

Swim Training Increases Ovalbumin Induced Active Systemic Anaphylaxis in Mice

Chan-Hoi Kim¹ and Yi-Sub Kwak, Ph.D.^{2,*}

¹Division of Sports and Well-Being, College of Human Movement Sciences,
Hanyang University-Ansan, Kyunggi-do, South Korea

²Institute of Sport Science, Dongeui University, Busan, South Korea

ABSTRACT

Aerobic training can be defined as any physical exercise that increases the heart rate and enhances the body's intake of oxygen long enough to benefit the condition of the body. Running, cycling, and swimming are examples of aerobic activities. In recent years, the importance of sports in everyday life has been rapidly increasing. Moderate exercise appears to stimulate the immune system. However, healthy elite runners often complain about bronchial symptoms after heavy exercise. Exercise-induced asthma and active systemic anaphylaxis are the most common problems seen in these individuals. The inter-relationship of exercise and the allergy response has not been well studied. This study was designed to examine the effects of regular swim training on body weight, spleen index, the number of lymphocytes, scoring of active systemic anaphylactic shock, proliferative activity of splenic lymphocytes and cytokine levels in BALB/c mice. Thirty mice (6 weeks old) were involved in this study and they were divided into 3 groups: a control group (Control, n = 10), a sensitized group (Sensitized, n = 10), and a sensitized-trained group (Sen-trained, n = 10). The sen-trained group was studied after 10 weeks of regular swim training. All data were expressed as mean and standard deviation by using SPSS (ver.10.0). The swim training caused a decrease in body weight ($p < 0.05$), an increase of spleen index, active

*Correspondence: Yi-Sub Kwak, Ph.D., Institute of Sport Science, Dong-eui University, 995 Eomgwangno, Busanjin-gu, Busan 614-714, South Korea; Fax: 82-51-890-2643; E-mail: ysk2003@deu.ac.kr.

systemic anaphylaxis, lymphocyte proliferation (stimulated with ovalbumin), and cytokine levels (especially IL-4) when comparing the sen-trained group to the sensitized group ($p < .05$). These data indicate that there is a link between allergy anaphylaxis and regular swim training. This may be due to increased lymphocyte proliferation (stimulated with ovalbumin), ASAS (active systemic anaphylactic shock) score, and IL-4 cytokine levels after exercise.

Key Words: Swim training; Active systemic Anaphylaxis; Ovalbumin; Immune system.

INTRODUCTION

There has been a growing interest in increased physical exercise in leisure time throughout the world. Various types of exercise, such as running, swimming, and tennis, are routinely done. These types of activities increase heart rate and the body's intake of oxygen long enough to benefit the condition of the human body. Thus, research concerning the impact of physical exercise on the body's immune system has become an important issue for the general public, athletes, and specific disease models using obese & hypercholesterolemic rats (Kwak et al., 2000). Long-term obesity may reduce the size of the T cell pool and impair the responsiveness of splenocytes in genetically obese rats (Tanaka et al., 1998). Experimental findings suggest that regular swim training may induce proper immune responses. It is generally believed that a program of moderate exercise has beneficial effects on immune function (Morimoto, 2000). However, very heavy and prolonged exercise can have adverse consequences (Boyum et al., 1996). This is the best documented as an increased vulnerability to viral infections and allergic reactions. The allergic reactions induced are exercise-induced asthma and exercise-induced anaphylaxis. Exercise-induced asthma is characterized by transient airway obstruction, occurring after strenuous exertion. A fall of 10% or more in the FEV1 (forced expiratory volume in one second) after exercise is diagnostic (Inman, 1999; Tan and Spector, 1998). The mechanism that causes exercise-induced asthma is thought to be related to the consequences of heating and humidifying large volume of air during exercise.

