Scientific Research and Essays Vol. 6(2), pp. 406-416, 18 January, 2011
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE10.919
ISSN 1992-2248 ©2011 Academic Journals

Full Length Research Paper

The pro-inflammatory and anti-inflammatory effects of human peripheral mononuclear cells against the human leukemia cells in middle age with habitual morning swimming

A. C. Huang[1], C. M. Jiang[2], C. C. Lee[3], T. S. Huang[4], M. C. Wu[1] and Y. Y. Chen[3]*

[1]Department of Food Science, National Ping-tung University of Science and Technology, 912, Taiwan (R.O.C.).
[2]Department of Seafood Science, National Kaohsiung Marine University, 811, Taiwan (R.O.C.)
[3]Department and Graduate School of Physical Education, Natl. Taiwan Sport University Taichung, 404, Taiwan (R.O.C.).
[4]Department of Nursing, National Taiwan University Hospital and National Taiwan University College of Medicine, 100, Taiwan (R.O.C.).

Accepted 24 November, 2010

In order to know if the physiologic stress under cool temperature, as well as the greater immunomodulatory effects of human peripheral mononuclear cells (PBMNC), will cause the pro-inflammatory and anti-inflammatory effects against the proliferation in human leukemia cells U937 in middle age with habitual morning swimming, 14 regular morning swimmers and 11 sedentary lifestyle subjects were enrolled with formal consents in winter season in Taiwan. The isolated PBMNC were stimulated by phytohemagglutinin to obtain the conditioned medium. We consumed no inflammatory status in the morning swimming group from the similar serum CPK, Ig-G, WBC and Ig-A in morning swimming and sedentary lifestyle groups. After being incubated with leukemia cells, the differential effects of the conditioned medium on growth inhibition in U937 were obtained. The cytokines, including IFN-γ, TNF-α, IL-10 and IL-4 secreted into the conditioned medium were higher in the morning swimming group than in the sedentary lifestyle group. In conclusion, the greater immunomodulatory effects of PBMNC against the proliferation in U937 in middle age was majorly caused by the effects of the regular moderate swimming exercise rather than the inflammatory effects in cool temperature. The cytokines, which are related to both the anti-inflammatory (such as IL-10 and IL-4) and pro-inflammatory effects, increased at the swimming exercise under cool temperature.

Key words: Morning swimming, middle age, leukemia, immunomodulatory, U937, human peripheral mononuclear cells.

INTRODUCTION

The naturally aging occurrence will promote the risk of infective diseases; of note, is the occurrence of the upper respiratory track infective diseases (Gotfried, 2001; Ruiz et al., 1999) and the influenza infection in humans (Bridges et al., 2003; Dussault and Miller, 1994; Potter et al., 1999). Moreover, the deficiency of an adequate secretion of IL-2 by the aging progression was found to be relative to the increased incidence of sepsis and death in older mice (Plackett et al., 2003). It was well documented that people who have habitual physical activities own greater immunity against cancer and other diseases with lower mortality (Frisch et al., 1985; Nieman et al., 1995). More obvious evidences about these activities are that the physical activities can lead to the elevating cytotoxic activities in natural killer cells. Also, other relative

*Corresponding author. E-mail: yu11.tw@yahoo.com.tw. Tel: 886-4-22213108*2070. Fax: 886-4-28617120.

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

Variable	MS (n = 14)	SL (n = 11)	p-value
Age (year)	49.8±4.5	49.7±4.7	0.965
Height (m)	1.69±0.0	1.65±0.0	0.247
Body mass (kg)	69.9±6.4	69.9±3.0	0.994
Resting heart rate (beats/min)	59.0±5.0	84.5±2.1	0.039*
Max. heart rate (beats/min)	214.5±3.5	146.0±14.1	0.021*
VO^2max (ml/[kg x min])	46.9±9.2	28.3±5.2	0.0004*

The middle age junior elderly with habitual morning swimming group were (MS) and the sedentary lifestyle control groups were (SL). Results are expressed as mean ± SEM. *: $p<0.05$.

cytokines have been observed to induce the secretion of cytokines in serum (Frisch et al., 1985, 1989; Nieman et al., 1993, 1995; Wang et al., 1991).

The promotion of the immunity responsibility against viruses and tumor by the regularly moderate exercise was well-publicized (Chiang et al., 2000; Frisch et al., 1985, 1989), and the immune responses such as the secretion of TNF-α and IFN-γ can exert the immunomodulatory effects against the leukemia cell lines, as U937 and HL-60, in humans (Brunda et al., 1993; Chen et al., 2008; Takei et al., 1984; Trinchieri, 1998). The immunity promoting effects of the exercise can also be proved by the greater natural killer cell cytotoxic activity (NKCA) in marathon runners than in sedentary controls (Nieman et al., 1995). Moreover, the incidences of the upper respiratory track infection (URTI) in regular calisthenic elderly women were lower than in sedentary control groups (Nieman et al., 1993). Besides the aging effect on diminishing immunity, the hyperthermal effects can also do that. We have observed that heat stress survives in a lower fraction and up-regulates the necrotic ratio of cyclists' human peripheral mononuclear cells (Chen et al., 2009). After the exercise pretreatment in 18°C water, the counting numbers of leukocyte, granulocyte and monocyte in humans were increased (Brenner et al., 1999). Other evidences about the repeated cold water immersions can increase the proportions of monocytes and lymphocytes, expressed in the plasma tumour necrosis factor alpha content (Jansky et al., 1996). Over 30 min exposure at cold room (4°C) in naked male volunteers, show that the natural killer cell (NK cell) activity was significantly augmented (Lackovic et al., 1988).

The inactive immune system often causes the failure of normal maturation to form the normal leukemia. In other words, the normal differentiation of leukemia to monocytes in an active immune system will render the great immune functions. In order to elucidate whether the effects of regular swimming activity is found under cool water in immunosenescence or not, we tried to conduct the *ex vivo* model system to compare the immuno-modulatory effects caused by phytohemagglutinin to human peripheral mononuclear cells in middle age with habitual morning swimming and other ones with sedentary lifestyle against the proliferation in U937. The secreting cytokines in the immunomodulatory effects by phytohemagglutinin to human peripheral mononuclear cells were also observed.

MATERIALS AND METHODS

Subject

The subjects were well informed of the total experimental purpose and details, and they completed the investigation form for individual healthy status. Fourteen healthy middle age (49.8 ± 4.5 years old) with customary morning swimming (MS group) and eleven sedentary lifestyle controls (49.7 ± 4.7 years old) without any other customary exercise types (SL group) were recruited, after being reviewed by a human ethics committee in Chinese culture university, Taiwan. All the MS groups performed about 51.6% of the heart rate reserve (HRR) at about 5:30 to 8:00 A.M. three days a week for at last three to four years. The heparinized tubes were used to collect the blood samples, while subjects had rested quietly for 30 min. After the collection of blood, samples in ice bath at 5°C were sent to laboratory immediately. Subsequently, they were centrifuged at 5°C to prepare the separation procedure. The isolated plasma was stored at -70°C before assay.

