

Tai Chi Chuan Increases Circulating Myeloid Dendritic Cells

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Dendritic cells, the most potent antigen-presenting cells linking innate and adoptive immunity, are thought to be important targets of immune modulators such as exercise. We examined the effect of Tai Chi Chuan (TCC) on dendritic cells. TCC practitioners were further divided to high-level practitioners (TCC-H) and low-level practitioners (TCC-L). The quantities of myeloid and plasmacytoid dendritic cells were estimated by flow cytometry. We examined parameters including age, body weight, body length, body fat, and serum albumin level, in the controls, TCC-H and TCC-L, which did not differ significantly. The mean peak $\dot{V}O_2$ (volume of O_2 utilization) of the TCC-H group was greater than that of the sedentary control group. White blood cell (WBC) count in the entire TCC group was greater than that of the controls. The quantity of myeloid dendritic cells was significantly greater in the TCC group, whereas the quantity of plasmacytoid dendritic cells was similar for both groups. Among the TCC subgroups, the quantity of myeloid dendritic cells, but not plasmacytoid dendritic cells, in the TCC-H group was greater than that of TCC-L practitioners. TCC could increase the number of circulating myeloid dendritic cells, but not plasmacytoid dendritic cells, in a performance level-dependent manner.

Keywords Tai Chi Chuan, Dendritic cell, Myeloid, Plasmacytoid.

Jasson Chiang and Yu-Yawn Chen contributed equally to this work.

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INTRODUCTION

Tai Chi Chuan (TCC), one of the ancient traditional Chinese martial arts, has been widely accepted and practiced since the 17th century, especially in Asia. TCC originated from the concept of promoting homeostasis of the body, physical fitness, and the capacity for self-defense (China Sports, 1983). Classical TCC consists of many complicated sets of postures that take approximately 30 minutes to complete. Previous studies showed that TCC improved cardio-respiratory function (Gong et al., 1981; Lai et al., 1995; Lan et al., 2001), muscular strength (Wolfson et al., 1996), humoral and cellular immunity (Sun et al., 1989, 1990), metabolic response (Zhuo et al., 1984), and mental control (Wang et al., 2008). The exercise intensity of TCC is defined as moderate (Lan et al., 2001). Although TCC modulates humoral and cellular immunity, the target effector cells specific to TCC remain undetermined. It has been reported that stress testing exercise before scheduled surgery increased the number of circulating dendritic cells (Ho et al., 2001). However, the investigation for correlation of TCC and dendritic cells is lacking.

Dendritic cells are specialized leukocytes that present antigens to naive and memory T cells and are characterized as the most potent antigen-presenting cells, linking innate and adoptive immunity. Immature dendritic cells possess the ability to capture antigen, migrate into lymphoid organs, and express high levels of immunostimulatory molecules, such as major histocompatibility complex (MHC) class II, B7-1, and B7-2 (Albert et al., 1998; Banchereau and Steinman, 1998; Hart, 1997). Upon exposure to various microbial and inflammatory products (e.g., lipopolysaccharide, TNF- α), immature dendritic cells mature and migrate into lymphoid tissues to present antigens to and interact with T cells (Cella et al., 1996; Jonuleit et al., 1997; Kato et al., 1997; Labeur et al., 1999).

In the circulation, there are various subsets of dendritic cells that perform different functions. Among these, myeloid dendritic cells (MDC) and plasmacytoid dendritic cells (PDC) are two major subpopulations. MDC express surface markers such as CD11c and exert immune responses against bacteria, tumors, and some viruses (Rissoan et al., 1999; Robinson et al., 1999). PDC express CD123, α subunit of IL-3 receptor, and produce type I interferon upon virus infection (Fanning et al., 2006). The quantities of circulating MDC and PDC have been used to examine the steady state of dendritic cell-related immunity against human pathogens (Hideo et al., 2005).

Given that dendritic cells are the most potent antigen presenting cells among WBCs and are characterized as a link between innate and adaptive immunity, we next examined the quantity of dendritic cells, including myeloid and plasmacytoid dendritic cells, in the circulation. We investigated the effect of TCC on the quantity of major dendritic cell subsets in the circulation. Healthy subjects living a sedentary lifestyle served as controls. Two

performance levels of TCC practitioners were recruited to examine any performance level-dependent effect.