Exercise-induced anaphylaxis is a unique physical allergy with increasing incidence as the exercising population increases. Clinical features are indistinguishable from IgE mediated anaphylaxis in which the offending allergens are known (Nelde et al., 2001). This is also characterized by airway obstruction, urticaria, and hypotension following physical exercise, which can be associated with food allergies (Okazaki et al., 1992).

Allergies are a member of a class of immune responses termed hypersensitivity reactions that produce tissue injuries and can cause serious disease. Allergic reactions are triggered when allergens cross-link performed IgE bound on the high-affinity FcεR1 of mast cells. Then, mast cells release stored mediators by granule exocytosis, and by synthesizing leukotrienes and cytokines (Tuner and Jean-Pierre, 1999).

There are many reports concerning exercise and allergic anaphylaxis (Harada et al., 2001; Hosey et al.; Huang et al., 2000; Karol, 2002; Medralla et al., 2001).

Exercise-induced anaphylaxis is a unique form of physical allergy that has been recognized with increasing frequency in recent years. The hallmarks of this disease are generalized pruritus with a flushing sensation, a feeling of warmth, and the development of urticaria in association with vigorous physical exertion. These symptoms tend to occur variably with exercise, but not with passive warming (Helenius et al., 1996). Management of these conditions includes patient education, antihistamines, and in some cases, immunomodulation of the response (Inman, 1999). Cessation of exercise with onset of symptoms and self-administration of epinephrine are recommended (Briner, 1993).

Taken together, exercise training is related with changes in allergic reactions. There are few research papers about exercise-induced allergy, especially related to the specific allergens. In order to know how exercise training is involved in allergic responses in mice which have exercised, we investigated the spleen index, anaphylactic score, total lymphocytes, proliferative activity of spleen lymphocytes, and cytokine levels in control, sensitized, and sen-trained group following peritoneal sensitization with ovalbumin.

MATERIALS AND METHODS

Animals

Male BALB/c mice (6 weeks old) were obtained from the Korean Institute of Science Technology (Daejeon, Korea). The animals were housed in a pathogen-free animal facility (22–24°C temperature, 44–55% relative humidity, 12 hr photo period) at University College of Medicine, Seoul, Korea. Food and water were available *ad libitum*. After 1 week of acclimatisation to the new environment, they were divided into control (Control, $n = 10$), sensitized (Sensitized, $n = 10$), and sensitized-trained (Sen-trained, $n = 10$) groups. The sensitized-trained group (Sen-trained, $n = 10$) swam for 10 minutes for the first week (5 days/week). During subsequent swim adaptation periods, they swam for 30 minutes for the second week in a tub measuring 63 cm $\times$ 40 cm and 18 cm depth. The size of the tub sufficiently assured the animals would swim freely and not float passively. The tub was deep enough so that they could not rest upon an extended tail.

Allergy Sensitization and Swim Training

Groups of mice were immunized by intraperitoneal sensitization with a single injection of mixture of ovalbumin (OVA, 1 mg/mouse), aluminum hydroxide (20 μ g/mouse) and *B. pertussis* (1×10^9 bacteria/mouse). The mice were then divided into 3 groups: the control, sensitized (allergy sensitization for the controlled mice), and sensitized-trained (allergy sensitization for the swim trained mice compare to sensitized group). The swim-trained mice underwent a 10 week endurance swim training including 2 weeks adaptation period (above mentioned) in water 30–35°C for 60 min. The fur of each mouse was washed with liquid soap prior to swimming and air bubbles trapped in the fur were removed periodically to reduce buoyancy and ensure the