The subjects, who did not participate in any intensive or competitive training programs, did not undergo any clinical surgery operation and were not supplied with any nutritional supplements for the past 4 months, at least prior to the experimental test. All the subjects were restricted from 45 to 55 years old, with their mean age, mean height and body weight as 49.8 years old, 1.6 m and 69.9 kg, respectively. These data have shown no obvious difference in age, body weight and height from the two groups (Table 1). However, exercise intensity (%) was determined using the method developed by Karvonen et al. (1957).

$$\text{Heart - rate reserve} = \text{maximal heart - rate} - \text{resting heart - rate}$$

intensity (%) = (Average heart rate during exercise - Resting heart rate) / Heart Rate Reserve

Measurement of cardiopulmonary fitness

The heart rates and the maximal heart rates values during test were measured and recorded by a polar pacer heart rate monitor. All the values of the test, including the maximal oxygen uptake were completed at 20 days before the experimental period. The maximal oxygen uptake (VO2max) values in subjects were determined by using the graded maximal treadmill protocol as our previous study

(Chiang et al., 2000). Shortly, the oxygen uptake and ventilation while treading on a treadmill (Quinton-645, USA) following the aforementioned protocol were performed, using a system for cardiopulmonary exercise testing (Q-plus IW/Corival 400, Seattle, WA). They ran at increasing speeds of 0.5 mph/min after the warming up step which was walking at 3.0 mph on a 10% grade initially.

Isolation of mononuclear cells and preparation of the conditioned media

The mononuclear cells (MNC) were isolated from the blood samples by centrifugation on a density gradient (Ficoll-Hypaque, 1.077 g/ml, Pharmacia Fine Chemicals), while the concentration of 1.5×106 cells/ml in MNC was incubated in RPMI 1640 medium (GIBCO, Grand Island, NY, USA) and supplemented with 0.1% penicillin/ streptomycin and 10% heat-inactivated fetal calf serum (FCS) (SAFC, USA). After being incubated with or without phytohemagglutinin (PHA, Sigma, St. Louis, USA) at a concentration of 10 µg/ml at 37°C in a 5% CO_2 incubator for 24 h, the cell-free supernatants were then collected, filtered and stored at -70°C to obtain the MNC-conditioned medium (MNC-CM). PHA, which is isolated from natural plants, is well known as a mitogen of T lymphocytes in published papers, and it has been used as an immune stimulant to observe the immune response. The MNC-CM obtained from treated PHA and untreated MNC cultures were termed as PHA-MNC-CM and normal MNC-CM, respectively.

The model as the stimulation of PBMNC by PHA was performed to stimulate the varying degree of the immune reaction for assessment of the drug-induced (Chen and Chang, 2004; Chen et al., 1997), exercise-mediated immunomodulation (Chiang et al., 2000; Liao et al., 2006). In this experimental model, the yield and release of various cytokines in the conditioned medium from the isolated human peripheral mononuclear cells (PBMNC) after been stimulated by PHA, including those related to anti-viral and anti-tumor immunity were observed. Particularly, both IFN-γ and TNF-α have been shown as critically relative cytokines on the anti-leukemic immunity (Geneva-Popova and Murdjeva, 1999; Mcclary et al., 2000; Suri et al., 2001). These cytokine levels mentioned previously were deemed as the index of anti-leukemic immunity.

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 which is cultured in RPMI 1640 medium containing 10% FCS, will keep the exponential growth curve. At first, the U937 seeded at an initial concentration of 1×10^5/ml, were incubated in Petri dishes that contained 30% (vol/vol) of PHA-MNC-CM or normal MNC-CM. The estimated concentration of PHA was no more than 1 µg/ml in MNC-CM, while the 30% (vol/vol) of CM was the optimal amount for treating U937 and it has no direct effect concentration of PHA on the proliferation and differentiation of U937 cells. To suspend the adherent cells and collect the number count on day 5, all the cells were gently rubbed on the dishes with a rubber policeman (Bellco Glass, Vineland, NJ, USA). The viable numbers of U937 cells were determined by using the trypan blue dye exclusion test. Nonetheless, the growth inhibition of U937 cells was determined in the following equation:

$$\text{Growth inhibition}(\%)=\left(1-\frac{\text{Number of viable cells treated with PHA-MNC-CM}}{\text{Number of viable cells treated with normal MNC-CM}}\right)\times100\%$$

Assay for cytokines

In subjects, the secreted cytokines in plasma were determined in duplication. The TNF-α, IFN-γ, IL-4 and IL-10, were measured by cytometric bead array (CBA) assays (human Th1/Th2 cytokine kit, BD Biosciences, San Diego, CA, USA). In short, for this assay, all cytokines were specifically captured by the anti-body bonded to microparticles and detected by using 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 for each cytokine standards was performed together with each samples' assay. All the data were analyzed in a FACS Caliburflow cytometer using the BD CBA (BD Biosciences, San Diego, CA, USA) analysis software.

Statistical analysis

Data were expressed as the mean standard error of the mean (SEM), while statistical significance was defined as a p-value of less than 0.05. The significant differences in the proliferation of cell numbers and the secretion of cytokines levels between various groups were assayed with one-way analysis of variance by SPSS 10.0 software (SPSS Inc, Chicago, IL, USA).

RESULTS

The exercising status in subjects over the experimental period

The average water temperature of the swimming pool during the experimental period was at about 15.7 ± 2.9°C. The training period of the MS group with habitual morning swimming was at 05:30 to 08:00 A.M. from March to May in our records. The exercise frequency in all the subjects was 5.2 ± 0.6 days per week with the exercise duration at 54.5 ± 6.8 min per day. The average measured heart rate of subjects in the exercise period was 141.4 ± 3.5 beats/min (Table 2).

The maximal oxygen uptake (VO2max) of MS and SL

The basic anthropometric and cardiopulmonary fitness data were listed in Table 2. There were no marked differences between the SL and MS groups in body mass, height and age ($p>0.05$). It was observed that the greater mean of VO2max was measured in the MS group as 46.9 ± 9.2 ml/kg/min (ranging from 33.37 to 56.53 ml/kg/min) compared to that in the SL group as 28.3±5.2 ml/kg/min (ranging from 20.76 to 35.34 ml/kg/min) ($p<0.0001$). The resting heart rates and the maximum heart rate during the exercise in the MS group were also greater than that of the SL group. The immunological data in both groups, such as IgA, IgG and WBC, have shown almost similar data. The biological data in serum including albumin, hemoglobin, BUN, CPK and lactic acid have shown no observable differences in the two groups (Table 3).

Growth inhibition on U937 by normal MNC-CM

The U937 were treated with normal MNC-CM at day 5 and growth inhibition was observed in order to compare the

Table 2. The exercise status during morning swimming.

Variable	Water temperature (℃)			Average temperature (℃)	Average heart rate during exercise (bpm)	Exercise intensity (%)	Exercise frequency (days/week)	Exercise duration (min/time)
	March	April	May					
value	12.9±0.5	15.6±0.8	18.8±1.2	15.7±2.9	141.4±3.5	51.8±0.7	5.2±0.6	54.5±6.8

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

Table 3. The immunological and biological data in the serum of subjects.

