

Effects of two types of inactivity on the number of white blood cells in rats

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Abstract Prolonged inactivity is known to induce changes in responses of many physiological defense systems such as the hypothalamo-hypophyseal-adrenocortical axis, the sympathetic nervous system, and immuno-responsive systems. However, effects of various types of inactivity on immuno-responsive systems are still unknown. Therefore, the effects of two types of inactivity (immobilization: IMM and whole body suspension: WBS) on the number of white blood cells were studied in rats. Rats were divided into the control group and each inactivity group to compare the number of total white blood cells, lymphocytes, monocyte, neutrophil, eosinophil, and basophil during the experimental periods. Both IMM and WBS were maintained for 11 days. IMM markedly increased the number of total white blood cells, monocyte, neutrophil, and eosinophil in the 1st to 10th day. However, the number of total white blood cells, monocyte, neutrophil, and eosinophil during the experiment of WBS were characterized by the presence of a lag phase followed by the significant increased actions. IMM did not change the

number of basophil during the experimental period. However, WBS increased the number of basophil in the 1st to 8th day to 2.8–4.8 times, compared with the values of the control. Both IMM and WBS did not change the number of lymphocytes. From these results, WBS increases the number of natural immunity cells without changing acquired immunity cells, and there are different responses in the number of total white blood cells, monocyte, neutrophil, eosinophil, and basophil between IMM and WBS.

Keywords Inactivity · Immobilization · Whole body suspension · White blood cells · Immuno-responsive system

Introduction

Physical exercises such as brisk walking, jogging, cycling, swimming, aerobic dancing/gymnastics, rowing and skiing for approximately 30–45 min/day three times per week are efficient to maintain and promote good health (Åstrand 1992). These habitual physical activities are known to be beneficial to chronic diseases such as coronary heart disease, hypertension, hyperlipidemia, obesity, diabetes type II, impaired glucose tolerance, osteoporosis, psychologic impairment, colon cancer, stroke and back injury (Åstrand 1992).

On the other hand, LaPerriere et al. (1994) showed that physical exercise was associated with improvements in mental health, neuroendocrine and immune functions. Further, Åstrand (1992) also indicated that physical exercise affected host defense mechanisms including the number of granulocytes, the number, functions, and cytotoxicity of lymphocytes, cytokine production, and

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secretory immunoglobulin levels (McFarlin et al. 2003). However, these changes are small in magnitude and brief in duration, and the physiological significance of these changes is uncertain. Therefore, it is necessary to have knowledge of the basic principle for eliminating stress-induced immunosuppression derived from social life or physical inactivity.

According to the recent World Health Organization (WHO) documents, over 60% of adult persons in the world are simply not active enough to benefit their health (Anonymous 2002). For example, approximately 250,000 persons per year in the United States are premature deaths due to physical inactivity (Booth et al. 2000). In fact, epidemiological data showed that physical inactivity increased the incidence of at least 17 unhealthy conditions, almost all of which are chronic diseases or considered risk factors for chronic diseases (Hoffman et al. 1996). Thus, the chronic diseases or lifestyle-related diseases may be categorized as the civilization diseases.

It is generally accepted that prolonged hypokinesia (i.e. reduction in limb movement) and/or hypodynamia (i.e. reduction in muscle loading) resulted from prolonged bed rest, life in a wheel chair, restricted movement, limited muscular function, and microgravity environment have been known for a long time to induce skeletal muscle atrophy and reduce physical work capacity. These physical conditions are known to induce activation of sympatho-adrenal axis associated with increases in circulating levels of noradrenalin and adrenalin from adrenal medulla (Miyamoto et al. 1992). Similarly, hypothalamo-hypophyseal-adrenocortical axis is activated associated with releases into peripheral blood of corticotropin releasing factor (CRF) from hypothalamus, adrenocorticotrophic hormone (ACTH) from pituitary, and glucocorticoids from adrenal cortex (Thomason and Booth 1990). These factors are well shown to regulate immuno-responsive systems.

Thus, physical activities-restricted and mechanical loading-reduced conditions have been shown to induce changes in responses of many physiological defense systems such as the hypothalamo-hypophyseal-adrenocortical axis, the sympathetic nervous system, and

immuno-responsive systems. However, physiological effects of various types of such physical inactivity on immuno-responsive systems are still unknown. It is crucial to summarize *in vivo* phenomena induced by various physical immobilizations such as inactivity as extremely restricted physical inactivity model and whole body suspension as weightlessness model. Therefore, the effects of two types of inactivity on the number of white blood cells and visceral organ mass were studied in rats.

Materials and methods

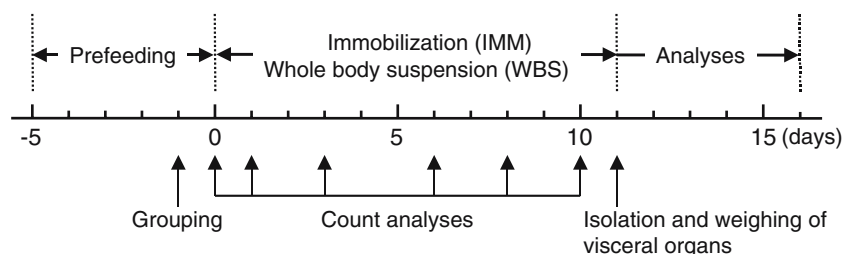
Experimental procedures and animal cares

Two parts of an experiment (1) the effects of immobilization (1st experiment), and (2) the effects of whole body suspension (2nd experiment) on the number of white blood cells such as lymphocytes, monocyte, neutrophil, eosinophil and basophil, and the weights of stress-responsive organs such as spleen, adrenals, and thymus were carried out in rats. The experimental protocol is shown in Fig. 1.