MATERIALS AND METHODS

Subjects

Forty-three males from TCC clubs located in Northern and central areas of Taiwan and 20 sedentary males in similar area were recruited. TCC practice was performed regularly in the early morning. During experimental period, all of subjects practicing the Yang style of TCC regularly, 5 times per week for at least 60 minutes each time at 7 am to 9 am. Each TCC session included a 20-minute warm-up (low back and hamstring stretches, gentle calisthenics, and balance training), followed by 24 minutes of TCC practice and a 20-minute cool-down. Each TCC set included 108 postures, with some repeated sequences. During the TCC practice, subjects were led by a TCC instructor and imitated the motions and postures at the same speed. Subjects performed each posture according to a prerecorded rhythm tape to ensure the same time course. Age- and gender-matched subjects with sedentary lifestyles were enrolled as controls.

As shown in Table 1, TCC practitioners and sedentary controls with comparable basic characteristics were enrolled. The questionnaires, about the life style, diseases history and habitual exercise activities, were completed before enrolled. With the permission, the registered forms of members in Tai Chi Chuan club were collected. The performance levels of practitioners were qualified by expertise coaches from a TCC association at the beginning of attendance to exclude senior practitioners whose immunity may already be affected by TCC. TCC practitioners were divided into two subgroups: high level (TCC-H), those who had performed TCC for more than 5 years, and low level (TCC-L), those who had performed TCC for 2–5 years.

Table 1: Characteristics and physiological parameters of subjects.

	TCC-H (n = 21)	TCC-L (n = 22)	SC (n = 20)
Age (years)	54.2 ± 8.4	53.8 ± 7.9	53.1 ± 7.1
Height (cm)	159.7 ± 11.3	162.0 ± 11.8	162.8 ± 9.8
Weight (kg)	58.9 ± 9.5	57.4 ± 10.3	63.1 ± 9.7
The years of club joined (years)	6.3 ± 1.1	2.8 ± 2.1	—
Body Fat (%)	26.8 ± 7.3	25.3 ± 8.6	29.1 ± 7.6
$\dot{V}O_{2peak}$ (ml/(kg × min))	33.6 ± 7.7*	30.4 ± 8.8	26.3 ± 8.6
Albumin (g/dl)(pre-experiment)	4.4 ± 0.6	4.3 ± 0.5	4.2 ± 0.8

TCC-H, high-performance practitioner of Tai Chi Chuan. TCC-L, low-performance practitioner of Tai Chi Chuan. SC, sedentary control. $\dot{V}O_{2peak}$ (ml/(kg × min)), peak volume of O_2 . One-way analysis of variance was used for comparison between various groups. * $p < 0.05$ means significant difference in comparison to the sedentary control group.

TCC practitioner qualifications were validated by assessments of a TCC coaching committee before subjected to statistical analysis. According to the registered forms for Tai Chi Chuan club, the years of club joined in TCC-H and TCC-L practitioner were 6.3 ± 1.1 and 2.8 ± 2.1 years, respectively (Tab. 1). Exclusion criteria for all subjects included infectious diseases, diabetes mellitus, and major cardiovascular, pulmonary, and musculoskeletal disorders. The study was approved by the Institutional Review Board of Mackay Memorial Hospital, and written informed consents were obtained from participants. The Clinical Trials.gov Identifier for this study is NCT00322959.

Measurement of Fitness Parameters during TCC

The fitness parameters of energy and metabolism were measured using a CORTEX Biophysik MetaMax 3B portable CPX system (Cortex, Leipzig, Germany). The parameters assessed included heart rate, minute ventilation, peak volume of oxygen consumption ($\dot{V}O_2$), relative oxygen uptake, relative carbon dioxide output, respiratory exchange ratio (RER), respiratory rate and metabolic equivalent (MET).