Variable	MS (n=14)	SL (n=11)	p-value
IgA (mg/dL)	253.6±75.0	232.6±36.2	0.649
IgG (mg/dL)	1300.9±215.0	1422.6±128.5	0.369
WBC (cumm)	7170.0±1010.7	6205.0±701.8	0.148
Albumin (g/dl) (pre-experiment)	4.6±0.2	4.5±0.2	0.284
HB (mg/dL)	15.2±1.1	14.7±0.7	0.404
BUN (mg/dL)	14.4±3.1	17.9±1.8	0.058
CPK (U/L)	141.0±61.4	137.3±54.8	0.924
LA (mmol/L) (pre-experiment)	1.4±0.2	1.6±0.1	0.413

The middle age junior elderly with habitual morning swimming group were (MS) and the sedentary lifestyle control groups were (SL). IgA, Immunoglobulin A; IgG, Immunoglobulin G; WBC, White blood cell; HB, Hemoglobin; BUN, blood urea nitrogen; CPK, creatine phospho kinase; LA, Lactic acid. Results are expressed as mean ± SEM

immunomodulatory effects by the MS and SL groups on leukemia. As shown in Figure 1a, the growth inhibition on U937 by normal MNC-CM at 18.4±0.8% in the MS group is greater than that of 4.2 ± 0.5% in the SL group ($p<0.0001$).

Growth inhibition on U937 by PHA-MNC-CM

In our preliminary experimental data, the growth inhibition on leukemia U937 by PHA-MNC-CM has been shown to exhibit a dose-response relationship to PHA. As shown in Figure 1b, the growth inhibition of U937 by PHA-MNC-CM (PHA, 10 µg) at 69.1±0.1% in the MS group was greater than that of 63.9 ± 0.7% in the SL group ($p = 0.001$).

Morphological observation of treated U937 cells

After 5 days of treatment with PHA-MNC-CM, the treated U937 cells were harvested and cytocentrifuged onto a microscope slide using a Cytospin2R (Shandon, Southern England), and then, they were stained with Liu's stain. Morphological observations of stained cells were viewed 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 b).

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

The levels of secreted cytokines in PHA-MNC-CM were measured after treatment of PHA over 24 h. The significantly higher levels of secreted IL-4 and IL-10 in PHA-MNC-CM from the MS group were found to be higher than that of the SL group

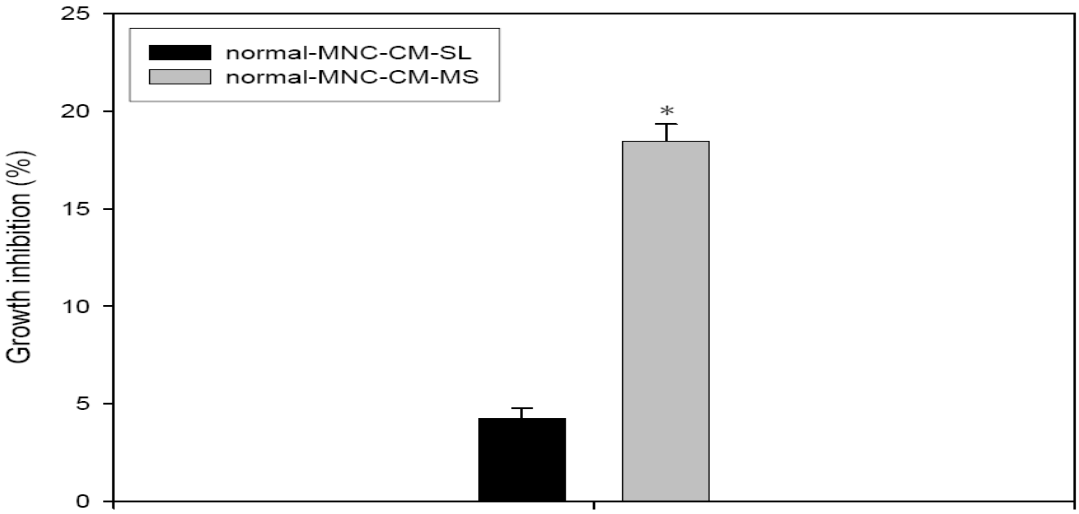

Figure 1a. Growth inhibition in U937 cells treated with normal MNC-CMs on day 5. Normal MNC-CM-MS was the conditioned medium, in which the mononeuclear cells were without any treatment in the middle age with the habitual morning swimming group, while normal MNC-CM-SL is found in the sedentary lifestyle control group. * is significantly different at $p < 0.05$.

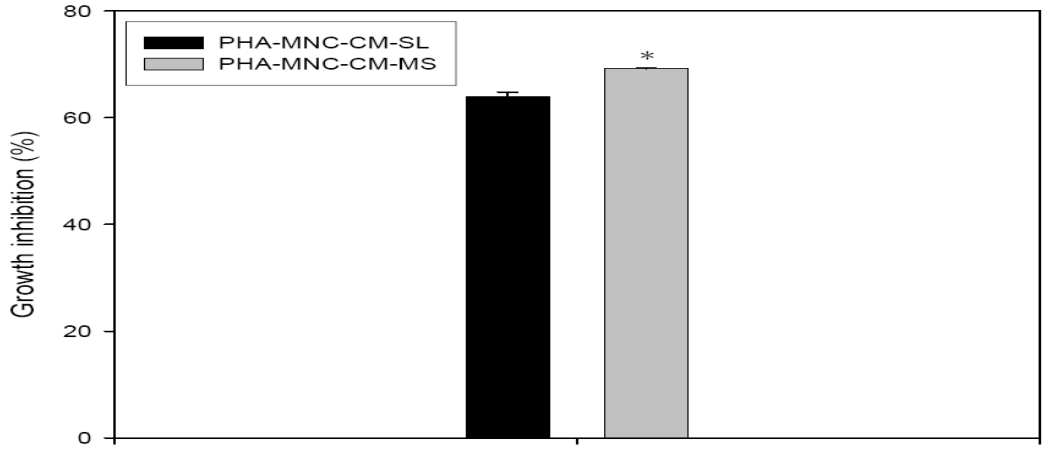

Figure 1b. Growth inhibition in U937 cells treated with PHA-MNC-CMs on day 5. PHA-MNC-CM-MS was the conditioned medium, in which the mononeuclear cells were treated with PHA in the middle age with the habitual morning swimming group, while PHA-MNC-CM-SL was found in the sedentary lifestyle control group. * is significantly different at $p < 0.05$.

(1681.1 ± 79.6 and 3448.0 ± 692.8 pg/ml vs. 51.0 ± 8.6 and 843.5 ± 22.6 pg/ml) ($p = 0.001$ and $p = 0.033$) (Figures 3 and 4). Particularly, in the MS group, IL-4 and IL-10 in PHA-MNC-CM from the middle age with habitual morning swimming were 32 and 4 folds more than that of the controls, which are without any habitual exercise types, respectively.