Male 7-week-old Sprague–Dawley rats ($n = 32$, CLEA Japan, Tokyo) were purchased. The rats were prefed for 5 days to allow adaptation to their new environment (Fig. 1). The rats were housed in cages at a temperature of 23–25°C and a relative humidity of 50–60%. Lighting was automatically provided from 7:00 to 19:00. Animal chow (CE-2, CLEA Japan, Tokyo) and once-boiled tap water were given to the rats *ad libitum* (Imaizumi et al. 1996; Harada et al. 1998). After the adaptation periods, the rats were randomly divided into two groups in each experiment. The present experiments were performed with the least possible pain or discomfort to the rats.

The present study was carried out according to the “Guiding Principle for the Care and Use of Animals in the Field of Physiological Sciences” of the Physiological Society of Japan (2004). The experimental protocols (Number 9) also were approved by the Animal Ethics Committee, Faculty of Human Sciences, Waseda University.

Fig. 1 Experimental protocols. *Up arrow*: Flow-cytometrical analyses of hematocrit, total white blood cells, lymphocytes, monocyte, neutrophil, eosinophil, and basophil



Immobilization (1st experiment)

After 5 days of prefeeding, the rats were divided into the immobilization (IMM: $n = 8$, initial body weight = 237 ± 3 g) group and the cage control (CON: $n = 8$, initial body weight = 237 ± 3 g) group. IMM group was consecutively immobilized for 11 days, whereas CON group was consecutively maintained in unrestricted cage activities for the same period. Animal immobilization was performed by using our self-made apparatus. The outlines of the apparatus are shown in Fig. 2. Figure 2 shows the diagram (A) and developmental drawings (B) of the immobilization apparatus. This experiment was carried out under diet-restricted feeding (chow intake/rat = 20 g/day) conditions. Once-boiled

tap water was given to the rats ad libitum. During the immobilization period, the number of white blood cells were analyzed according to the protocol (Fig. 1).

Whole body suspension (2nd experiment)

The rats were divided into the whole body suspension (WBS: $n = 8$, initial body weight = 213 ± 1 g) group and the cage control (CON: $n = 8$, initial body weight = 213 ± 2 g) group. WBS group was consecutively held in suspension harnesses for 11 days, whereas CON group was consecutively maintained in unrestricted cage activities for the same period.

The suspension apparatus used in this study was a modified version of a type developed by Musacchia

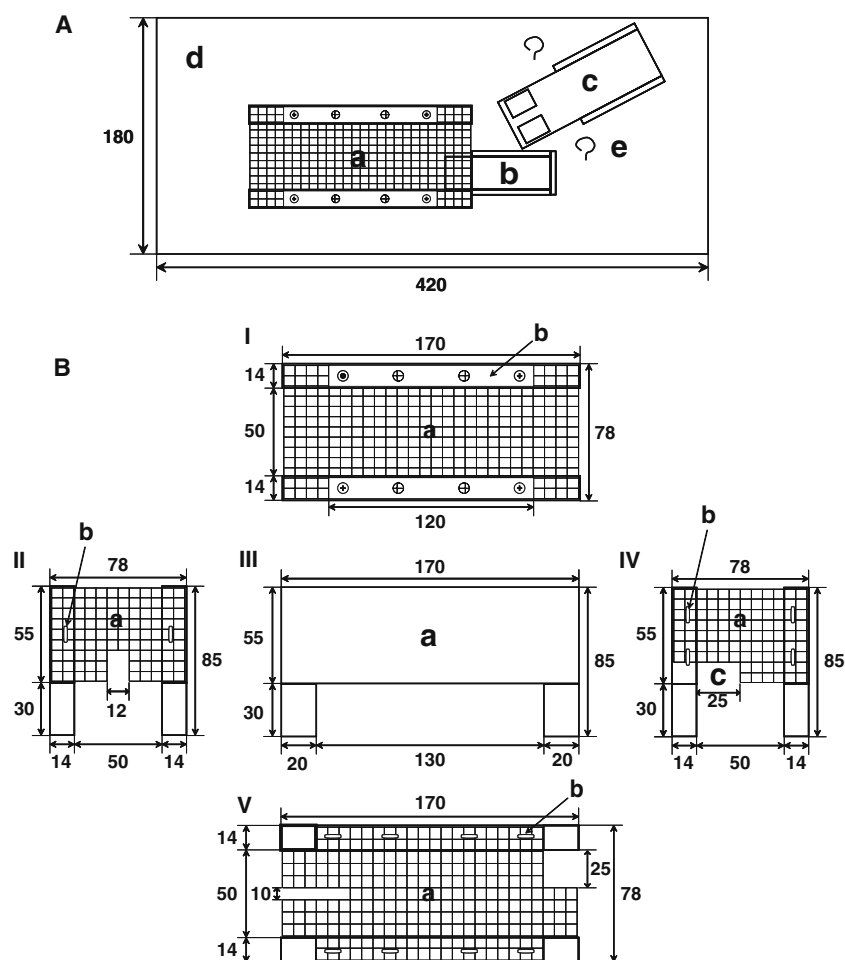


Fig. 2 Diagram (A) and developmental drawings (B) of immobilization apparatus. **A:** a, rat immobilization cage; b, feeding box stand; c, water bottle stand; d, wooden board (14 mm thick) and e, metal hook for fitting water bottle. Unit of length: mm. **B:** I, top-surface view; II, back surface view; III, side view; IV, frontal view and V, base view. Unit of length: mm. I-a, iron metallic mesh for covering cage; I-b, metals and screws for securing cover iron metallic mesh to wall woods; II-a, iron metallic mesh for preventing rat from backing and this open space is capable to put tail out;

