African Journal of Biotechnology Vol. 9(50), pp. 8555-8565, 13 December, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1206
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

The comparison of immunomodulatory effects of peripheral mononuclear cells against proliferation in U937 in junior elderly habitual morning swimming in Taiwan cohort

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Accepted 12 November, 2010

The present investigation was carried out to evaluate the effect of greater immunomodulatory effects of human peripheral blood mononuclear cells against proliferation in human leukemia cells. To achieve this, cells U937 in junior elderly (with cool environmental physical activities) subjects with habitual morning swimming and sedentary lifestyle were recruited in relatively cool season in Taiwan; the isolated human peripheral blood mononuclear cells were stimulated by phytohemagglutinin to obtain the conditioned medium which contains various cytokines. However, the differential effects of the conditioned medium on growth inhibition in U937 leukemia cells were observed. The cytokines, including interferon-gamma, tumor necrosis factors-alpha and interleukine-2 secreted into conditioned medium were higher in the morning-swimming subjects than in the sedentary-lifestyle ones. Similarly, serum white blood cell, creatine phosphokinase, immunoglobulin G and immunoglobulin A in the morning-swimming and sedentary-lifestyle groups indicated that no further inflammatory status existed in the morning-swimming group. In summary, greater immunomodulatory effects of human peripheral blood mononuclear cells against proliferation in human leukemia cells U937 in junior elderly subjects came from the effects of regular moderate exercise in cool temperature rather than of inflammatory effects.

Key words: Immunomodulatory, junior elderly, morning swimming, leukemia, U937, human peripheral blood mononuclear cells.

INTRODUCTION

A healthy man with fitness by physical activities exhibits greater immunity against cancer and disease mortality. Other evidences abound that exercise can induce the promotion of cytotoxic activities in natural killer cells, while other relative cytokines that can induce the secretion of cytokines in serum have also been observed (Frisch et al., 1985, 1989; Nieman et al., 1993, 1995; Wang et al., 1991). The naturally aging progression will increase the risk of infective diseases, especially the upper respiratory track infective (URTI) diseases (Gotfried, 2001; Ruiz et al., 1999) and influenza infection in humans (Bridges et al., 2003; Dussault and Miller, 1994; Potter et al., 1999). Other evidences on weakened cell-mediated immune-surveillance in natural killer in aging mice have been reported. More- over, the deficiency of an adequate secretion of IL-2 by aging progression was found in relation to the increased incidence of sepsis and death in older mice (Plackett et al., 2003).

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Table 1. Basic physiological characteristics and cardiopulmonary fitness of subjects.

Variable	MS (n = 15)	SL (n = 12)	p-value
Age (year)	50.8±5.1	51.0±5.7	0.963
Height (m)	1.68±0.0	1.65±0.0	0.322
Body mass (kg)	67.3±9.2	71.3±5.2	0.285
RHR (beats/min)	62.3±7.6	82±4.5	0.018*
MHR (beats/min)	211.6±5.5	142.6±11.5	0.000*
$\dot{V}O_{2max}$ (ml/ [kg x min])	44.9±10.1	28.0±4.9	0.000*
Albumin (g/dl) (pre-experiment)	4.6±0.2	4.5±0.1	0.189
WBC (cumm)	7038.5±986.0	6272.0±701.9	0.169
HB (mg/dL)	15.3±1.1	14.6±0.7	0.210
BUN (mg/dL)	14.2±3.1	16.9±2.7	0.106
CPK (U/L)	135.6±62.4	124.2±51.8	0.744
LA (mmol/L) (pre-experiment)	1.6±0.5	1.5±0.2	0.687
IgG (mg/dL)	1274.7±228.6	1294.5±277.0	0.885
IgA (mg/dL)	268.5±91.0	287.7±114.0	0.728

MS, Morning swimming; SL, sedentary lifestyle. RHR, resting heart rate; MHR, maximal heart rate; $\dot{V}O_{2max}$, maximal oxygen uptake; WBC, white blood cell; HB, hemoglobin; BUN, blood urea nitrogen; CPK, creatine phospho kinase; LA, lactic acid; IgG, immunoglobulin G; IgA, immunoglobulin A. Results are expressed as mean ± SEM. *$p < 0.05$.

It was well-documented that regularly moderate exercise can promote the immunity responsibility against viruses and tumor (Chiang et al., 2000; Frisch et al., 1985, 1989) and as such, the greater natural killer cell cytotoxic activity (NKCA) in marathon runners indicated the immunity promoting effects of exercise than in sedentary controls (Nieman et al., 1995). However, the incidences of URTI were lower in regular calisthenic elderly women than in sedentary controls (Nieman et al., 1993).

Heat stress will decrease the surviving fraction and increase the necrotic ratio of cyclists' mononuclear cells (MNC) from cyclists' human peripheral mononuclear cells (Chen et al., 2009). After the pretreatment with exercise in 18°C water, the count numbers of leukocyte, granulocyte and monocyte in humans were augmented (Brenner et al., 1999). Other evidences show that repeated cold-water immersions can increase the proportions of monocytes and lymphocytes with expressed IL2 receptors (CD25) in plasma tumor necrosis factor alpha content (Jansky et al., 1996). However, in over 30 min exposure at cold room (4°C), the nature killer (NK) cell activity in naked male volunteers was obviously increased (Lackovic et al., 1988).