Comparing this with the normal-MNC-CM in both groups, the secreted TNF-α in the MS group (415.9 ±106.7 pg/ml) is greater than that in the SL group (2.5 ± 3.5 pg/ml) ($p = 0.021$). Likewise, when it is compared with the PHA-MNC-CM in both groups, the secreted TNF-α in the MS group (2983.0 ± 919.1 pg/ml) is greater than that in the SL group (763.0 ± 331.4 pg/ml) ($p = 0.051$) (Figure 5). In the normal-MNC-CM, the secreted IFN-γ in the MS group (2.1 ± 0.1 pg/ml) were higher than that in the SL group (0.3 ± 0.3 pg/ml) ($p = 0.003$). Also, in PHA-MNC-CM, IFN-γ in the MS group (46.4 ± 1.1 pg/ml) were higher than that in the SL group (8.4 ± 2.6 pg/ml) ($p= 0.001$) (Figure 6).

DISCUSSION

Viewing the *ex vivo* experiment as an immunomodulatory model, we observed that the middle age with habitual

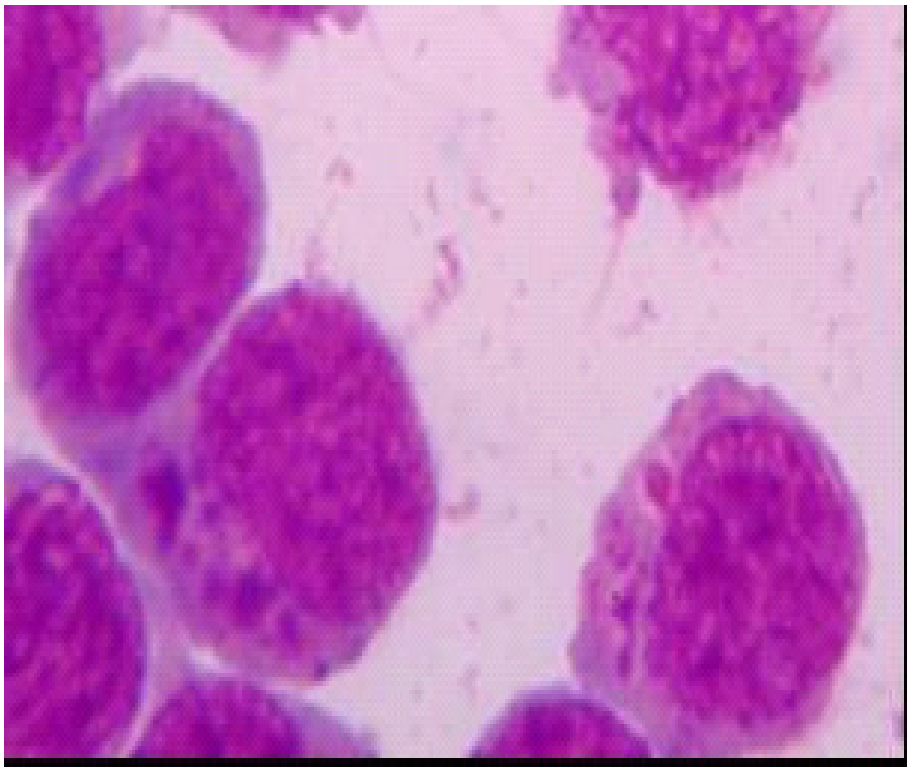

Figure 2a. Morphological features of the untreated U937 cells on day 5 (Magnification 1000×). After 5 days of treatment with normal-MNC-CM, the untreated U937 cells were harvested and cytocentrifuged onto a microscope slide, using a Cytospin2R, and were then stained with Liu's stain.

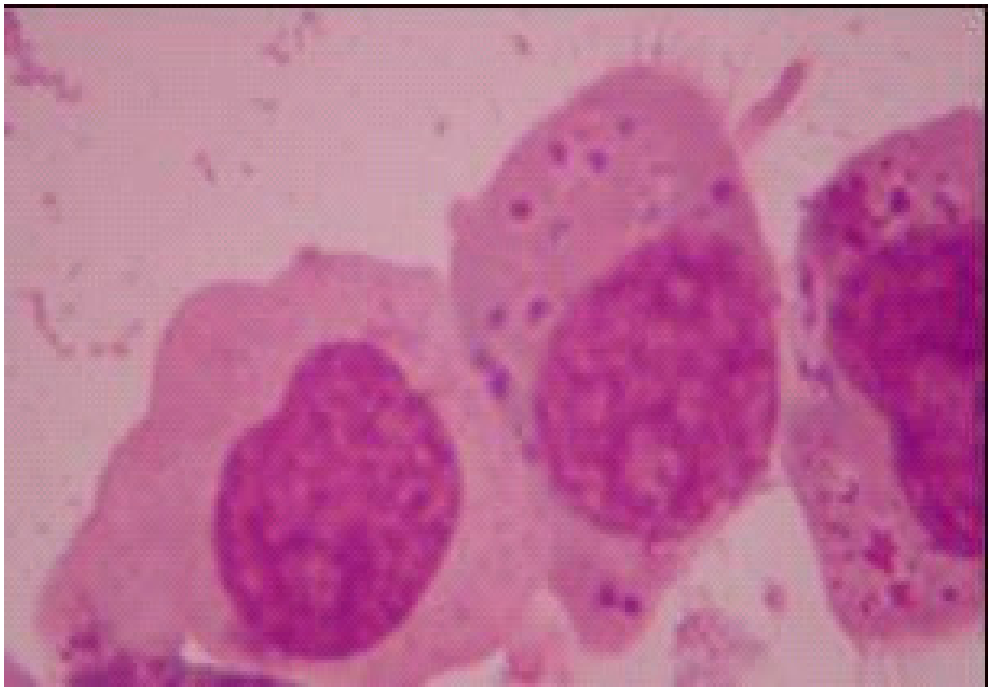

Figure 2b. Morphological features of U937 cells on day 5, differentiated after treatment of PHA-MNC-CM (Magnification 1000×). After 5 days of treatment with PHA-MNC-CM, the treated U937 cells were harvested and cytocentrifuged onto a microscope slide using a Cytospin2R, and were then stained with Liu's stain.