II-b, double-crossing nails for securing iron metallic mesh to wall woods; II-c, space position for putting tail out; III-a, cover walls made of lauan woods; IV-a, cover iron metallic mesh preventing rats from advancing; IV-b, contact points between metallic mesh and wall wood; IV-c, positions for drinking and eating water and food; V-a, metallic mesh cut for attaching feed stand and ensuring removal of urine and feces; and V-b, contact points between metallic mesh and wall wood

et al. (1980), Morey-Holten and Wronski (1981), and Imaizumi et al. (1996). The rats were checked daily for signs of leg, nose and eye lesions, unusual breathing patterns, or undue discomfort. Feeding conditions were in the same manner as described above. The number of white blood cells were analyzed at the same intervals with the 1st experiment (Fig. 1).

Blood samplings

Whole blood samples (60–70 μ l) were collected with microcapillary tubes coated with an anticoagulant, 0.3 M EDTA-2K (ethylenediamine tetraacetic acid, dipotassium salt, Wako Co., Osaka, Japan) from the tail vein according to the protocol (Fig. 1). Fifty-microliter blood samples were immediately prepared with a microsyringe, and then diluted by 20% with the cell pack (whole blood diluent for use in hematology analyzers, Sysmex Co., Hyogo, Japan). These samples were used for count analyses of white blood cells.

Count analyses of white blood cells

Count analyses of white blood cells were carried out by the hematology analyzer (Model SF-3000, Sysmex Co.) based on a flow cytometry technique with light-emitting diode. The SF-3000 type analyzer is known to be a fully automated hematology analyzer (Zahorec 2001). White blood cells are flown with fluid in the flow cell. A laser beam emitted from diode irradiates them and then forward-scattered lights are generated. These lights are collected by photodiodes and transformed to electric pulses. As a result, the number of white blood cells are analyzed. On the other hand, forward-scattered lights are separated into low- and high-angle forward-scattered lights, standing for white blood cell sizes and nucleus forms, respectively. These scattered angles are different among white blood cells. As these

result, white blood cells are fractionated into lymphocytes, monocyte, neutrophil, eosinophil, and basophil.

Isolation and weighing of stress-responsive organs

As shown in Fig. 1, the stress-responsive organs (spleen, adrenals, and thymus) were isolated on the final days of the IMM and WBS and promptly weighed (Shimadzu LIBROR Model EB-330D).

Statistical analyses

Experimental data were presented as mean \pm standard error of the mean (SEM). The effects of IMM and WBS on the body weight and the weights of visceral organs were tested by one-way analysis of variance (ANOVA). The effects of IMM and WBS on the hematocrit values and the number of white blood cells were evaluated by a two-way ANOVA for repeated measures. Subsequent post hoc analyses to determine significant differences between two groups and from day 0 in each group were performed by Fisher's protected least significant difference (PLSD) test and Dunnett's test, respectively. The differences were considered significant when P was <0.05 .

Results

Body weights and the weights of stress-responsive organs

We investigated the effects of immobilization (IMM) and whole body suspension (WBS) on the body weight (BW) and the weights of stress-responsive visceral organs in rats. As shown in Table 1, the body weights in IMM and WBS groups were 0.85 ($P < 0.001$) and 0.79 times ($P < 0.001$) clearly lower than those in CON

Table 1 Effects of immobilization (IMM) and whole body suspension (WBS) on the body weight (BW) and the weights of stress-responsive visceral organs

BW and visceral organs	1st experiment		2nd experiment	
	CON	IMM	CON	WBS
Body weight (BW) (g)	268 \pm 3	229 \pm 5*** (0.85)	254 \pm 4	200 \pm 5*** (0.79)
Spleen (mg)	642 \pm 44	509 \pm 32* (0.79)	627 \pm 12	368 \pm 19*** (0.59)
Spleen (mg/g BW)	2.40 \pm 0.16	2.22 \pm 0.13 (0.93)	2.48 \pm 0.06	1.84 \pm 0.07*** (0.74)
Adrenals (mg/pair)	45 \pm 1	44 \pm 3 (0.97)	50 \pm 2	52 \pm 2 (1.04)
Adrenals (mg/pair/g BW)	0.17 \pm 0.01	0.19 \pm 0.01 (1.13)	0.20 \pm 0.01	0.26 \pm 0.01*** (1.33)
Thymus (mg)	352 \pm 22	251 \pm 23** (0.71)	426 \pm 27	214 \pm 12*** (0.50)
Thymus (mg/g BW)	1.31 \pm 0.07	1.09 \pm 0.09 (0.83)	1.67 \pm 0.09	1.07 \pm 0.05*** (0.65)

The values are mean \pm SEM. Values in parentheses are shown as the relative ratio of IMM and WBS group to each CON group

CON control group, IMM immobilization group, WBS whole body suspension group

Statistics: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (vs. CON, by one-way ANOVA)