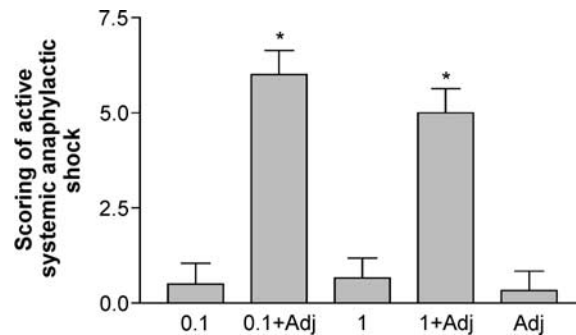


Figure 1. Scoring of active systemic anaphylactic shock. The mice were intraperitoneally sensitized with several dose of OVA (0.1, 1 mg/mouse) with and without adjuvants, aluminium hydroxide (20 μ g/mouse) and *B. pertussis* (1×10^9 bacteria/mouse) and single injection of adjuvants. Induction of active systemic anaphylactic shock in the sensitized mice was performed by a single intravenous injection of OVA (100 μ g/mouse) on the 15th days after sensitization.

imposed work load. Exhaustion was determined by the inability of the mice to remain on the surface of water. At the time of exhaustion, mice were given a 3 min recess. At the end of the training period, 48 h after last training session, the mice were anaesthetized with CO₂ and sacrificed.

Sensitization and Active Systemic Anaphylactic Shock (ASAS)

For OVA sensitization, BALB/c mice were injected once intraperitoneally with a mixture of OVA (0.1 mg, 1 mg with and without adjuvant/mouse), aluminum hydroxide (20 μ g/mouse) and *B. pertussis* (1×10^9 bacteria/mouse). The results (Fig. 1), showed that 1 mg + adjuvant was the optimal dose for mice sensitization. ASAS score was measured by intravenous injection with 100 μ g of OVA at the time of 15th days after the peritoneal sensitization (1 mg + adjuvant) (Table 1) (Haffner, 1929).

Table 1. Scoring of active systemic anaphylactic shock.

Shock score	Sign
0	No sign
1	Decreasing bouts of spontaneous activity and piloerection
2	Loss of coordination and dyspnea
3	No activity following whisker stimuli and slight activity after prodding with ball-point pen
4	No activity following whisker stimuli, progressive paresis beginning with the behind leg
5	No activity following Haffner's tail pinching stimuli by forcep
6	Brief but violent convulsion, prostration, coma or substantial recovery
7	Fatal shock(died within 30–60 min)
8	Fatal shock(died within 15 min)

Reagents

Ovalbumin, concanavalin A (ConA) and lipopolysaccharide (LPS, L 4005) were obtained from Sigma Chemical Co. (ST. Louis, MO, USA), and formalin-inactivated *Bordetella pertussis* was obtained from the Green -Cross Co. (Seoul, Korea). The ELISA kits (IFN- γ and IL-4) were purchased from the Endogen Inc. (Boston, MA, USA).

Spleen Index

Spleens from the treated animals were removed and weighed to examine the development of splenomegaly compare to control groups. The spleen index was calculated as the weight of the spleen divided by the weight of the animal. The spleen index was calculated according to a formula (Chandra et al., 1991).

Isolation of Spleen Lymphocytes

The spleen was removed and a single- cell suspension was prepared by gently teasing between two sterilized slide glasses (frosted end). The mesh and culture dish were rinsed with medium, and the suspension collected in sterile 15 ml conical tubes. Red blood cells were lysed using an ACK lysing buffer [8.28 g NH_4Cl /1 g KHCO_3 /37.2 mg $\text{Na}_2\text{EDTA}/\text{H}_2\text{O}$ 1 liter/pH 7.2–7.4]. The spleen lymphocytes were prepared in an RPMI 1640 medium, supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 2.2 mM sodium bicarbonate, 100 units/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin.

Proliferation Assays

Splenic lymphocytes were used at a final density of 1×10^6 cells/ml. Two days after sensitization, the mice were sacrificed. Lymphocytes were adjusted to 1×10^6 cells/ml in a complete RPMI medium and 200 μl of cell suspensions were plated in 96-well rounded bottom micro titer plates. OVA (20 $\mu\text{g}/\text{well}$) was added to the wells and cultures were maintained for 4 days in an atmosphere of 95% air, 5% carbon dioxide, and in a humidified CO_2 incubator at 37°C . Cell proliferation was measured using ^3H -thymidine incorporation (^3H -TdR, New England Nuclear Boston, MA, USA).