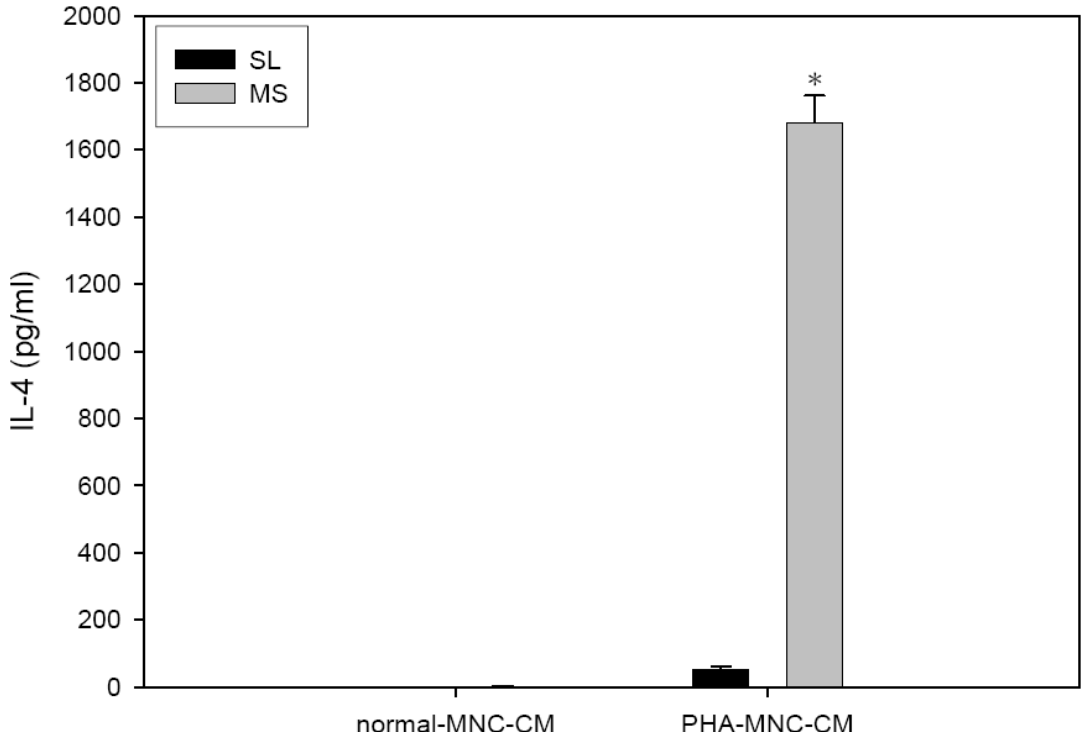

Figure 3. The levels of secreted IL-4 in PHA-MNC-CMs and normal MNC-CM in the MS and SL groups. The levels of secreted IL-4 in the PHA-MNC-CM and normal-MNC-CM of middle age are found in the habitual morning swimming group and sedentary lifestyle control group. The middle age with habitual morning swimming group is (MS) and the sedentary lifestyle control group is (SL). * is significantly different at $p < 0.05$.

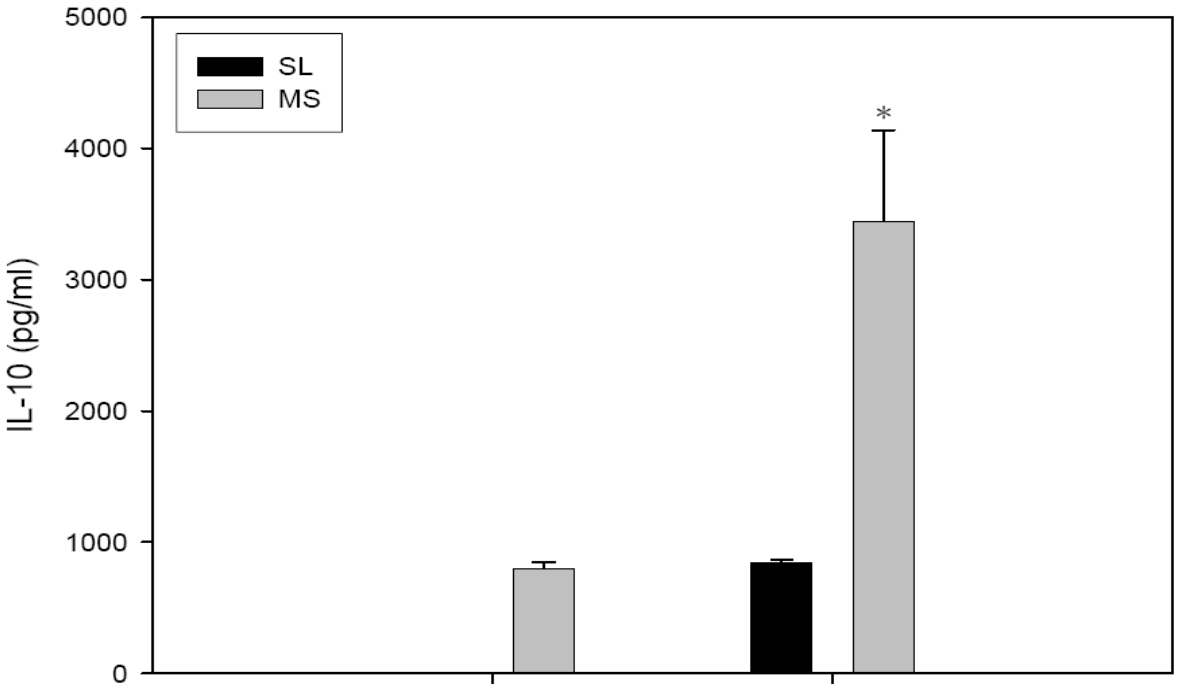

Figure 4. The levels of secreted IL-10 in PHA-MNC-CM and normal MNC-CM in the MS and SL groups. The levels of secreted IL-10 in PHA-MNC-CM and normal-MNC-CM of middle age are found in the habitual morning swimming group and sedentary lifestyle control group. The middle age with habitual morning swimming group is (MS) and the sedentary lifestyle control group is (SL). * is significantly different at $p < 0.05$.

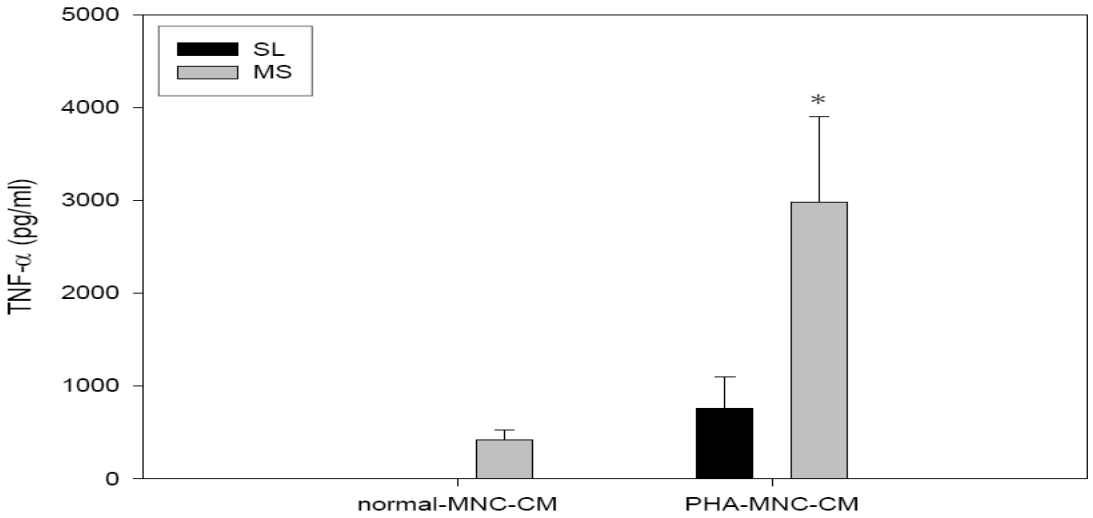

Figure 5. The levels of secreted TNF-α in PHA-MNC-CM and normal MNC-CMs in the MS and SL groups. The levels of secreted TNF-α in PHA-MNC-CM and normal-MNC-CM of middle age are found in the habitual morning swimming group and sedentary lifestyle control group. The middle age with habitual morning swimming group is (MS) and the sedentary lifestyle control group is (SL). * is significantly different at $p < 0.05$).