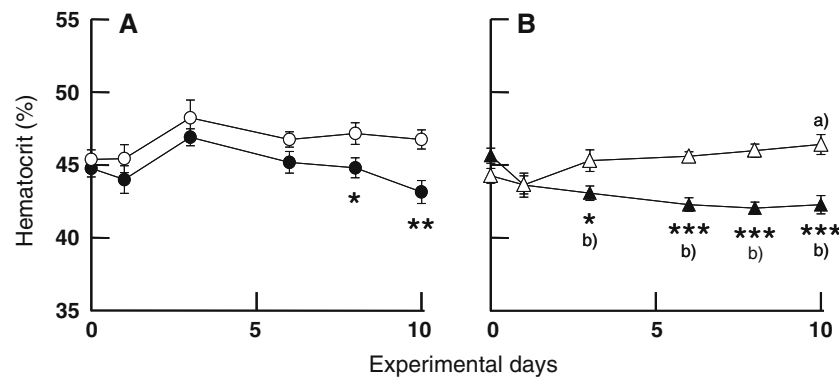


Fig. 3 Effects of IMM (**A**) and WBS (**B**) on the hematocrit values during the experimental periods. **A** open circle, control (CON) group and filled circle, IMM group. **B** open triangle, CON group and filled triangle, WBS group. Values: mean \pm SEM ($n = 8$)

groups, respectively. The relative weights of spleen per body weight in IMM and WBS groups were 0.93 and 0.74 ($P < 0.001$) times lower than those in CON groups, respectively. The relative weights of adrenals per body weight in IMM and WBS groups were 1.13 and 1.33 ($P < 0.001$) times higher than those in CON groups, respectively. The relative weights of thymus per body weight in IMM and WBS groups were 0.83 and 0.64 ($P < 0.001$) times lower than those in CON groups, respectively. From these results, the effects of two types of inactivity on the body weight and the weights of stress-responsive organs are relatively higher in WBS condition than in IMM condition, indicating that there are different responses in the body weight and stress-responsive organs between IMM and WBS.

Hematocrit values

Figure 3 shows the changes of the hematocrit value during the experimental period. As shown in Fig. 3A, the hematocrit values in IMM group were 0.95 ($P < 0.05$) and 0.92 times ($P < 0.01$) lower than those in CON group in the 8th and 10th day of IMM, respectively. On the other hand, hematocrit values in WBS group were 0.95 ($P < 0.05$), 0.93 ($P < 0.001$), 0.91 ($P < 0.001$), and 0.91 times ($P < 0.001$) lower than those in CON group in the 3rd, 6th, 8th, and 10th day of WBS, respectively (Fig. 3B). These results suggest that extracellular fluid volumes are relatively higher in both IMM and WBS conditions than in each control group, and the number of white blood cells are apparently diluted in the IMM and WBS groups, as compared with the corresponding CON group. In the present study, therefore, the number of white blood cells were corrected by corresponding hematocrit values.

group). Statistics: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (vs. CON group, by two-way ANOVA and Fisher's PLSD test), and ^a $P < 0.05$ and ^b $P < 0.01$ (vs. 0 day, by Dunnett's test)

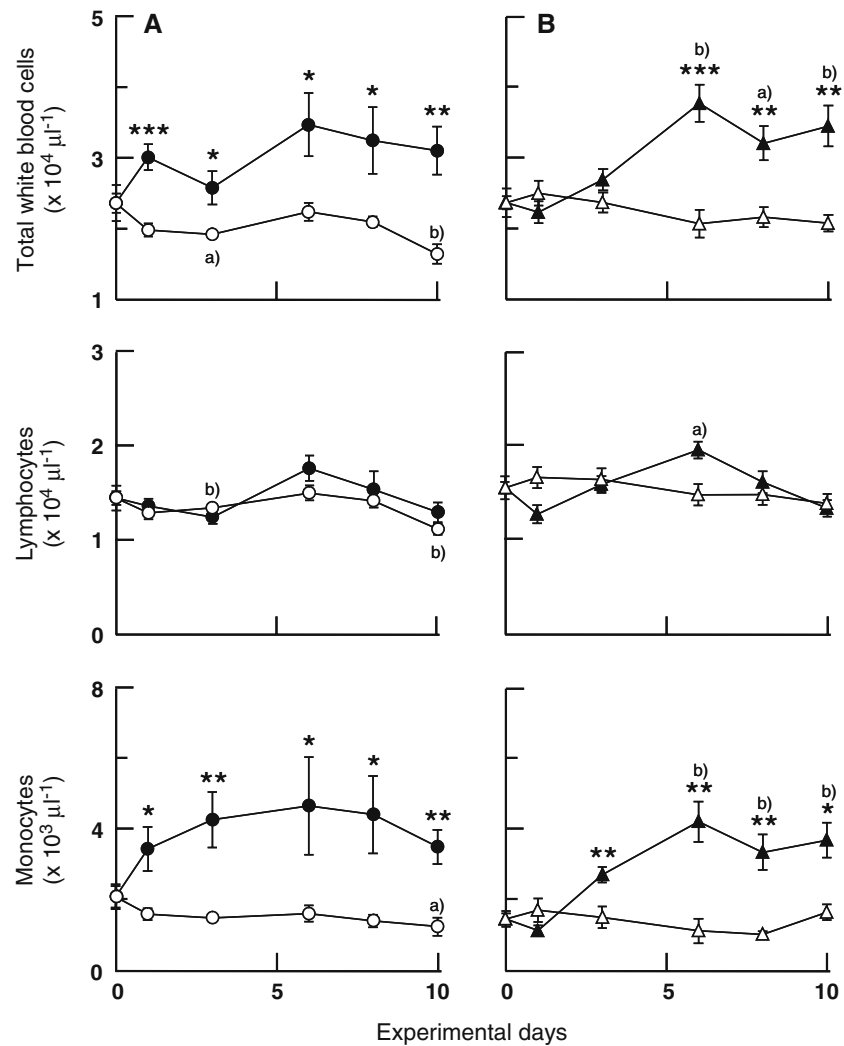
The number of total white blood cells, lymphocytes, and monocyte