Two weeks before the experimental period, the tests of maximal oxygen uptake ($\dot{V}O_{2peak}$) were completed by using an initially incremental maximal exercise test. In short, peak oxygen uptake was evaluated while the subjects were exercising on electronically braked cycle ergometer (Lode Excalibur, Quinton Instruments, Seattle, WA, USA). After warming up by loading set at 25 W for 2 min, they began to increase loading set at 15 W every 2 min until exhaustion. A polar pacer heart rate meter was used to monitor and record the heart rate.

Blood Collection and Analysis

The subjects had no clinical illnesses or surgical treatment, which were examined by physician, during the 4 weeks prior to blood collection and the experimental period. All subjects were well informed to refrain from ingesting any caffeinated beverages, drugs, drinks alcoholic, smoking, and vegetarian diet for one month prior to the study, as well as during the experimental period. All subjects did not perform any exercise, including TCC practice, on the days received blood sampling. Each fasting blood sample (50 ml) was taken between 8 am and 9 am, after 12-hour fasting, at morning after the subjects had rested quietly for 30 min." The total white blood cell (WBC) count was calculated on a hemacytometer. Lactate responses before and immediately after TCC practice were measured; 20 ml of plasma was analyzed by using the YSI2300 STAT glucose/lactate analyzer (Yellow Springs Instrument Co., Yellow Springs, OH) to determine the blood glucose and lactate concentrations. Serum albumin concentrations were determined using the bromocresol green method (Pinnel and Northain, 1978).

Circulating Myeloid Dendritic and Plasmacytoid Cells

Peripheral blood mononuclear cells were isolated by centrifugation on a density gradient (Ficoll-Hypaque, 1.077 gm/ml, Pharmacia Fine Chemicals, Inc, Uppsala, Sweden). Multicolor immunolabelling was performed using fluorescein-conjugated antibodies. Before surface marker analysis, the cell debris was excluded by forward and side scattering. To assess the distribution of dendritic cell subpopulations, non-dendritic cells were first excluded using lineage markers (CD3, CD14, CD16, CD19, CD20, and CD56). Myeloid dendritic cells were characterized as having HLA-DR⁺CD11c⁺ lineage⁻ and plasmacytoid cells were characterized as having HLA-DR⁺CD123⁺ lineage⁻ (Fig. 1).

The antibodies conjugated with Phycoerythrin (PE) (Serotec, Oxford, UK) used for CD11c and CD123 were anti-CD11c-PE and anti-CD123-PE (Serotec). For example, for analysis of CD11c expression, cells were incubated with saturating concentrations of primary mouse anti-human CD11c monoclonal antibodies followed by F(ab')₂ goat anti-mouse IgG-PE at 4°C for 30 min. Isotype controls were purchased from Serotec. After washing twice with PBS, 10⁶ cells were placed in the FACS Caliber Flow Cytometer (BD Biosciences, San Jose, CA). Data were collected and analyzed using CellQuest software (BD Biosciences).

Statistical Analysis

The Statistical Package for the Social Sciences, version 10.0 (SPSS Inc, Chicago, IL) was used to analyze all data. Student *t* test and paired *t* test were used for comparison between various groups as indicated. Data are expressed as means ± standard errors of the means.

RESULTS

Characteristics and Parameters of Fitness and Metabolism

The basic characteristics of the two groups of subjects are summarized in Table 1. No significant difference between controls and TCC group were noted in terms of age, body weight, body length, body fat, or serum albumin level. Mean peak $\dot{V}O_2$ in the TCC-H group was greater than in the sedentary control group (33.6 ± 7.7 ml/min/kg vs. 26.3 ± 8.6 ml/min/kg, $p < 0.05$). The mean $\dot{V}O_2$ peak did not differ between the TCC-H and TCC-L group or between the TCC-L group and sedentary control group.