The failure of maturation in blood cells causes the formation of leukemia in the inactive immune system and as such, the successful differentiation of leukemia and normal monocytes in the active immune system will occur. To observe the effects of regular swimming exercise under cool water in immunosenescence, we tried to apply the *ex vivo* model system to compare the phytohemagglutinin immunomodulatory effects of human peripheral mono- nuclear cells against proliferation in human leukemia cells U937 in junior elderly with habitual morning swimming (MS) and those with sedentary lifestyle (SL). More so, the secreting cytokines, by phytohemagglutinin immuno- modulatory effects of human peripheral mononuclear cells, were also observed.

MATERIALS AND METHODS

Subject

Fifteen healthy junior elderly (50.8 ± 5.1 years old) subjects with habitual morning swimming group and twelve sedentary-lifestyle controls (51.0 ± 5.7 years old) without any other type of habitual exercise (SL group), were recruited after being reviewed by a human ethics committee and the well informed subjects were asked to complete the investigation form for individual healthy status. All the MS performed about 51.5% of heart rate reserve (HRR) thrice a week at about 5:30 to 8:00 a.m. for at last three to four years. The blood samples were taken into heparinized tubes after subjects had rested quietly for 30 min. After the collection of blood, the samples in ice bath were sent to the laboratory immediately and centrifuged at 5°C to prepare the separation procedure. The plasma was stored at -70°C until it was assayed. None of the subjects participated in any competitions or intensive training programs, underwent clinical surgery nor was supplied with any nutritional supplements over the past 4 months prior to the study. All the healthy junior elderly subjects (male) ranged from 45 to 55 years old (mean age of 50.9 years) with a mean height of 1.6 m and a mean mass of 69.3 kg. Consequently, no significant difference was observed between the two groups (Table 1) and as such, exercise intensity (%) was determined using the method developed by Karvonen et al. (1957).

Heart- rate reserve = maximal heart -rate –resting heart -rate

$$\text{Exercise intensity}(\%) = \frac{(\text{Average heart rate during exercise} - \text{Resting heart rate})}{\text{Heart rate reserve}}$$

Measurement of cardiopulmonary fitness

The values of maximal oxygen uptake ($\dot{V}O_{2max}$) in subjects were evaluated by using the graded maximal treadmill protocol similar to our previous study (Chiang et al., 2000). Briefly, we used a system for cardiopulmonary exercise testing (Q-plus IW/Corival 400, Seattle, WA) to measure the oxygen uptake and ventilation, while treading on a treadmill (Quinton-645) following the above mentioned protocol. After warming up by walking at 3.0 mph on a 10% grade initially, they ran at increasing speeds of 0.5 mph / min. The heart rates and the maximal heart rates during the test were monitored and recorded by a polar pacer heart rate monitor. As such, the $\dot{V}O_{2max}$ test in entirety was accomplished 20 days before the experimental period.

Isolation of mononuclear cells and preparation of conditioned media

MNCs were separated from the blood samples of subjects by centrifugation on a density gradient (Ficoll-Hypaque, 1.077 g/ml, Pharmacia Fine Chemicals). Subsequently, the mononuclear cells at a concentration of 1.5×10^6 cells/ml was incubated in Roswell Park Memorial Institute medium (RPMI) 1640 medium (GIBCO, Grand Island, NY) supplemented with penicillin/streptomycin and 10% heat-inactivated fetal calf serum (FCS) (Hyclone, Logan, UT). After incubating the cells with or without concentrations of phytohemagglutinin (PHA, Sigma, St. Louis) (10 µg/ml) at 37°C in a 5% CO_2 incubator for 24 h, the cell-free supernatant was then collected, filtered and stored at -70°C to obtain the MNC-conditioned medium (MNC-CM).

The MNC-CM collected from PHA-treated and from untreated MNC cultures was termed as PHA-MNC-CM and normal MNC-CM, respectively. PHA, isolated from natural plants, is a mitogen of T lymphocytes. In many published papers, it has been used as an immune stimulant to observe the immune response. A model used as the stimulation of PBMNC by PHA was performed to mimic the varying degree of immune reaction for evaluation of drug- induced (Chen et al., 1997; Chen and Chang, 2004), exercise- mediated immunomodulation in our studies (Chiang et al., 2000; Liao et al., 2006). In this model, the production and release of various cytokines from peripheral blood mononuclear cells (PBMNC) after been stimulated by phytohemagglutinin (PHA), including those related to anti-viral and anti-tumor immunity were observed. Particularly, IFN-γ, TNF-α and IFN-α have been shown as closely relative cytokines on the anti-leukemic immunity (Geneva-Popova and Murdjeva, 1999; Mcclary et al., 2000; Suri et al., 2001). The cytokine levels in PHA-MNC-CM were used as the index of anti-leukemic capacities, while the U937, which is a human myeloid leukemic cell line, obtained from the American Type Culture Collection (Catalog No. CRL-1593.2, Rockville, MD) and cultured in RPMI 1640 medium containing 10% FCS was used to keep the exponential growth intact.

To observe the induction of differentiation by immunomodulatory effects, the U937 at an initial concentration of 1×10^5/ml was incubated in Petri dishes containing 30% (vol/vol) of PHA-MNC-CM or normal MNC-CM. The 30% (vol/vol) CM, determined by our preliminary test, was the optimal amount for treating U937 and had no direct concentration effect of PHA on proliferation and differentiation of U937 cells. Moreover, the estimated concentration of PHA was 0-1 µg/ml in MNC-CM.