Next we studied the effects of two types of inactivity on the number of total white blood cells, lymphocytes, monocyte, neutrophil, eosinophil, and basophil during experimental periods. Figure 4 shows the changes of the number of total white blood cells, lymphocytes, and monocyte during the experimental periods. As shown in Fig. 4A, the number of total white blood cells in IMM group were 1.52 ($P < 0.001$), 1.34 ($P < 0.05$), 1.55 ($P < 0.05$), 1.55 ($P < 0.05$), and 1.89 times ($P < 0.01$) higher than those in CON group in the 1st, 3rd, 6th, 8th, and 10th day of IMM, respectively. On the other hand, WBS was independent of the number of total white blood cells within the 1st to 3rd day. However, the number of total white blood cells in WBS group were 1.82 ($P < 0.001$), 1.48 ($P < 0.01$), and 1.66 times ($P < 0.001$) higher than those in CON group in the 6th, 8th, and 10th day of WBS, respectively (Fig. 4B).

No differences in the number of lymphocytes between both IMM and CON groups were observed during the experimental periods (Fig. 4A). Similar phenomena were also observed in WBS condition (Fig. 4B).

On the other hand, the number of monocyte in IMM group were 2.15 ($P < 0.05$), 2.85 ($P < 0.01$), 2.88 ($P < 0.05$), 3.13 ($P < 0.05$), and 2.80 times ($P < 0.01$) higher than those in CON group in the 1st, 3rd, 6th, 8th, and 10th day of IMM, respectively (Fig. 4A). The number of monocyte during the experimental periods showed the increased actions by WBS except the data of the 1st day. As shown in Fig. 4B, the number of monocyte in WBS group were 1.80 ($P < 0.01$), 3.75 ($P < 0.01$), 3.27 ($P < 0.01$), and 2.24 ($P < 0.05$) times

Fig. 4 Effects of IMM (A) and WBS (B) on the number of total white blood cells, lymphocytes, and monocyte during the experimental periods. **A** open circle, CON group and filled circle, IMM group. **B** open triangle, CON group and filled triangle, WBS group. Values: mean \pm SEM ($n = 8$ /group). Statistics: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (vs. CON group, by two-way ANOVA and Fisher's PLSD test), and ^{a)} $P < 0.05$ and ^{b)} $P < 0.01$ (vs. 0 day, by Dunnett's test)



markedly higher than those in the CON group in the 3rd, 6th, 8th, and 10th day of WBS, respectively.

The number of neutrophil, eosinophil, and basophil

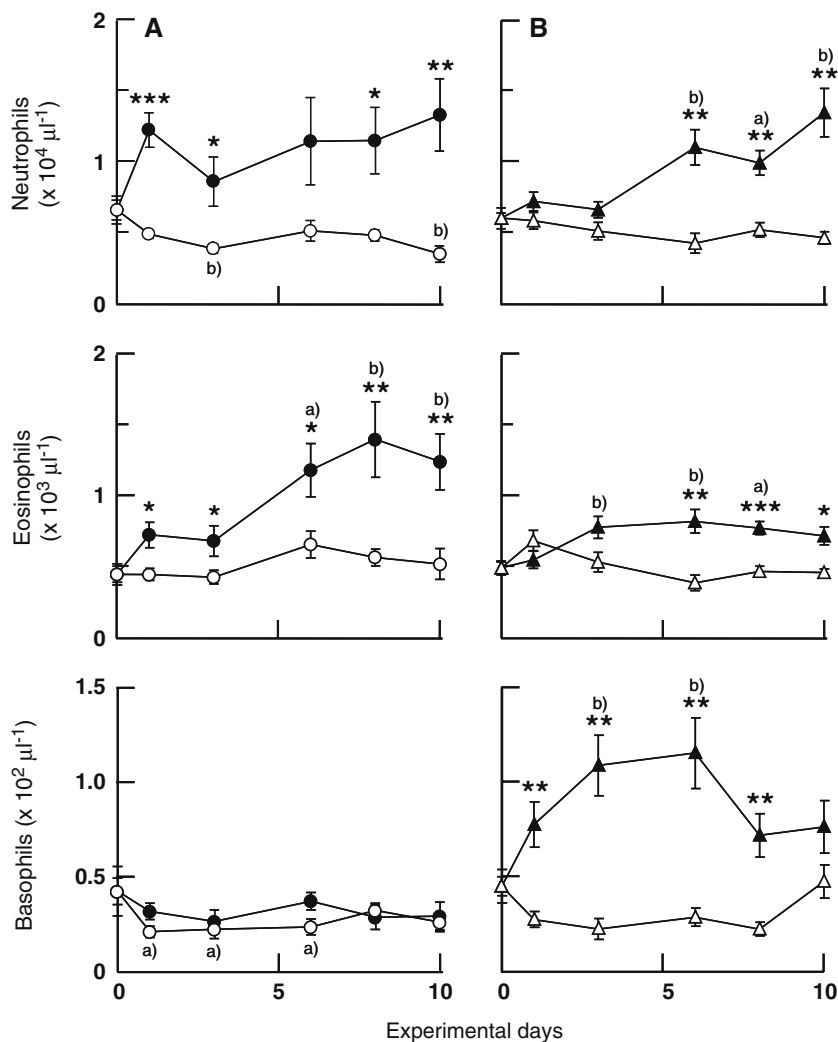
Figure 5 shows the changes of the number of granulocytes (neutrophil, eosinophil, and basophil) during the experimental periods. As shown in Fig. 5A, the number of neutrophil in IMM group were 2.49 ($P < 0.001$), 2.21 ($P < 0.05$), 2.38 ($P < 0.05$), and 3.79 times ($P < 0.01$) markedly higher than those in CON group in the 1st, 3rd, 8th, and 10th day of IMM, respectively. Although WBS was independent of the number of neutrophil within the 3rd day, the number of neutrophil in WBS group were 2.57 ($P < 0.01$), 1.90 ($P < 0.01$), and 2.89 times ($P < 0.01$) markedly higher than those in CON group in the 6th, 8th, and 10th day of WBS, respectively (Fig. 5B).