Analysis of TCC Practitioner Circulating WBCs and Myeloid Dendritic Cells

Initially, we analyzed the WBC counts of all subjects. All TCC practitioners (including TCC-H and TCC-L) had more circulating WBCs than the

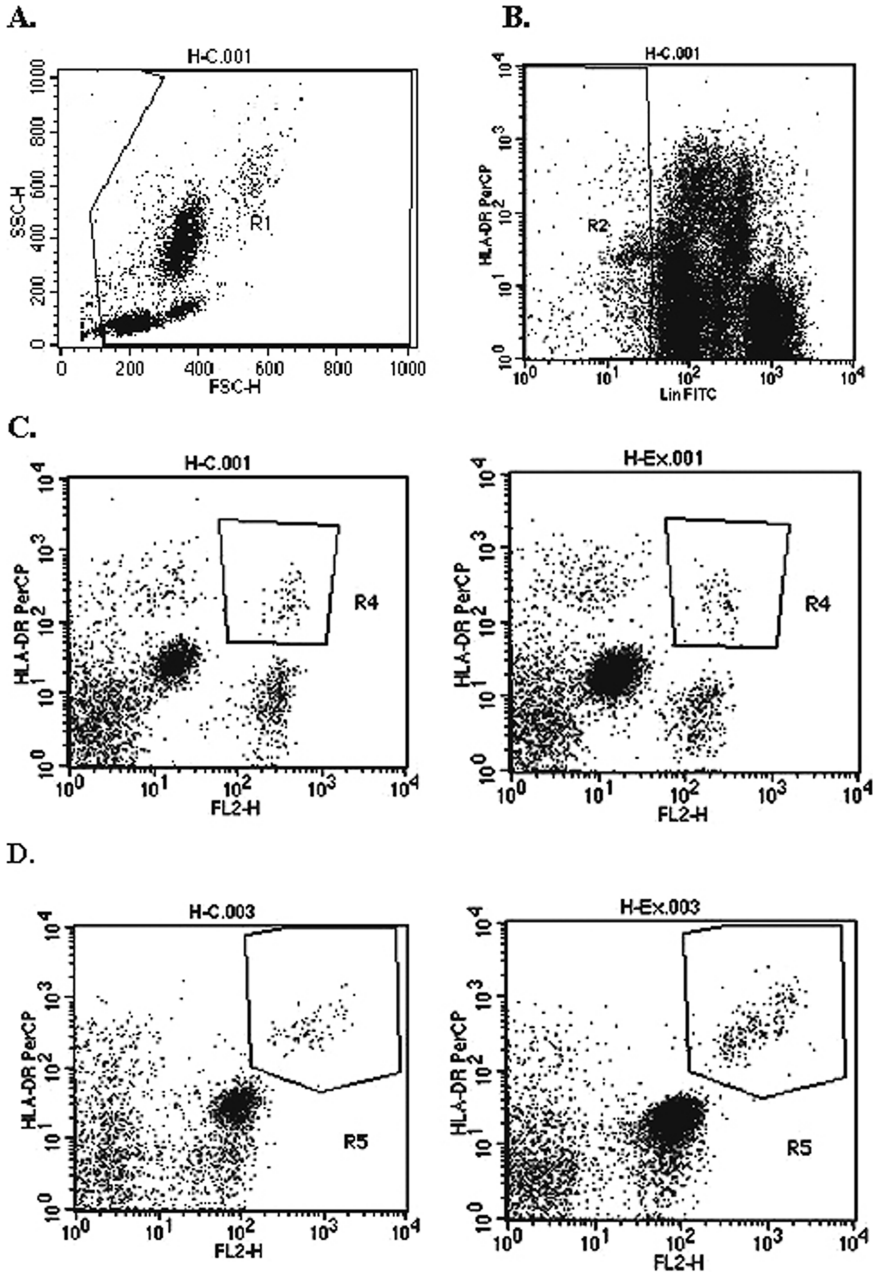


Figure 1: Flow cytometric analysis of myeloid and plasmacytoid dendritic cells base on detection of cell surface molecule expression. (A) Representative dot plot of forward and side scattering. (B) Representative plot for excluding lineage marker (CD3, CD14, CD16, CD19, CD20, and CD56) positive cells. (C) Comparison of gated distribution of HLA-DR⁺CD123⁺ cells (plasmacytoid dendritic cells) from a control (left plot) and a TCC practitioner (right plot). (D) Comparison of gated distribution of HLA-DR⁺CD11c⁺ cells (myeloid dendritic cells) from a control (left plot) and a TCC practitioner (right plot).