After gently rubbing the dishes with a rubber policeman (Bellco Glass, Vineland, NJ, USA) to suspend the adherent cells, all the cells were collected in order to count their numbers on day 5. As such, the trypan blue dye exclusion test was performed to determine the numbers of viable U937 cells and the growth inhibition of U937cells was calculated as follows:

$$\text{Growth inhibition}(\%) = \left(1 - \frac{\text{Number of viable cells treated with PHA - MNC - CM}}{\text{Number of viable cells treated with normal MNC - CM}}\right) \times 100\ \%$$

Assay for cytokines

In subjects, the secreted cytokines in plasma were determined in duplicates, whereas, the tumour necrosis factor-alpha (TNF-α), interleukin 2 (IL-2) and interferon-gamma (IFN-γ were measured by cytometric bead array (CBA) assays (human Th1/Th2 cytokine kit, BD Biosciences, San Diego, CA, USA). In short, for this assay, the above cytokines were specifically captured by an anti-body, bonded to microparticles and detected by the use of a fluorescence-based detection system and flow cytometric analysis. In order to create the standard curves to determine the quantities, a series of 10 times dilutions of each cytokine standard was performed together with each sample assay. All the data were analyzed in a FACS Calibur-flow cytometer (BD Biosciences, San Diego, CA, USA) using the BD CBA analysis software.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and as such, the significant changes in the proliferation of cell numbers and the secretion of cytokines levels between various groups were evaluated with one-way analysis of variance by SPSS 10.0 software (SPSS Inc, Chicago, Ill, USA). However, the statistical significances were defined as a p-value of less than 0.05.

RESULTS

The exercising status in subjects over the experimental period

The training period of MS group with habitual morning swimming was at 05:30 - 08:00 a.m. from March to May, while the average water temperature at this interval was at 15.7 ± 2.9°C. The frequency and duration of exercise were 5.0 ± 1.2 days per week and 80.0 ± 8.6 min per day, respectively, whereas the average heart rate in the exercise period was 139.3 ± 4.5 beats/min (Table 2).

The maximal oxygen uptake ($\dot{V}O_{2max}$) of MS and SL

The basic physiologic characteristics and cardiopulmonary fitness data are shown in Table 1 and it was seen that no observable difference exist between MS group and SL group in age, height and body mass ($p > 0.05$). The mean $\dot{V}O_{2max}$ measured in the MS group was 44.9 ± 10.1 ml/ [kg × min] (ranging from 31.2 to 56.5 ml/ [kg × min]) and was obviously greater than that in the SL group (mean: 28.0 ± 4.9 ml/ [kg × min], range: from 20.7 to 35.3 ml/ [kg × min]) ($p < 0.001$). The resting heart rate and maximum heart rate in MS group were also greater than that of the SL group. The serological data that were present in the two groups including albumin, hemoglobin

(HB), blood urea nitrogen (BUN), creatine phosphokinase (CPK) and lactic acid (LA) showed no differences, while the immunological data such as white blood cells (WBC),

Table 2. The exercise status during morning swimming.

Variable	Exercise intensity (%)	Exercise frequency (days/week)	Exercise duration (min/time)	Average heart rate during exercise (bpm)	Water temperature (℃) (05:30 - 08:00 AM)			Average temperature (℃)
					March	April	May	
Value	51.5±0.6	5.0±1.2	80.0±8.6	139.3±4.5	12.9±0.5	15.6±0.8	18.8±1.2	15.7±2.9

Exercise intensity = the target heart rate determined by HRR (heart rate reserve); Exercise frequency = the average days per week over last 3 years; Exercise duration = the average minutes in exercising period over last 3 years; Average heart rate during exercise = the average heart rate in exercising period over last 3 years.