Cytokine Assay

For in vitro production of IFN- γ and IL-4, the number of lymphocytes was adjusted to 1×10^6 cells/ml in a complete RPMI medium, and 1 ml of cell suspensions were plated in each well of 24-well tissue culture plates. OVA (100 $\mu\text{g}/\text{well}$) was added to the wells and the cells were cultured for 4 days in a humidified 5% CO_2 incubator at 37°C . The culture supernatants were harvested from the plates and stored at -20°C .

The concentration of IFN- γ and IL-4 in the supernatants was determined with murine IFN- γ and IL-4 ELISA kits (Endogen Inc., Boston, MA, USA).

Analysis of Data

All data were expressed as the mean $\pm$ SD. Statistical analysis of weight changes was performed using an unpaired t test in the comparison study. Applicability of the t test to the data was verified by the F test and Welch's correction. Statistical analysis of differences was performed using a one-way ANOVA in the comparison study to evaluate the effects of swim training and OVA sensitization. Significance was measured using Fisher's least significant for the exact p values. Differences were considered statistically significant at p value less than 0.05.

RESULTS

Body Weight and Spleen Index

As shown in Fig. 2, the body weight after 10-week swim training was significantly lower than the control group ($p < .05$). After 10 week swim training, the body weight of the control and trained groups were 33.7 ± 0.327 g and 31.0 ± 0.147 g respectively. This difference was 2.65 g was lower in the trained compared to the control group. After 10 week training an increase in the size of the spleens was seen in the trained group. The spleen indexes of the mice treated with sen-trained group was the highest, followed by the sensitized group, and the control group had the lowest spleen

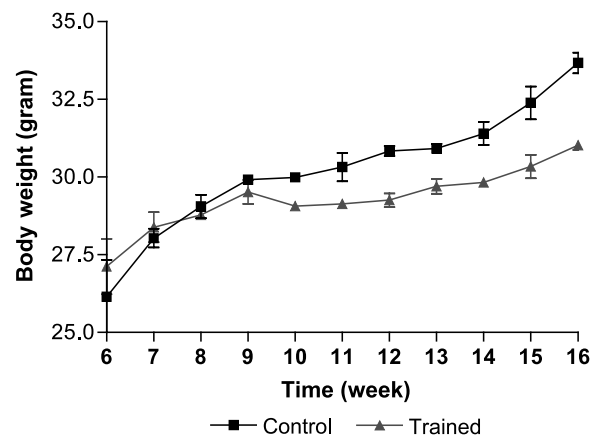


Figure 2. The change of body weight. The mice underwent a 10-wk swimming program (1 h/day, 5 days/wk) in water at 30–35°C. We measured the body weight every week at the end of last day before swim training.

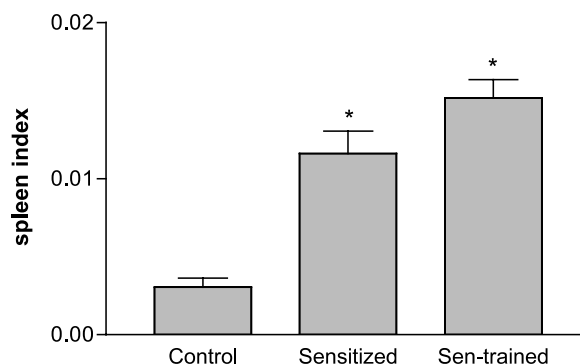


Figure 3. The change of spleen index. The mice underwent a 10-wk swimming program (1 h/day, 5 days/wk) in water at 30–35°C. After the swimming program, the spleens were removed and weighed. The spleen index was calculated as described in materials and methods.

size (Fig. 3). The spleen indexes were 0.014 ± 0.002 , 0.012 ± 0.001 , and 0.003 ± 0.001 respectively.