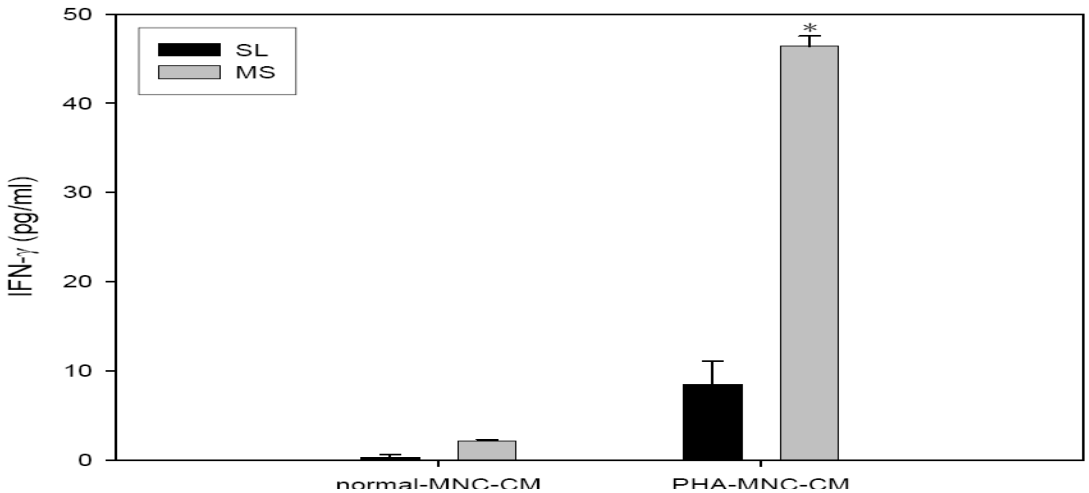

Figure 6. The levels of secreted IFN-γ in PHA-MNC-CM and normal MNC-CM in MS and SL groups. The levels of secreted IFN-□ in the PHA-MNC-CMs and normal-MNC-CM of middle age are found in the habitual morning swimming group and sedentary lifestyle control group. The middle age with habitual morning swimming group is (MS) and the sedentary lifestyle control group is (SL). * is significantly different at $p < 0.05$.

morning swimming have greater immunomodulatory potential to produce more cytokines such as TNF-α, IFN-γ, IL-4 and IL-10 than that of sedentary controls, which are without any other habitual exercise types. In addition, the greater growth inhibition of U937 stimulated by PHA immunomodulatory effects via the MS group can be the other evidences for the greater immunomodulatory potential. In our enrolled health, by the MS and SL groups, the similar anthropometric data without markedly statistic differences in height, weight and age between each other have shown that they have similar cohort. However, all the physical fitness parameters such as maximal oxygen uptake (VO2max), maximal heart rate (MHR) and resting heart rate (RHR), which are greater in the WS group than in the SL group, have shown the effects of exercise on the physiologic properties.

In analysis of the serum BUN, the normal data in both groups have indicated the balance of nitrogen under normal nutritional status in these two groups. From both groups, the normal ranges in serum hemoglobin (Forster and Gariballa, 2005) and albumin (Abbasi and Rudman, 1994; Thalacker-Mercer et al., 2007) have revealed that

they have the normal nutritional status without diseases. We can assume that there is no inflammatory and mal-nutritional status in both groups by the WBC in normal ranges (Abbasi and Rudman, 1994; Thalacker-Mercer et al., 2007). Therefore, our observed immnomodulatory phenomena should not be attributed to the existence of inflammation. Both the similar serum IgG (Maddison and Reimer, 1976) and IgA (Schenkel and Holdren, 2004) which provided the evidence about the abnormal inflammatory status should be ruled out. In other words, no other inflammatory excitation on immune cells can contribute to the immuno-effects in our experimental data in these two groups. Moreover, between both groups, no markedly different CPK values in serum can reveal that any other tissues' damage, such as heart, liver or muscle, occurred to induce the inflammatory effects (Ama et al., 1986; Brewster et al., 2007). We can also assume that the contribution of the immune system responses by the tissues' damage may not be considered in our study. Although the subtropical Taiwan area has a warm weather, the ambient temperatures at winter to spring are relatively cool, especially in the early morning. The swimming exercise intensity can be defined as an aerobically moderate exercise when it is less than 52% of the target heart rate in our study, while it can be defined as a habitually regular exercise when the exercise frequency per week is over 5 days in the MS group. The average water temperatures in swimming pool in our experimental period were about 12.9 ± 0.5 to 18.8 ± 1.2°C during March to May (mean as 15.7 ± 2.9°C). In our prior study, it was shown that the heat stress will deteriorate the immune cells (Chen et al., 2009); yet, the mildly cooling water temperature under 18°C in the morning in Taiwan, may protect all the subjects from overheating and from the deteriorating to immune cells during the swimming exercise periods.

In the study, the recorded exercise frequency for over 5 days per week has shown the habitually regular exercise in the MS group. The habitual swimming exercise done very early in the morning with relatively lower temperature (at about 15°C) may provide a protective environment against the heat stress damage to immune cells to keep the greater effects of immunomodulatory effects by PHA in the elderly ones.

It was well known that the whole immune system *in vivo* is a team work by various immune cells and cytokines. In our experimental model, the immune cells in PBMNC, used as the *ex vivo* model, secrete various cytokines, which may modulate both the secreting immune cells themselves and other sub-populations; in other words, the real immune system in the human body is simulated as the *in vivo* network. Therefore, the isolated PBMNC which contain a heterogeneous population of leukocytes and monocytes in naivety are more proper to simulate the *in vivo* immune status than any other isolated single subset of the immune cells alone. From many published papers, it was well known that treatment of PHA majorly activates T cells to secret different cytokines, which is also fed back to stimulate the proliferation of T cells themselves in combination with B and NK cells (Tvede et al., 1989). The PBMNC, PHA-MNC-CM and secreted cytokines in our experiment can also observe the phenomena as immunomodulatory effects, which have been mentioned previously. This immunomodulatory model for the evaluation of human anti-leukemic cellular immunity has been widely presented in our previous studies (Chen et al., 1997; Lieu et al., 1992; Taetle et al., 1983).

We knew that immunity will decrease with age, for example, the NK cells cytotoxtic activities (Borrego et al., 1999). However, the greater growth inhibition in U937, which are treated with PHA-MNC-CM from the MS group at day 5, than that from the SL group in mild elderly, revealed that greater immunomodulatory effects might be caused by habitual swimming in lightly cool temperature. From the other evidence in our study, which is relatively in greater viability in leukemia cells (about 90%), we can assume that the growth inhibition in U937 is caused by the immunomodulatory effects rather than the killing effects directly. This means that the effects are from immunity. From the morphological observation of treated U937 cells in telescope, the much greater ratio of immature blasts were found in the SL group, while the matured monocytes-macrophages were found in the MS group. This indicated the greater occurrence of differentiation in leukemia cells by immunomodulatory effects in the MS group.