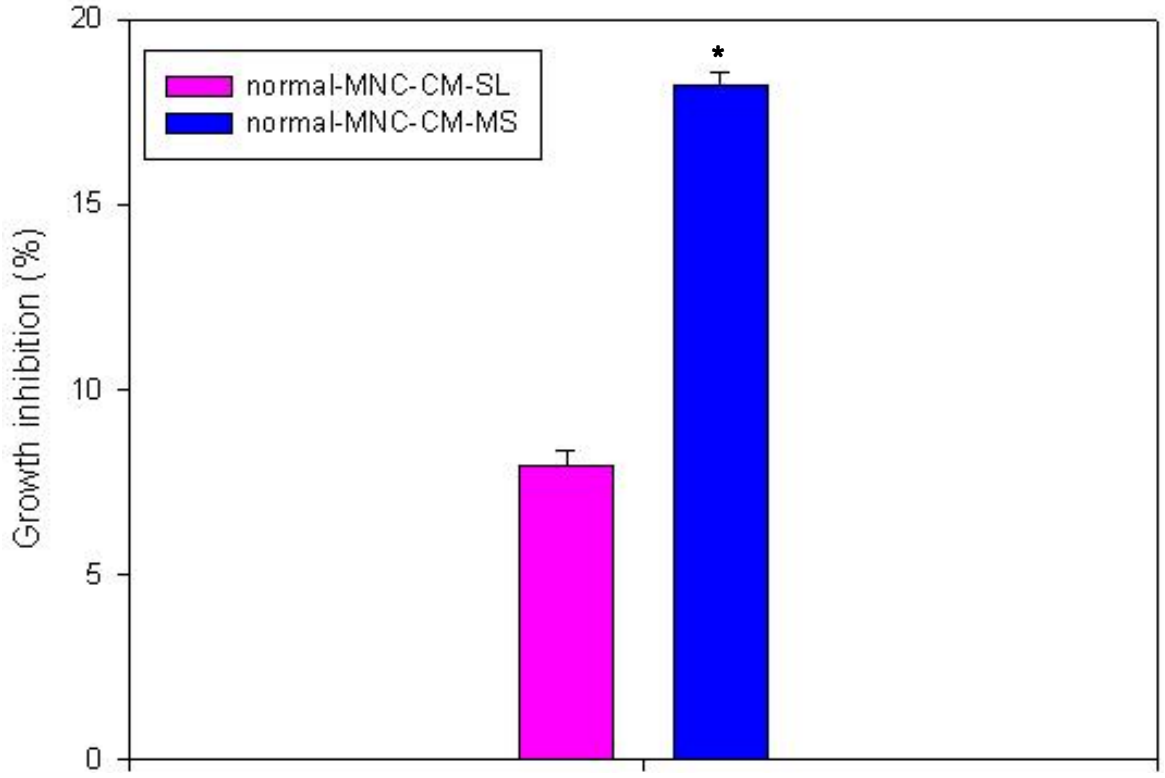

Figure 1a. Growth inhibition in U937 cells treated with normal MNC-CMs on day 5. Normal MNC-CM-MS was the conditioned medium, in which the mononuclear cells were without any treatment in the junior elderly with habitual morning swimming group and normal MNC-CM-SL was that of the sedentary lifestyle control group. *Significant difference ($p < 0.05$).

immunoglobulin G (IgG) and immunoglobulin A (IgA) exhibited the same status.

Growth inhibition on leukemia U937 by normal MNC-CM

To compare the immunomodulatory effects of the MS and SL group on leukemia, the U937 were treated with normal MNC-CM on day 5 and the growth inhibition was observed. As shown in Figure 1a, the growth inhibition of 18.2 ± 0.4% in the MS group was greater than that of 7.9 ± 0.2% in the SL group ($p = 0.004$).

Growth inhibition on leukemia U937 by PHA-MNC-CM

Our preliminary experimental data have shown that the growth inhibition on leukemia U937 by PHA-MNC-CM exhibit dose-response relationship to PHA. As shown in Figure 1b, the growth inhibition at 68.7 ± 0.1% in MS group was greater than that of 64.2 ± 0.2% in the SL group ($p < 0.001$).

Morphological observation of treated U937 cells After 5 days of treatment with PHA-MNC-CM, the treated

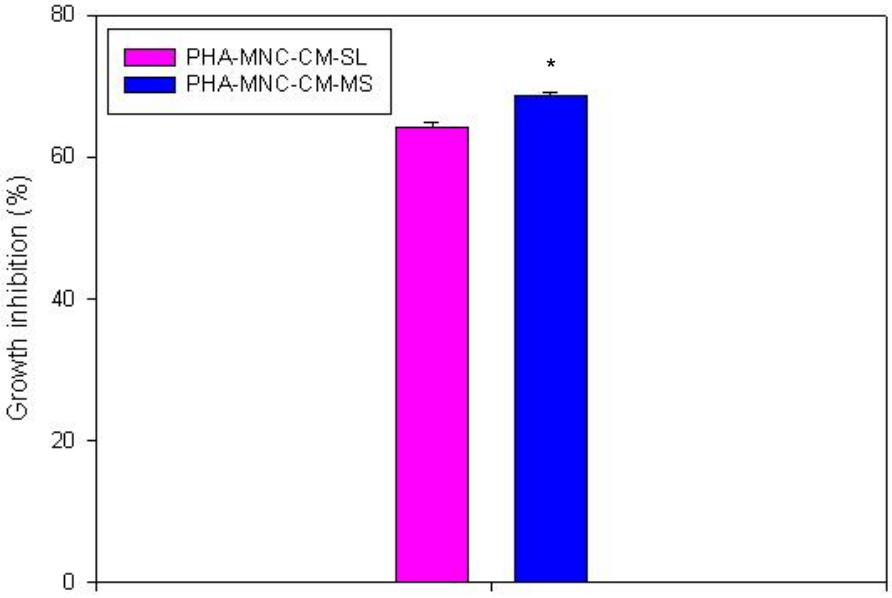

Figure 1b. Growth inhibition in U937 cells treated with PHA-MNC-CM on day 5. PHA-MNC-CM-MS was the conditioned medium, in which the mononuclear cells were treated with PHA in the junior elderly with habitual morning swimming group and PHA-MNC-CM-SL was that in the sedentary lifestyle control group. *Significant difference ($p < 0.05$).

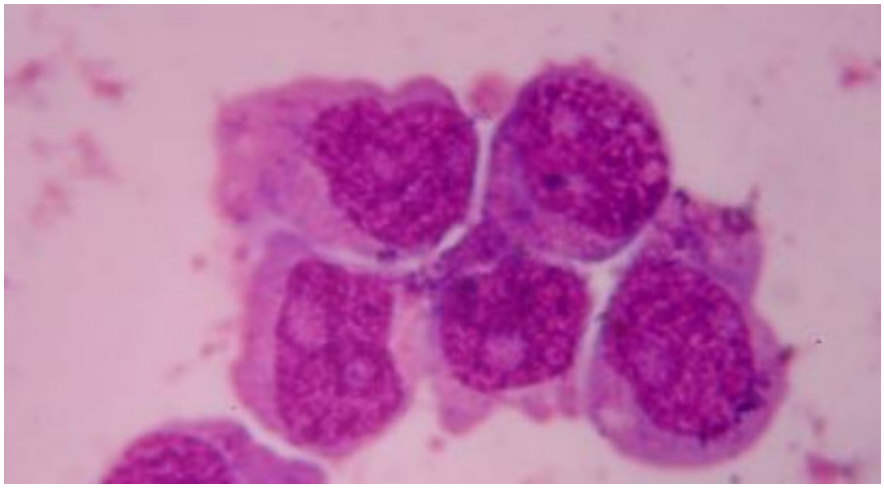

Figure 2a. Morphological features of U937 cells on day 5 (untreated, magnification 1000 ×). After 5 days of treatment with normal-MNC-CM, the untreated U937 cells were harvested and cytocentrifuged onto a microscope slide by using a Cytospin^{2R} and then were stained with Liu's stain.