Swim Training Increases the Active Systemic Anaphylaxis (ASAS)

Control, sensitized, and sen-trained groups were used to evaluate the effect of swim training on allergic responses. The mice were challenged with OVA 0.1 mg via tail vein to induce ASAS to comparatively evaluate the ASAS scores. We tested two doses of OVA (0.1 and 1 mg/mouse) and adjuvant (aluminum hydroxide; 20 μ g/mouse & *B. pertussis*; 1×10^9 /mouse), as shown in Fig. 1. Single treatment of OVA without adjuvant (0.1 mg and 1 mg) and adjuvant alone resulted in lower ASAS scores than the mixture of OVA and adjuvant. A 1 mg dose of OVA with adjuvant yielded the optimum score in ASAS,

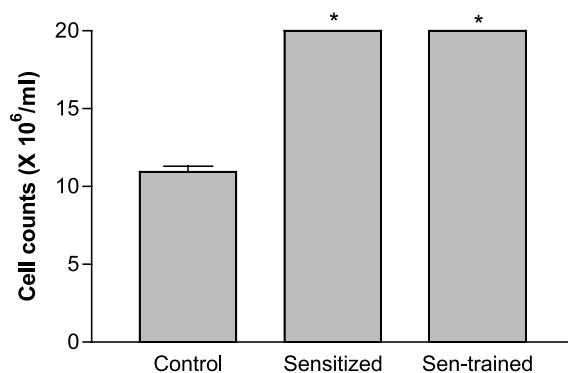


Figure 4. The change of total lymphocytes following the swimming training. The mice underwent a 10-wk swimming program (1 h/day, 5 days/wk) in water at 30–35°C. After the swimming program, the spleens were removed and counted with trypan blue.

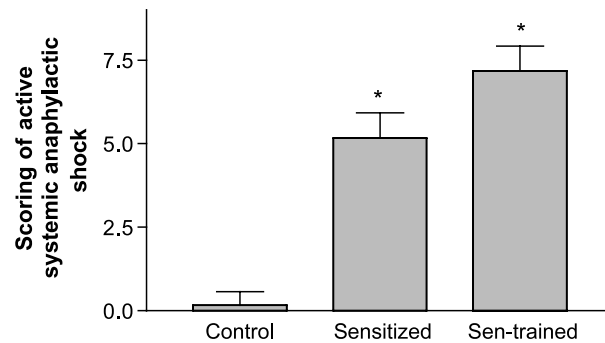


Figure 5. The scoring of active systemic anaphylactic shock following the swim training. The mice were intraperitoneally sensitized with a single injection of mixture of OVA (1 mg/mouse) and adjuvant, aluminum hydroxide (20 µg/mouse) and *B. pertussis* (1×10^9 bacteria/mouse). Induction of active systemic anaphylactic shock in the control, sensitized, and sen-trained mice was performed by a single intravenous injection of OVA (1 mg/mouse) on the 15th days after sensitization. Shock score was determined as described in Table 1. Results are the mean $\pm$ S.D. * $P < .05$ vs. control group.

so this dose was chosen to induce allergy response. After 10 weeks of swim training, ASAS was induced using OVA with adjuvant (as above). The sen-trained group had the highest ASAS score (Fig. 5). The score of ASAS were 0.167 ± 0.408 , 5.167 ± 0.753 and 7.167 ± 0.753 for the control, sensitized and sen-trained group. These results suggest that swim training appears to increase the ASAS.