The habitual swimming exercise in elderly persons with lower temperature will modulate the immunity in PBMNC against the proliferation of leukemia cell (Woods et al., 1999). It has pointed out that the moderate aerobic exercise, in long term, can promote the immune function in elderly persons. It was worthy of note that the immunomodulatory effects by PHA against hepatitis B virus are found in elderly persons (Chen et al., 2008). In fact, the prevalence of some specific types of cancers does exist reversely to the degree of physical activity (Hsieh et al., 2010). The TNF-α secretion is induced less in young donors, while immunomodulatory effects by lipopoly-saccharide (LPS) are observed more in monocytes purified from the ones that are over 65 years old (Anne et al., 1998). In other words, immunosenescence will lessen the immune response of LPS stimulation to induce less TNF-α., while the observed differentiation effects and colonies inhibition can indicate the immunomodulatory effects against leukemia by TNF-α. In the test of promyelocytes, HL-60 is exposed to TNF-α *in-vitro* (Kikuchi et al., 1996; Munker and Koeffler, 1987). From our results about the dramatically higher secretion of TNF-α in the MS group than in the SL group, another evidence has been shown for the elevation of immunomodulatory effects by regular swimming exercise with cool temperature in elderly persons. The aged mice, which significantly secreted lower levels of TNF-α in macrophages, were also revealed by other immunosenescence phenomena (Renshaw et al., 2002).

The INF-γ which acts as a major central mediator of the immunoregulatory and antitumor effects has been well

documented (Brunda et al., 1993; Trinchieri, 1998), while the decreases observed in secreting INF-γ by stimulators with the progression of age have been well mentioned in the paper by Rink et al. (1998). From the present experimental data, the manifested higher concentrations of secreted INF-γ in the MS group conditioned medium provided more evidence for the elevation of immunomodulatory effects by regular swimming exercise with cool temperature in elderly persons. The roles played by INF-γ in the growth inhibition of leukemia can be assumed to be the differentiating effects rather than the killing effects. Similar evidence can also be seen in the induction of differentiation by highly purified natural INF-γ in the human promyelocytic leukemia HL-60 cell line (Takei et al., 1984).

Fundamentally, similar roles are played by TNF-α and IFN-γ as differentiation promoters, rather than killing agents in leukemia U937. In fact, the combined treatment of both TNF-α and IFN-γ can effectively modulate the differentiation of U937 cells (Kikuchi et al., 1996). The immune responses of PHA in producing both greater TNF-α and IFN-γ amount in elderly persons with Tai Chi Chuan exercises in the morning are seen to be less than 50% VO2max from mild to moderate intensity. Moreover, the sedentary control of elderly persons has shown a relationship between these two cytokines and the amelioration of the immunosenescence with advancing age (Chen et al., 2008).

The IL-4 and IL-10 are usually deemed as anti-inflammatory cytokines (Zaldivar et al., 2006). In our study, after the stimulation by PHA, the greater secreted amount in IL-4 and IL-10 in the MS group was found to be 32 and 4 folds than that of the SL group, indicating the occurrence of anti-inflammatory effects. It is well known that the physiologic stress during exercise can activate the leukocytosis via inflammatory effects in the human body (Zaldivar et al., 2006). However, the unique physiologic characteristics (as physiologic stress) under cool condition may induce both the pro-inflammatory and anti-inflammatory effects.

Conclusions

The greater response of human peripheral mononuclear cells via a stimulation of phytohemagglutinin may render the greater differentiation in U937 leukemia cells. From the afore-mentioned results, we can assume that the greater immunomodulatory effects of human peripheral mononuclear cells in middle age habitual morning swimming against the proliferation in leukemia cells U937 may be attributed to the greater cytokines secretion. In summary, the swimming exercise as regular, moderate and gentle cool temperature exerted greater immunomodulatory effects on elderly persons.

REFERENCES

Abbasi AA, Rudman D (1994). Undernutrition in the nursing home: Prevalence, consequences, causes and prevention. Nutr. Rev., 5: 113-122.

Ama PF, Simoneau JA, Boulay MR, Serresse O, Theriault G, Bouchard C (1986). Skeletal muscle characteristics in sedentary black and Caucasian males. J. Appl. Physiol., 61: 1758-1761.

Anne DD, Marc JB, Zahra M, Sophie F, Ouahid B (1998). Signal Transduction in LPS-Activated Aged and Young Monocytes. J. Interf Cytokine Res., 8(6): 429-437.

Borrego F, Alonso MC, Galiani MD, Carracedo J, Ramirez R, Ostos B, Pena J, Solana R (1999). NK phenotypic markers and IL-2 response in NK cells from elderly people. Exp. Gerontol., 34: 253-265.

Brenner IKM, Castellani JW, Gabaree C, Young AJ, Zamecnik J, Shephard RJ, Shek PN (1999). Immune changes in humans during cold exposure: effects of prior heating and exercise. J. Appl. Physiol., 87: 699-710.

Brewster LM, Mairuhu G, Sturk A,van Montfrans GA (2007). Distribution of creatine kinase in the general population: implications for statin therapy. Am. Heart J., 154: 655-661.

Bridges CB, Harper SA, Fukuda K, Uyeki TM, Cox NJ, Singleton JA (2003). Advisory Committee on Immunization Practices. Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP). Morbidity Mortality Weekly Rep., 52: 1-34.

Brunda MJ, Luistro L, Warrier RR, Wright RB, Hubbaro BR, Murphy M, Wolf SF, Gately MK (1993). Antitumor and antimetastatic activity of interleukin 12 against murine tumors. J. Exp. Med., 178: 1223-1230.

Chen YJ, Huang AC, Chang HH, Liao HF, Jiang CM, Lai LY, Chan JT, Chen YY, Chiang J (2009). Caffeic acid phenethyl ester, an antioxidant from propolis, protects peripheral blood mononuclear cells of competitive cyclists against hyperthermal stress. J. Food Sci., 74(6): 162-167.

Chen YJ, Shiao MS, Lee SS, Wang SY (1997). Effect of Cordyceps Sinensis on the proliferation and differentiation of human leukemic U937 cells. Life Sci., 60: 2349-2359.

Chen YY, Chang HM (2004). Antiproliferative and Differentiating Effects of polysaccharide Fraction from Fu-Ling (Poria cocos) on Human Leukemic U937 and HL-60 Cells source. Food Chem. Toxicol., 42: 759-769.

Chen YY, Chiang J, Chen YJ, Chen KT, Yang RS, Lin JG (2008). Cycling and Tai Chi Chuan exercises exert greater immunomo-dulatory effect on surface antigen expression of human hepatitis B virus. Chinese Med. J., 121(21): 2172-2179.

Chiang J, Huang YW, Chen ML, Wang SY, Huang AC, Chen YJ (2000). Comparison of anti-leukemic immunity against U937 cells in endurance athletes versus sedentary controls. Int. J. Sports Med., 21: 602-607.

Dussault I, Miller SC (1994). Decline in natural killer cell-mediated immunosurveillance in aging mice-a consequence of reduced cell production and tumor binding capacity. Mech. Ageing Dev., 75: 115-129.