U937 cells were harvested and cytocentrifuged onto a microscope slide using a Cytospin^{2R} (Shandon Southern Instrument Inc.) and were then stained with Liu's stain. Morphological observations of stained cells were done under a microscope at a magnification of 1000×. Under observation by a light microscope, U937 cell differentiation was observed on the fifth day of incubation (Figures 2a and 2b).

Comparison of cytokine secreted levels in PHA-MNC-CM of two groups

The cytokine secreted levels in PHA-MNC-CM were

determined after treatment of PHA for 24 h and the greater secreted levels of IFN-γ and TNF-α in PHA-MNC-CM from the MS group were found mostly than those from the SL group (76.5 ± 2.8 pg/ml and 7.1 ± 1.4

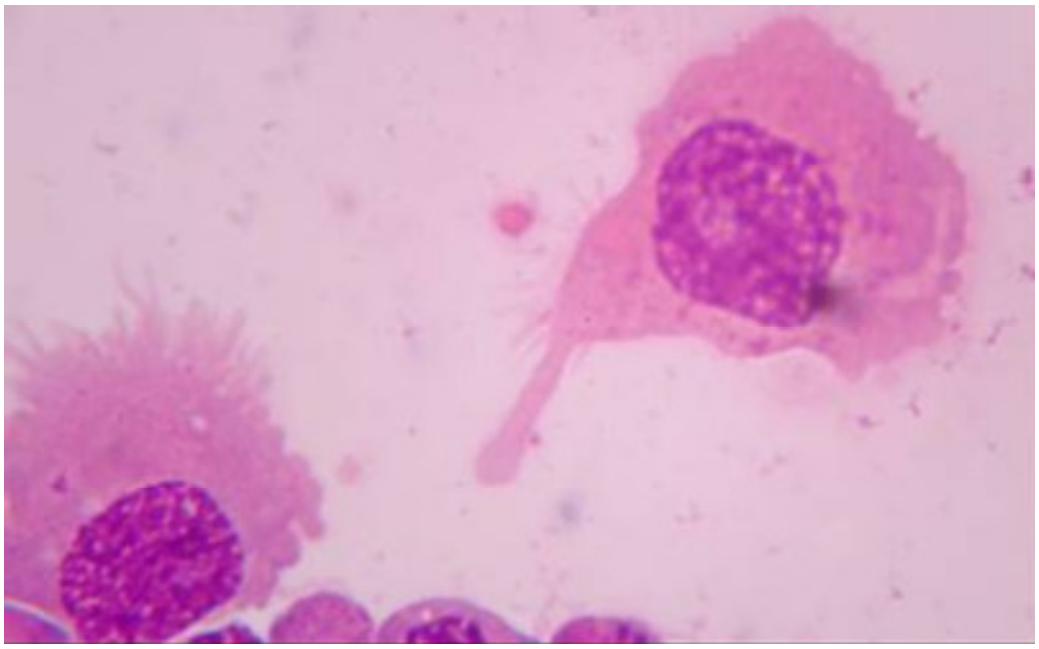

Figure 2b. Morphological features of U937 cells on day 5 (differentiated after treatment of PHA-MNC-CM, magnification 1000 ×). After 5 days of treatment with PHA-MNC-CM, the treated U937 cells were harvested and cytocentrifuged onto a microscope slide by using a Cytospin^{2R} and then were stained with Liu's stain.

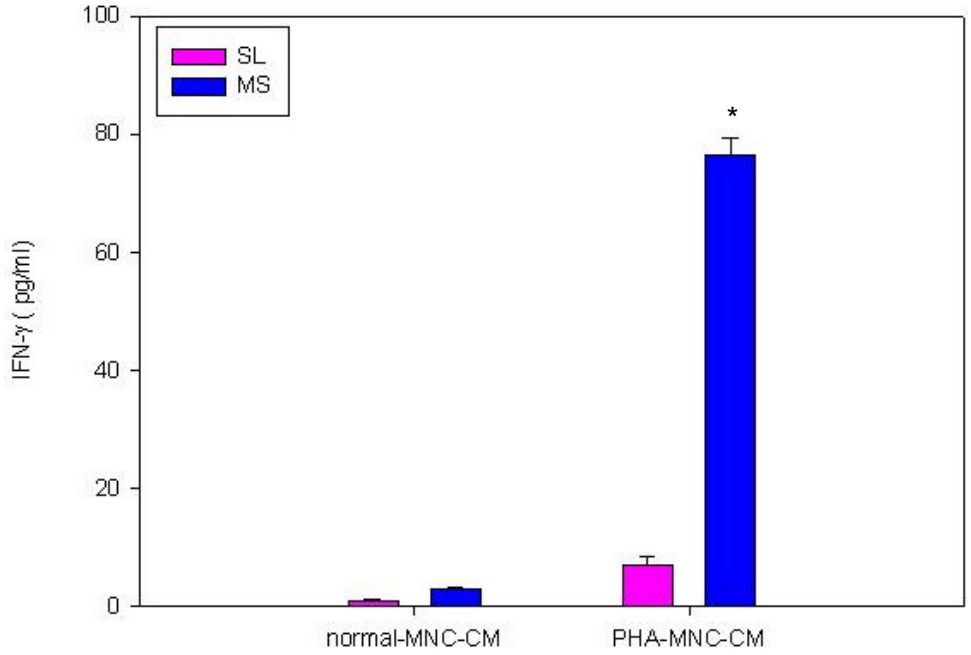

Figure 3. The levels of secreted IFN-γ in PHA-MNC-CMs and normal MNC-CM in MS and SL groups. Levels of secreted IFN-γ in the PHA-MNC-CM and normal-MNC-CM of junior elderly with habitual morning swimming group and that of sedentary lifestyle control group. *Significant difference ($p < 0.05$).