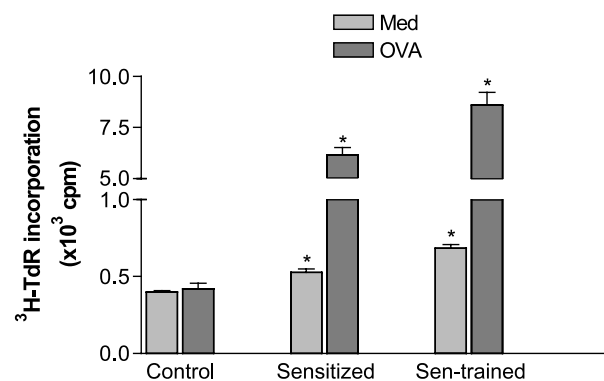


Figure 6. The effect of swim training on lymphocyte proliferation of spleen lymphocyte from Mice sensitized with OVA. The mice were intraperitoneally sensitized with a single injection of a mixture of OVA (1mg/mouse) and adjuvant, aluminum hydroxide (20 µg/mouse) and *B. pertussis* (1×10^9 bacteria/mouse), and thirteen days later, the mice were sacrificed and the spleen lymphocytes were isolated. Cells were seeded in 96 well round bottomed microtiter plates (2×10^5 cells/200 µl/well) and cultured in the absence or the presence of OVA (100 µg/well) for 4 days. Cell proliferation was determined by the technique of ³H-thymidine incorporation. Results are the mean $\pm$ S.D. * $P < .05$ vs. control group.

Treatment of Swim Training and Stimulation with OVA Does Not Increase the Cell Counts

Sen-trained mice, which were sensitized with OVA, showed the highest levels of spleen cell counts. The sensitized mice also showed higher levels of cell counts compared to the control group. The score of total lymphocytes were $11.0 \times 10^6/\text{ml}$, $23.3 \times 10^6/\text{ml}$, and $20.3 \times 10^6/\text{ml}$ for the control, sensitized and sen-trained groups (Fig. 4). And proliferation of spleen lymphocyte showed the highest in sen-trained stimulated with Med and OVA (Fig. 6). These results suggest that sensitization of OVA and swim training induce splenomegaly caused by spleen cell proliferation.

Swim Training Induces IL-4 Production More than INF- γ in the OVA Sensitized Mice

The production of IL-4 and INF- γ by spleen lymphocytes from OVA sensitized mice with regular swim training was studied. OVA sensitized mice were treated with OVA and adjuvant following the end of swim training. 15 days later spleen lymphocytes were isolated and cultured in RPMI media with OVA for 4 days. IL-4 and INF- γ levels in the cultured supernatants were assayed by ELISA. As shown in Fig. 7, after OVA stimulation, the production of IL-4 ($142.4 \pm 9.94 \text{ pg/ml}$) by spleen lymphocytes from sensitized-trained mice were higher than production by the cells from the sensitized ($98.8 \pm 12.90 \text{ pg/ml}$) and control groups ($25.4 \pm 7.46 \text{ pg/ml}$), and these differences were statistically significant ($p < .05$). However, the production of INF- γ ($137.4 \pm 9.35 \text{ pg/ml}$) by spleen lymphocytes from the sensitized mice were higher than the cells from sensitized-trained ($108.6 \pm 13.70 \text{ pg/ml}$) and control groups ($44.9 \pm 10.80 \text{ pg/ml}$), which were also statistically significant ($p < .05$).

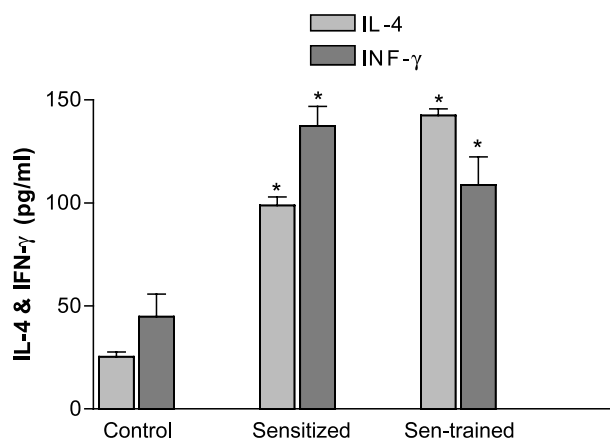


Figure 7. The effect of swim training on the production of IL-4 and INF- γ by spleen lymphocytes from BALB/c mice sensitized with OVA. The cells ($1 \times 10^6/\text{ml}$) were cultured with OVA ($500 \mu\text{g}/\text{well}$) for 4 days. The levels of cytokines in the culture supernatants were determined using murine IL-4 and INF- γ ELISA kits. Results are the mean $\pm$ S.D. * $P < .05$ vs. control group.

