IL-17 production, which were regulated by different T cells. Thus, we speculated that EA regulated T cell differentiation to modulate immune function. However, these findings warrant further study.

In conclusion, this present study demonstrated that EA stimulation at CV4 and ST36 in SAMP8 mice significantly promoted the expressions of the surface costimulatory molecules in DCs, and promoted proliferation and cytotoxicity of DC-CIKs against SW480. Simultaneously the cytokines produced by DC-CIKs and DC-CIK-SW480 were markedly increased in response to EA stimulation. Additionally, acupuncture had similar effects with EA, but these effects are weaker than EA. This study highlighted that EA could enhance immunocompetence of DCs and CIKs by enhancing the function of DCs, promoting proliferation and cytotoxicity of CIKs and DC-CIKs, and promoting the release of inflammatory cytokines. Our findings provided new insights into the mechanism of improving immunity by EA administration and supported the use of EA as a preventive health measure in the elderly.

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Disclosure of conflict of interest

None.

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