pg/ml vs. 3500.0 ± 293.0 pg/ml and 1852.6 ± 136.4 pg/ml) ($p = 0.001$ and $p = 0.018$) (Figures 3 and 4). In addition, they were 10.7 and 1.8 folds greater in IFN-γ and TNF-α, respectively in the MS group.

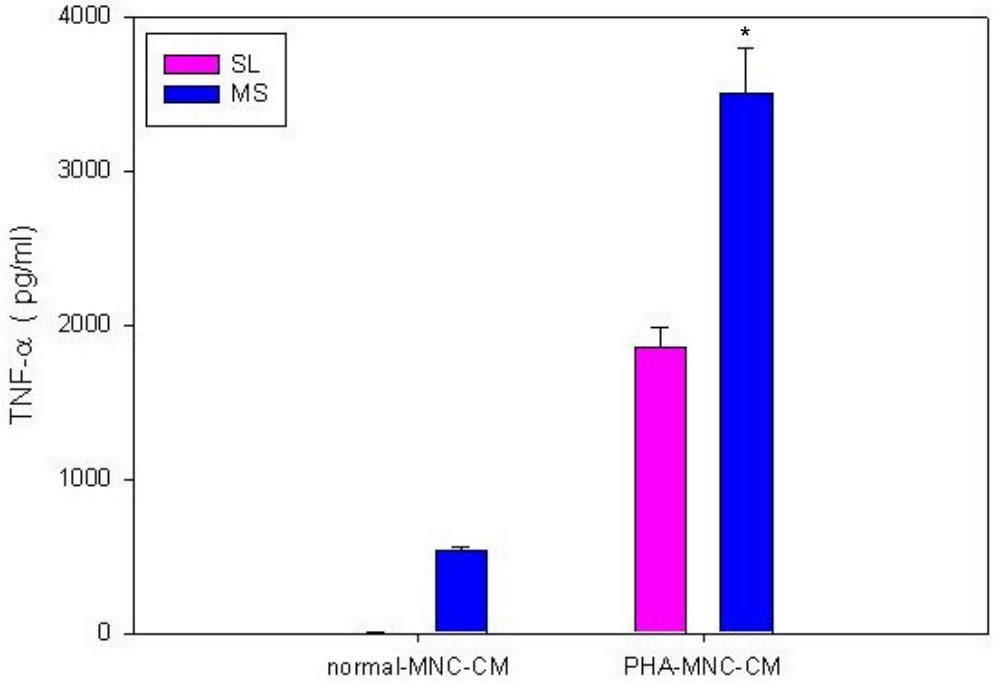

Figure 4. The levels of secreted TNF-α in PHA-MNC-CMs and normal MNC-CM in MS and SL groups. Levels of secreted TNF-α in the PHA-MNC-CM and normal-MNC-CM of junior elderly with habitual morning swimming group and that of sedentary lifestyle control group. *Significant difference ($p < 0.05$).

In the MS group, IL-2 (1433.8 ± 154.1 pg/ml) in junior elderly with habitual morning swimming was over 3 folds as compared to that in controls without any other habitual exercise types ($p = 0.012$) (Figure 5).

DISCUSSION

By comparing the *ex vivo* experiment with the immuno-modulatory model, we found that the junior elderly with habitual morning swimming exhibited greater immuno-modulatory potential to produce more cytokines such as IFN-γ, TNF-α and IL-2 than that of controls without any other habitual exercise types. Also, the greater growth inhibition of leukemia cells U937 by PHA immuno-modulatory can be observed.

In our healthy MS and SL groups, similarity without markedly statistic differences in height, weight and age showed a similar cohort. The measurement of physical fitness values such as resting heart rate (RHR), maximal heart rate (MHR) and maximal oxygen uptake ($\dot{V}O_{2max}$) were all greater in the MS group than in the SL group. Both the normal ranges of serum albumin (Abbasi and Rudman, 1994; Thalacker-Mercer et al., 2007) and hemoglobin (Forster and Gariballa, 2005) in our two groups have indicated that they have the normal nutritional status and as such, the normal BUN values showed normal nitrogen balance and similar nutritional status in these two groups. It should be noted that both groups exhibited WBC in normal ranges, where no inflammatory and mal-nutritional status was shown (Forster and Gariballa, 2005; Idem, 1991). However, the inflammatory factor cannot be attri- buted to our observed immunomodulatory phenomenon, because it is similar to both IgG (Maddison and Reimer, 1976) and IgA (Schenkel and Holdren, 2004). In these two groups, the study also pointed out that the abnormal inflammatory status should be ruled out. In other words, there would be no other inflammatory excitation on immune cells in these two groups. Furthermore, it is obvious that none of the different CPK values indicated that no other organ such as heart, liver or muscle damage occurred between the two groups (Ama et al., 1986; Brewster et al., 2007). As such, the contribution of immuno-response from organ damage would not be considered in our study.