DISCUSSION

Exercise -induced anaphylaxis frequently is related to food ingestion, so that it receives the name of 'food-dependent exercise-induced anaphylaxis.' It is an uncommon condition. However, it is increasing reported, possibly in relation to an augmentation of physical exercise. In one-third of the patients, anaphylactic reactions are associated with a specific food ingested before exercise (Tamarcarz et al., 2001). The diagnosis of exercise-induced anaphylaxis is based on screening for potential food allergens by anaphylaxis score, skin prick tests (Romano et al., 1995), and changes of serum INF- γ , interleukin- 4. Elevated IL-4 levels regulate IgE synthesis and this is well characterized by the Food-induced anaphylaxis patients (Fireman, 2003; Testa et al., 2001).

In the recent years, the importance of regular exercise in everyday life has rapidly increased. However, exercise-induced asthma and exercise-induced allergies are among the most common problems for those individuals practicing exercise. The prevalence of these diseases is increasing worldwide. The increase has been found in both sexes, and all ethnic and age groups. Therefore, the identifying mechanisms of regular exercise and allergy response in recent times are of great interest.

From the results, lower levels of allergens with adjuvant could induce ASAS (active systemic anaphylaxis) easier than the other doses. We used the optimum level of allergens (1 mg OVA with adjuvant) to induce allergies (Fig. 1).

Swim training decreases body weight, compared to the control group by weeks. This was consistent with the results reported from other studies (Boghossian et al., 2000; Huang et al., 2003; Kwak et al., 2002; Lemitsu et al., 2002; Tanabe et al., 2003). Swim training increased spleen indexes compared to the control and sensitized groups. Swim-training results in splenomegaly, this was also consistent with the results reported from the other studies (Kwak et al., 2000, 2002). From the results, swim training induces lymphocyte proliferation, especially in the Th2 cells, as opposed to Th1 cells. This was confirmed by increased cell counts and higher ASAS scores which was induced by OVA allergen.

Swim training also increased the number of spleen lymphocytes compared to the control and sensitized groups. The ASAS score was the highest for the sensitized-trained mice group. From the results, swim training induced more allergic anaphylaxis through increased levels of Th2 lymphocytes proliferation, as opposed to the other groups, which was induced by OVA allergen.

This was also supported by the other research data for the swim trained group. For the swim trained, MTB (mycobacterium tuberculosis) counts were much higher than that of the controlled group after aerosol infection, with *M. tuberculosis* H37Rv used an inhalation device (Glass-Col, Terre Haute, Ind). This was caused by lowered levels of INF- γ stimulated by media and CFP (culture filtrated protein) (Kwak and Paik, 2002). In this study, the final data (Fig. 7) shows that although the INF- γ level (Th-1 skewed cytokine) was not the highest, the IL-4 (Th-2 skewed cytokine) level was the highest for sen-trained group than in the other groups. This finding was also consistent with the results reported from other studies (Karol, 2002).

In conclusion, the Sen-trained group has the highest score of allergy anaphylaxis. This result may be explained by the information obtained that regular swim training induces Th2 lymphocyte proliferation, which was caused by increased IL-4 levels for

the Sen-trained group. However, our results demonstrate that there is a link between allergy anaphylaxis and regular swim training. We were not able to demonstrate a direct mechanism linking ASAS and swim training intensity and duration related with several kinds of food (allergen). Further study is urgently needed in this area.

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