The number of eosinophil during the experimental periods were also significantly higher in IMM (A) and

WBS (B) groups than in the corresponding CON groups (Fig. 5A, B). As shown in Fig. 5A, the number of eosinophil in IMM group were 1.62 ($P < 0.05$), 1.59 ($P < 0.05$), 1.79 ($P < 0.05$), 2.46 ($P < 0.01$), and 2.38 times ($P < 0.01$) markedly higher than those in CON group in the 1st, 3rd, 6th, 8th, and 10th day of IMM, respectively. The number of eosinophil in WBS group also were 2.12 ($P < 0.01$), 1.66 ($P < 0.001$), and 1.56 times ($P < 0.05$) higher than those in CON group in the 6th, 8th, and 10th day of WBS, respectively (Fig. 5B).

Clearly, different responses of the number of basophil were observed between IMM and WBS. The results are shown in Fig. 5A, B. There were no significant changes in the number of basophil when IMM group was compared with the corresponding CON group (Fig. 5A). However, the number of basophil in WBS group were 2.82 ($P < 0.01$), 4.83 ($P < 0.01$), 4.01 ($P < 0.01$), and 3.19 ($P < 0.01$) times distinctly higher than those in the CON group in the 1st, 3rd, 6th, and 8th day of WBS, respectively (Fig. 5B).

Fig. 5 Effects of IMM (**A**) and WBS (**B**) on the number of neutrophil, eosinophil and basophil during the experimental periods. **A** open circle, CON group and filled circle, IMM group. **B** open triangle, CON group and filled triangle, WBS group. Values: mean \pm SEM ($n = 8/\text{group}$). Statistics: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (vs. CON group, by two-way ANOVA and Fisher's PLSD test), and ^{a)} $P < 0.05$ and ^{b)} $P < 0.01$ (vs. 0 day, by Dunnett's test)



Relationship between two types of inactivity and the number of white blood cells

The effects of two types of inactivity on the relative ratio of IMM group and WBS group to CON group in the number of white blood cells as plotted against the experimental days were summarized in Fig. 6. As shown in Fig. 6, the relative ratios of IMM group to CON group in the number of total white blood cells (A), monocyte (C), neutrophil (D), and eosinophil (E) during the experimental periods were characterized by relatively increased actions. However, the relative ratios of WBS group to the CON group in total white blood cells (A), monocyte (C), neutrophil (D), and eosinophil (E) during experiments were characterized by the presence of a lag phase followed by significantly increased actions. These results show that there is a different response in the number of total white blood cells, monocyte, neutrophil, and eosinophil between IMM and WBS.