Forster S, Gariballa S (2005). Age as a determinant of nutritional status: a cross sectional study. Nutr. J., 27(4): 28.

Frisch RE, Wyshak G, Albright NL (1985). Lower prevalence of breast cancer and cancers of the reproductive system among former college athletes compared to non-athletes. British J. Cancer., 52: 885-891.

Frisch RE, Wyshak G, Albright NL, Albright TE, Schiff I (1989). Lower prevalance of non-reproductive system cancers among female former college athletes. Med. Sci. Sports Exercise, 21: 250-253.

Geneva-Popova M, Murdjeva M (1999). Study on proinflammatory cytokines (IL-1 beta, IL-6, TNF-alpha) and IL-2 in patients with acute hepatitis B. Folia Medica (Plovdiv)., 41: 478-481.

Gotfried MH (2001). Epidemiology of clinically diagnosed communityacquired pneumonia in the primary care setting: results from the 1999–2000 respiratory surveillance programs. Am. J. Med., 111: 25-29.

Hsieh KC, Chen YY, Jang TR, Chen YJ (2010). Middle-aged Subjects With Habitual Low-speed Cycling Exercise Have Greater Mononuclear Cell Responsiveness Against Human Hepatitis B Virus Surface Antigen. Gerontol., 4(2): 82-88.

Jansky L, Pospisilova D, Honzova S (1996). Immune system of cold-exposed and cold-adapted humans. Eur. J. Appl. Physiol., 72: 445-450.

Karvonen MJ, Kentala E, Mustala O (1957). The effects of training on heart rate: a longitudinal study. Annales medicinae Experimentalis Et Biologiae Fenniae., 35(3): 307-315.

Kikuchi HR, Iizuka S, Sugiyama GN, Mori H, Arai M, Mizumoto K, Imajoh-Ohmi S (1996). Monocytic differentiation modulates apoptotic response to cytotoxic anti-Fas antibody and tumor necrosis factor alpha in human monoblast U937 cells. J. Leukocyte Biol., 60: 778-783.

Lackovic V, Borecky L, Vigas M, Rovensky J (1988). Activation of NK cells in subjects exposed to mild hyper- or hypothermic load. J. Interf. Res. 8: 393-402.

Liao HF, Chiang LM, Yen CC, Chen YY, Zhuang RR, Lai LY (2006). Effect of a periodized exercise training and active recovery program on antitumor activity and development of dendritic cells. J. Sports Med. Phys. Fitness. 46: 307-314.

Lieu CW, Lee SS, Wang SY (1992). The effect of Ganoderma lucidum on induction of differentiation in leukemic U937 cells. Anticancer Res., 12: 1211-1215.

Maddison SE, Reimer CB (1976). Normative values of serum immunoglobulins by single radial immunodiffusion: a review. Clin. Chem., 22: 594-601.

Mcclary H, Koch R, Chisari FV, Guidotti LG (2000). Relative sensitivity of hepatitis B virus and other hepatotropic viruses to the antiviral effects of cytokines. J. Virol., 74: 2255-2264.

Munker R, Koeffler HP (1987). Tumor necrosis factor : recent advances. Klin Wochenschr., 65(8): 345-352.

Nieman DC, Buckley KS, Henson DA, Warren BJ Suttles J, Ahle JC, Simandle S, Fagoaga OR, Nehlsen-Cannarella SL (1995). Immune function in marathon runners versus sedentary controls. Med. Sci. Sports Exercise, 27: 986-992.

Nieman DC, Henson DA, Gusewitch G, Warren BJ, Dotson RC, Butterworyh DE, Nehlsen-Cannarella SL (1993). Physical activity and immune function in elderly women. Med. Sci Sports Exercise., 25: 823-831.

Plackett TP, Schilling EM, Faunce DE, Choudhry MA, Witte PL, Kovacs EJ (2003). Aging enhances lymphocyte cytokine defects after injury. The FASEB journal: official publication of the Federation of American Soc. Exp. Biol., 17: 688-689.

Potter JM, O'Donnell B, Carman WF, Roberts MA, Stott DJ (1999). Serological response to influenza vaccination and nutritional and functional status of patients in geriatric medical long-term care. Age Ageing., 28: 141-145.

Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz, J, Sambhara S (2002). Cutting edge: impaired Toll-like receptor expression and function in aging. J. Immunol., 169: 4697-4701.

Rink L, Cakman I, Kirchner H (1998). Altered cytokine production in the elderly. Mech. Ageing Dev., 102: 199-209.

Ruiz M, Santiago E, Marcos MA, Martinez JA, Arancibia F, Mensa J, Torres A (1999). Etiology of community-acquired pneumonia: impact of age, comorbidity, and severity. Am. J. Respiratory Critical Care Med., 160: 397-405.

Schenkel EJ, Holdren KA (2004). Family members of patients with selective IgA deficiency should be routinely screened for primary immunodeficiency. J. Allergy Clin. Immunol., 1 13(2): 126.

Suri D, Schilling R, Lopes AR, Mullerova I, Colucci G, Williams R, Naoumov, NV (2001). Non-cytolytic inhibition of hepatitis B virus replication in human hepatocytes. J. Hepatol., 35: 790-797.

Taetle R, Caviles A, Koziol J (1983). Response of human myeloid leukemia cells to various sources of colony-stimulating activity and phytohemagglutinin-conditionedmedium. CancerRes., 43: 2350-2357.

Takei M, Takedak K, Konno K (1984). The role of interferon-gamma in induction of differentiation of human myeloid leukemia cell lines, ML-1 and HL-60. Biochem. Biophys. Res. Commun., 124: 100-105.

Thalacker-Mercer AE, Johnson CA, Yarasheski KE, Carnell NS, Campbell WW (2007). Nutrient ingestion, protein intake, and sex, but not age, affect the albumin synthesis rate in humans. J. Nutr., 137(7): 1734-1740.

Trinchieri, G (1998). Interleukin-12: a cytokine at the interface of inflammation and immunity. Adv. Immunol., 70: 83-243.

Tvede N, Pedersen BK, Hansen FR, Bendix T, Christensen LD, Halkjaer-Kristensen J (1989). Effect of physical exercise on blood mononuclear cell subpopulation and in vitro proliferative responses. Scandinavian J. Immunol., 29: 383-389.

Wang SY, Chen LY, Wang SJ, Lin CK, Ho CK (1991). Growth inhibition and differentiation in HL-60 leukemia cells induced by 1, 25-dihydroxyvitamin D3 and tumor necrosis factor alpha. Exp. Hematol., 19: 122-127.

Woods JA, Ceddia MA, Wolters BW, Evans JK, Lu Q, McAuley E (1999). Effects of 6 months of moderate aerobic exercise training on immune function in the elderly. Mechan. Ageing Dev., 109: 1-19.

Zaldivar F, Wang-Rodriguez J, Nemet D, Schwindt C, Galassetti P, Mills PJ, Wilson LD, Cooper DM (2006). Constitutive pro- and anti-inflammatory cytokine and growth factor response to exercise in leukocytes. J. Appl. Physiol., 100: 1124-1133.