Table 2: Counts of circulating white blood cells and dendritic cells among the Tai Chi Chuan practitioners and sedentary controls.

	TCC-H (n = 21)	TCC-L (n = 22)	SC (n = 20)
WBC count (per μL)	9102 \pm 1042	8954 \pm 986	7647 \pm 963
Dendritic cell subgroup (per μL)			
CD11c ⁺ cells	10.35 \pm 1.66*#	7.52 \pm 1.83*	6.23 \pm 1.68
CD123 ⁺ cells	4.63 \pm 1.37	4.31 \pm 1.45	3.92 \pm 1.32

TCC-H, high-performance practitioner of Tai Chi Chuan. TCC-L, low-performance practitioner of Tai Chi Chuan. SC, sedentary control. One-way analysis of variance was used for comparison between various groups. * $p < 0.05$ in comparison to the sedentary control group. # $p < 0.05$ in comparison to the TCC-L group.

sedentary controls (9026 \pm 1004 vs. 7647 \pm 963 / μL , $p < 0.05$). Intriguingly, we found the quantity of myeloid dendritic cells was significantly greater in total TCC practitioner groups than in the control group (8.91 \pm 2.24 cell/ μL vs. 6.23 \pm 1.68 cell/ μL , $p < 0.05$), whereas the quantities of plasmacytoid dendritic cells were comparable (4.47 \pm 1.63 cell/ μL vs. 3.92 \pm 1.32 cell/ μL , $p > 0.05$) (Table 2).

To verify whether the differences in dendritic cells were relative to the various performance level of the TCC practitioner, the PBMNC (peripheral blood mononuclear cells) in TCC-H and TCC-L groups before and after TCC were examined by flow cytometry. Respiratory exchange ratios (RERs) obtained before and after TCC did not differ. As demonstrated in Table 3 the mean % VO_2 peak during TCC exercise increased from 21.76 \pm 7.51 % to 41.83 \pm 12.81 % in the TCC-H group ($p < 0.001$) and, similarly, from 23.52 \pm 12.14 to 46.35 \pm 13.41 % in TCC-L group ($p < 0.001$). The % HRmax (the percentage of maximum heart rate) after TCC exercise moderately increased [63.21 \pm 7.93 % in the TCC-H group ($p < 0.001$) and 66.43 \pm 8.12 % in the TCC-L group ($p < 0.001$)]. The serum lactate concentrations did not differ in TCC-H group ($p = 0.354$) after TCC but differ in TCC-L ($p = 0.015$) group (Table 3). MET increased in both the TCC-H and TCC-L groups after TCC exercise ($p < 0.001$) (Table 3).

The fitness parameters indicated that TCC performance intensity is moderate and TCC should be considered an aerobic exercise. At the point along the physiological exercise gradient where TCC is, we found the quantity of myeloid dendritic cells, but not plasmacytoid dendritic cells, in the TCC-H group was greater than in the TCC-L group (Table 2). This performance level-dependent increment suggests that myeloid dendritic cells, among WBC, might be one of the effector cells modulated by TCC exercise.

DISCUSSION

Our results indicate that TCC could increase the number of total white blood cells and circulating myeloid dendritic cells, but not plasmacytoid dendritic

Table 3: Changes in physiological parameters before and after TCC.

	Before TCC exercise		After TCC exercise		<i>p</i> values between Before and After	
	TCC-H (n = 21)	TCC-L (n = 22)	TCC-H (n = 21)	TCC-L (n = 22)	TCC-H (n = 21)	TCC-L (n = 22)
Blood lactate (mmol/L)	1.84 ± 0.55	1.93 ± 0.44	1.68 ± 0.32*	1.62 ± 0.51*	0.345	0.015
% HRmax	47.11 ± 7.36	51.21 ± 8.32	63.21 ± 7.93	66.43 ± 8.12	<0.01	<0.01
% $\dot{V}O_2$ peak	21.76 ± 7.51	23.52 ± 12.14	41.83 ± 12.81	46.35 ± 13.41	<0.01	<0.01
MET	1.5 ± 0.6	1.4 ± 0.7	4.3 ± 0.8	3.7 ± 0.8	<0.01	<0.01
RER	0.79 ± 0.09	0.78 ± 0.09	0.76 ± 0.10*	0.78 ± 0.07*	0.390	1.000