In Taiwan (located in subtropical area with warm weather), the ambient temperature from winter to spring

exists relatively cool, especially in the morning. It is well known that the heat stress damages the immune cells (Chen et al., 2009); however, the gently cool water temperature at about 15°C in the morning in Taiwan may protect all subjects from overheating and damage to immune cells over the swimming exercise periods. As a result, the exercise frequency for 5 days per week indicated the regular exercise in the MS group.

In our experimental model, the immune cells of PBMNC

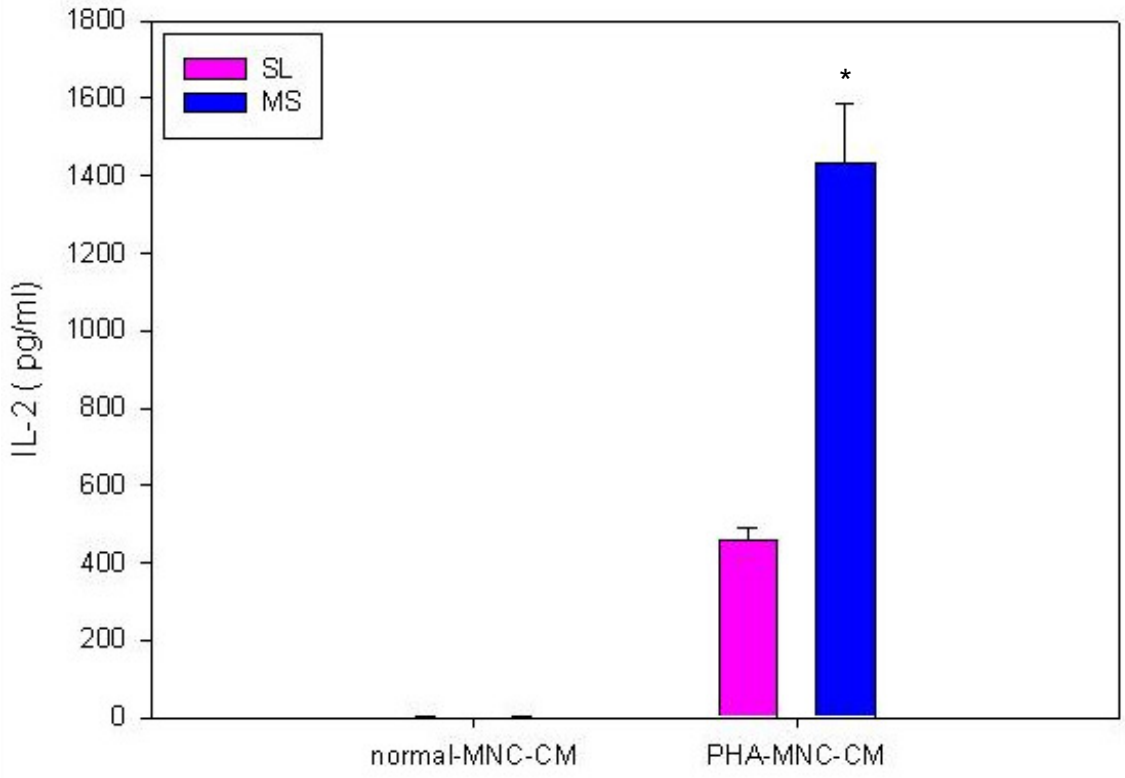

Figure 5. The levels of secreted IL-2 in PHA-MNC-CM and normal MNC-CM in MS and SL groups. Levels of secreted IL-2 in the PHA-MNC-CM and normal-MNC-CM of junior elderly with habitual morning swimming group and that of sedentary lifestyle control group. *Significant difference ($p < 0.05$).

secreted different cytokines that may modulate both the secreting cells themselves and other sub-populations that may just simulate to an *in vivo* network in the real immune system. Consequently, PBMNC, which contains a heterogeneous population of leukocytes and monocytes in *ex vivo* model, are more suitable to simulate *in vivo* immune status than any isolated single subset alone. It is well known that treatment of PHA mainly activates T cells to release various cytokines, which also stimulates the proliferation of T cells themselves together with B cells and NK cells (Tvede et al., 1989). The immunomodulatory effects mentioned above can be observed in our PBMNC, PHA-MNC-CM and secreted cytokines, since this immunomodulatory model for evaluation of human anti-leukemic cellular immunity has been widely accepted in our previous pharmacological studies (Chen et al., 1997; Lieu et al., 1992).

The greater growth inhibition in leukemia cells U937 treated with PHA-MNC-CM from the MS group (on day 5) than from the SL group demonstrated that greater immunomodulatory effects may come from the regular swimming with lightly cool temperature. It is well known that the diminishing immunity would be undergone with age, for example, the NK cells cytotoxic activities (Borrego et al., 1999). By judging from the relatively greater viability in leukemia cells up to 90% (data not shown here) in our study, the growth inhibition in leukemia cells U937 did come from the immunomodulatory effects rather than the killing effects directly. From the morphological observation of treated U937 cells, the much greater mature monocytes-macrophages were found (data not shown here) in the MS group and the immature blasts in the SL group. This can be assumed to be an occurrence of differentiation in leukemia cells, while the regular swimming with lower temperature in this study will promote the immunity against the proliferation of leukemia cells. The moderate aerobic exercise in long term can promote the immune function in elderly subjects (Woods et al., 1999), especially the immunomodulatory effects by PHA against hepatitis B virus (Chen et al., 2008; Hsieh et al., 2010). In fact, the prevalence of some site-specific types of cancers exists reversely in

dose-response to physical activity (Thune and Furberg, 2001). The exercise intensity in regular swimming at a target heart rate of less than 52% was defined as a moderate exercise, whereas the exercise frequency per week was over 5 days in MS group and was defined as the aerobic regular exercise. The average water temperatures among the experimental period were about 12.9 ± 0.5 to 18.8 ± 1.2℃ from March to May (mean as 15.7 ± 2.9℃) and as such, the heat diminishes the activities in the immune cells (Chen et al., 2009). The regular swimming done very early in the morning with

lower temperature may provide a protective environment against the heat stress for the immune cells to enhance the immunomodulatory effects by PHA in elderly subjects.