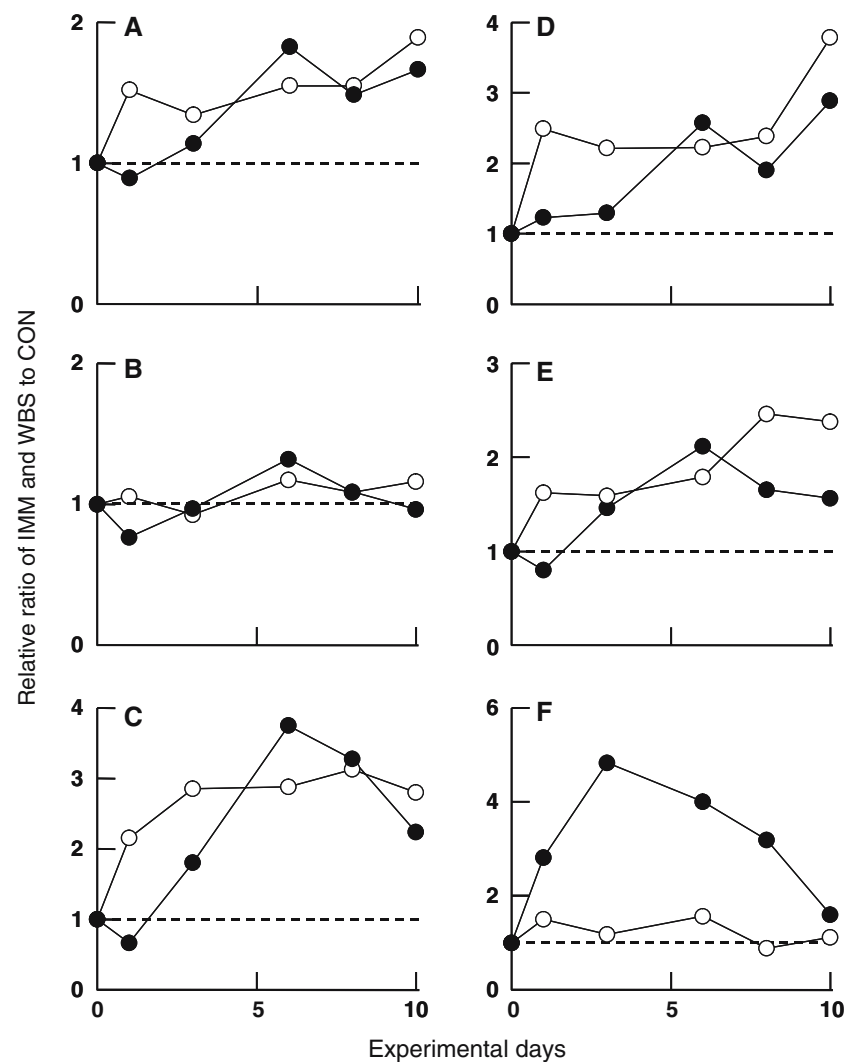
On the other hand, as shown in Fig. 6F, IMM did not change the relative ratio of IMM group to CON group in the number of basophil. However, WBS increased the relative ratio of WBS group to CON group in the number of basophil in the 1st to 8th day of WBS to 2.8–4.8 times. These results indicate that there is a different response in the number of basophil between IMM and WBS.

Discussion

The purpose of the present study was to elucidate the effects of two types of inactivity (immobilization: IMM and whole body suspension: WBS) on the number of total white blood cells, lymphocytes, monocyte, neutrophil, eosinophil, and basophil in rats.

The main findings of the present study are summarized as follows: (1) IMM significantly increased the number of total white blood cells, monocyte, neutrophil, and eosinophil in the 1st to 10th day (Figs. 4A, 5A). How-

Fig. 6 Changes of the relative ration of IMM (*open circle*) group and WBS (*filled circle*) group to CON group in the number of total white blood cells (**A**), lymphocytes (**B**), monocyte (**C**), neutrophil (**D**), eosinophil (**E**), and basophil (**F**). Values are calculated from Figs. 3 and 4



ever, the number of total white blood cells, monocyte, neutrophil, and eosinophil during the experiment of WBS were characterized by the presence of a lag phase followed by the significant increased actions (Figs. 4B, 5B). (2) IMM did not change the number of basophil during the experimental period (Figs. 5A, 6F). However, the WBS increased the number of basophil in the 1st to 8th day to 2.8–4.8 times, compared with the values of the control (Figs. 5B, 6F). (3) Both IMM and WBS did not change the number of lymphocytes (Figs. 4A, B, 6B). These results suggest that WBS increases the number of natural immunity cells without changing acquired immunity cells, and there are different responses in the number of total white blood cells, monocyte, neutrophil, eosinophil, and basophil between IMM and WBS.

Stress-responsive organ weights

The present study showed that the relative weights of thymus, one of the central lymphoid organs and the

relative weights of spleen, one of the peripheral lymphoid organs in IMM and WBS groups were also relatively lower than those in CON groups (Table 1). On the other hand, the weights of adrenals, one of the endocrine organs, in IMM and WBS groups were non-specifically higher as compared with CON groups (Table 1). These results suggest that IMM and WBS induced stress responses during the experiments. This suggestion accords with those of Hayase and Yokogoshi (1991), Imaizumi and Tachiyashiki (1994), and Imaizumi et al. (1996).

Steffen and Musacchia (1987) reported that plasma corticosterone concentrations were transiently increased during the experiment of WBS in rats. It is well known that glucocorticoids are adrenal steroid hormones with antiinflammatory effects, and induce immature T lymphocytes and thymus cell apoptosis. As already reported, for instance, glucocorticoids increased during restraint stress, produce thymic involution, decrease CD4⁺/CD8⁺ thymocytes, and cause

DNA fragmentation (Tarcic et al. 1998; Mcconkey et al. 1990). From these observations, thymus hypotrophy with WBS and IMM would be caused by increased plasma glucocorticoid levels during two types of inactivity.

Adrenal hypertrophy under both experimental conditions would be caused by stress-induced activation of hypothalamo–hypophyseal–adrenocortical axis stimulated by IMM and WBS (Madden and Felten 1995). These results indicate that WBS-induced stress responses, particularly glucocorticoid effects, would be more significant than those of IMM, suggesting that additional stress by inactivity conditions affect these phenomena. Therefore, WBS may be more apt to activate physiological defense system via endocrine systems than IMM condition. These suggestions do not contradict with results shown in Table 1.

Inactivity-induced changes of the number of white blood cells

The present studies demonstrate that IMM and WBS significantly increased the circulating number of total white blood cells (Fig. 4A, B). These results clearly suggest that various white blood cells in prolonged two types of inactivity conditions were as a whole mobilized into the circulating blood from the marginal pool or outside of blood vessel.