TCC-H, high-performance practitioner of Tai Chi Chuan. TCC-L, low-performance practitioner of Tai Chi Chuan. HR, heart rate; MET, metabolic equivalent; RER, respiratory exchange ratio. Unpaired student *t* test was used for comparison between these two groups **p* < 0.01 means significant difference.

cells, in a performance level-dependent manner. TCC is considered an aerobic exercise of moderate intensity that can modulate immunity. This study assessed possible target effector cells of TCC in the circulation. This suggests that myeloid dendritic cells could be one of the target cell lineages affected by TCC exercise.

A similar approach to assess the target effector cells of the other types of exercise has been reported. For example, stress testing exercise prior to scheduled surgery resulted in increases in dendritic cells number and subsets (Ho et al., 2001). Another example is that periodized exercise training with active recovery promoted dendritic cell differentiation, maturation, and antigen presentation ability in rats (Liao et al., 2006).

The assessment of TCC exercise intensity was validated by measurement of peak % $\dot{V}O_2$ and other physiologic parameters. Mean $\dot{V}O_2$ peak was greater in the TCC-H group than in the sedentary control group. The % $\dot{V}O_2$ peak, measured after TCC exercise, was not more than about 50% in both the TCC-H and TCC-L groups, which also indicating low to moderate-intensity exercise. After TCC exercise, the measured %HRmax was 66.43 %, which also showed that TCC performed in the study was moderately intense exercise. Similar results for %HRmax of TCC practitioners were also found in TCC exercise among younger adults (Wang et al., 2004), middle-aged adults (Chen et al., 2008), and among elderly adults (Lan et al., 1996).

Changes of RER, and serum lactate did not differ significantly before and after TCC practice, which suggests that TCC as performed in our study was aerobic exercise and did not affect immunity through induction of anabolic stress. RER obtained during exercise was close to 0.8, which was indicative of enhanced fat oxidation. Moreover, the similar plasma albumin concentration in all groups indicated no participant was malnourished during the study.

Although the immunobiologic effect of TCC has been extensively explored, the target cell ontogeny has not been elucidated, especially those cells linking innate and adaptive immunity such as the dendritic cell lineage. To our knowledge, no investigation assessed the effect of TCC on dendritic cell immunobiology. Our study showed that TCC appeared to increase the number of WBCs and myeloid dendritic cells, but not plasmacytoid dendritic cells. This raises two interesting issues of human immunity. First, the increase in total WBC in TCC practitioners may not mean a general augmentation of immunity. Instead, only ontogenesis of highly specific myeloid dendritic cells increased.

Thus, TCC could have an impact on a specific immunomodulation pathway. The myeloid dendritic cells are well known to capture foreign antigens and migrate into the regional lymph nodes through the afferent lymphatic system, where they present the antigens to T cells (Jonuleit et al., 1997). Second, the comparable quantities of plasmacytoid dendritic cells, which produce type I interferon in response to viral infections, among both groups of TCC practitioners and controls might not correlate with other studies reporting that TCC significantly increased in the magnitude and duration of the antibody response to influenza vaccine (Yang et al., 2008). A greater response of mononuclear cells against surface antigen expression of human hepatitis B virus among middle-aged people performing TCC exercise is also reported (Chen et al., 2008). This conflicting concept for TCC concerning antiviral immunity might be because the enhancement of anti-viral immunity by TCC comes from pathways or cell populations other than plasmacytoid dendritic cells. Nonetheless, the other putative immune effector cell populations participating in human immunity remain undetermined; they could be assessed using our experimental model to elucidate the effective cellular mediators of TCC exercise.

In conclusion, TCC, an aerobic exercise of moderate exercise intensity, promoted the number of circulating myeloid dendritic cells, but not plasmacytoid dendritic cells, in a performance level-dependent manner among our study participants. This suggests that TCC may have a role in potentiating human immunity against tumors and bacterial pathogens.

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