It has been well documented that the IFN-γ acts majorly, as a central mediator of the immunoregulatory (Trinchieri, 1998) and antitumor effects (Brunda et al., 1993). The evidences about the alleviation of induction in IFN-γ by stimulators with the progression of age have been well mentioned (Rink et al., 1998). In our experimental data, the dramatically higher amount of secreted IFN-γ in MS group provided the other evidence for the promotion of immunomodulatory effects by regular swimming exercise with cool temperature in elderly subjects. The roles played by IFN-γ in the growth inhibition of leukemia U937 came from the differentiating effects rather than the killing effects. A similar evidence can also be seen in induction of differentiation by highly purified natural INF-γ in the human promyelocytic leukemia HL-60 cell line (Takei et al., 1984).

While observing the immunomodulatory effects by lipopolysaccharide (LPS) in monocytes purified from donors over 65 years old, a less production of TNF-α was induced than the young ones did (Anne et al., 1998). In other words, immunosenescence will lessen the immune response to induce the TNF-α. In the test of promyelocytes HL-60 *in-vitro* exposure to TNF-α, the observed differentiation effects and colonies inhibition can reveal the immuno- modulatory effects against leukemia by TNF-α (Kikuchi et al., 1996; Munker and Koeffler, 1987). In our data about the obviously greater secreted TNF-α in MS group than in SL group, we have shown another evidence for the promotion of immunomodulatory effects by regular swimming exercise with cool temperature in elderly subjects. More so, the significantly secreted lower levels of TNF-α in macrophages from aged mice were observed (Renshaw et al., 2002). The similar roles of IFN-γ and TNF-α act as a differentiation promoter in leukemia U937 rather than a killing agent. In fact, the combined treatment of TNF-α and IFN-γ can effectively modulate the differentiation of U 937 cells (Kikuchi et al., 1996). The treatment of recombinant IL-2 on patients with leukemias in clinic use has been reported (Cortes et al., 1999; Foa et al., 2008) and it is well documented that the production of interleukin IL-2 in the *in vitro* function of T cells decline with age (Miller, 1996). After being stimulated by concanavalin in splenocytes, the production of IL-2 and IFN-γ were obviously lower in the aged injured mice compared with the young ones (Plackett et al., 2003).

The higher percentage of lymphocytes producing intracellular IL-2 in elderly women attending 2-years physical activity program may play a crucial role in amelioration of immunosenescence than in the control group of elderly sedentary women (Drela et al., 2004). The significantly greater secreted IL-2 in WS group in the present experimental data may also support the evidence that the greater growth inhibition in U937 cells by regular swimming exercise with cool temperature in elderly subjects are attributed to the immunomodulatory effects.

Comparing this to young adults, the immune responses by mitogen induction of both IL-2 and IFN-γ in elderly persons were attenuated (Rink et al., 1998). To ameliorate the immunosenescence with advancing age, the introductory moderate exercise in older mice enhanced the multiple immune responses of increment in antigen-specific IL-2 and IFN-γ production against viral challenge (Kohut et al., 2001). The immune responses of PHA to produce both greater TNF-α and IFN-γ amount in elderly subjects with Tai Chi Chuan exercises in the morning, which is 50% HRR less in mild to moderate intensity than sedentary control elderly subjects, has shown the relationship between these two cytokines and the amelioration of immunosenescence with advancing age (Chen et al., 2008).

Conclusion

Collectively, it is noteworthy that the augmentations of IL-2, TNF-α and IFN-γ in all the elderly subjects in our immunomodulatory experiment are in agreement with issues that are previously explained. It can be assumed that the greater immunomodulatory effects of human peripheral mononuclear cells against the proliferation of human leukemia cells U937 in junior elderly habitual morning swimming may be attributed to the greater cytokines secretion. In brief, the swimming exercise as regular, moderate and gentle cool temperature exerted greater immunomodulatory effects on elderly habitual morning swimming.

Abbreviations

URTI, Upper respiratory track infective diseases; **NKCA,** natural killer cell cytotoxic activity; **MNC,** mononuclear cells; **NK,** nature killer; **MS,** morning swimming; **SL,** sedentary lifestyle; **HRR,** heart rate reserve; VO_{2max}; values of maximal oxygen uptake; **RPMI,** Roswell Park Memorial Institute medium; **FCS,** fetal calf serum; **PHA,** phytohemagglutinin; **CBA,** cytometric bead array; **HB,** albumin, hemoglobin; **BUN,** blood urea nitrogen; **CPK,**

creatine phosphokinase; **LA,** lactic acid; **WBC,** white blood cells; **IgG,** immunoglobulin G; **IgA,** immunoglobulin A; **MHR,** maximal heart rate; **LPS,** lipopolysaccharide.

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