As demonstrated in the present study, IMM and WBS increased the circulating number of granulocytes such as neutrophil and monocyte (Figs. 4, 5, 6). These findings are clearly important. These neutrophilia and monocytosis are also known to be induced by exhaustive exercises in human (Nieman 1989; Suzuki et al. 2003), which are physically opposed stimuli of prolonged inactivity [hypokinesia (i.e. decreased motor activity)/hypodynamia (i.e. decreased mechanical loading)] conditions. These results suggest that neutrophilia and monocytosis are one of the general-adaptation reactions during stresses, and must play fundamental roles in the defense system of living body.

Suzuki et al. (2003) showed that exhaustive exercise-induced increase of plasma interleukin (IL)-6 level mediates bone marrow release of neutrophil. In the present study, although it was not investigated whether syntheses of the hematopoietic cytokines such as IL-6, granulocyte colony-stimulating factor (G-CSF), or macrophage colony-stimulating factor (M-CSF) are induced by prolonged inactivity conditions, at least stress hormones such as glucocorticoids and catecholamines are likely involved in the neutrophilia due to stress-responsive changes of visceral organ weights and previous study (Miyamoto et al. 1992). Glucocorticoids

are known to induce mobilization of neutrophil from the bone marrow into the circulating blood (Bishop et al. 1968), and inhibit apoptosis of the circulating neutrophil (Cox 1995) and migration of neutrophil into the tissues (Bishop et al. 1968; Dale et al. 1974). Further, catecholamines also are known to induce mobilization of neutrophil from the margin of vessel into the circulating blood by the arterial shearing force through β -adrenergic enhancements of heart functions (Suzuki et al. 1996).

On the other hand, van Furth and Cohen (1968) reported on monocyte distributions in mice that the circulating monocyte accounts for 40% and the marginated monocyte accounts for 60% of the peripheral blood monocyte. These results suggest that the circulating number of monocyte are capable to be increased up to 2.5 times when all of the marginated monocyte are mobilized into the circulating blood. In the present study, because the circulating number of monocyte were increased to 3.13 and 3.75 times, respectively, at the peak days of IMM and WBS (Figs. 4A, B, 6C), differentiations of hematopoietic stem cells into monocyte may be accelerated in bone marrow, and released into the circulating blood (van Furth and Sluiter 1986).

On the other hand, Miller et al. (1994) showed that WBS did not alter rat neutrophil functions such as the oxidative burst in peripheral blood of rats. Suzuki et al. (2003) showed that postexercise plasma neutrophil and monocyte were not functionally activated despite increased plasma levels of cytokines acting as recruiting and priming them. These findings suggest that neutrophil and monocyte, nonspecifically mobilized from the margin of vessel and bone marrow, would be not activated in the circulating blood, indicating the role of significant prevention against useless injury of living body. It is also meaningful to stock these phagocytes sleeping in the circulating blood, pump them systemically, and send them into the tissues injured by stresses during these physical conditions.

IMM and WBS increased significantly the circulating number of eosinophil (Figs. 5A, B, 6E). However, according to our unpublished data, the administration of β_2 -agonist, clenbuterol (1 mg/kg BW/day, for 30 days) to rats decreased relatively the circulating number of eosinophil. These results indicate that mechanisms of eosinophil redistributions are different from those of the neutrophil and monocyte redistributions during three experimental conditions, suggesting that physiological significances of eosinophil during stresses would be different from those of other granulocytes. In fact, glucocorticoids are reported to enhance eosinophil apoptosis but inhibit neutrophil apoptosis in rats (Nittoh et al. 1998). However, although clenbu-

terol administration induces β -adrenergic promotion of arterial shearing force, the circulating number of eosinophils clearly decreased (our unpublished data). Therefore, mechanisms of the eosinophil redistributions in prolonged inactivity conditions would not be directly caused by the effects of stress hormones such as glucocorticoids and catecholamines. Rather, β -adrenergic stimuli may induce eosinophil removals from the circulating blood.

On the other hand, cytokines, involved in peripheral eosinophil survivals, such as IL-3 (Rothenberg et al. 1988), IL-5 (Rothenberg et al. 1989), granulocyte-macrophage colony-stimulating factor (GM-CSF) (Lopez et al. 1986; Owen et al. 1987), and interferon- γ (IFN- γ) (Valerius et al. 1990; Collota et al. 1992) were produced systemically or locally in prolonged inactivity conditions. However, Wallen et al. (1991) also reported that eosinophil survival promoted by these factors is inhibited by glucocorticoids, so more detailed investigations are necessary to clarify this paradox, mechanisms of eosinophil redistributions and roles of them in the living body during stresses.

The main findings of the present study are that the circulating number of basophil were drastically increased during the experiment of WBS (Figs. 5B, 6F). However, IMM did not change the number of basophil during the experimental period (Figs. 5A, 6F). Glucocorticoids-induced apoptosis has been shown in basophil as well as eosinophil (Yoshimura et al. 2001). Thus, syntheses of other factors that overcome glucocorticoid effects and promote differentiations of the hematopoietic stem cells into basophil would be specifically induced during WBS. WBS may induce intensive damages of the tissues such as some skeletal muscles or visceral organs. However, the causes of different responses of the number of basophil between IMM and WBS are still uncertain. Therefore, further studies are required in these questions.

As shown in the present paper, drastic changes of the circulating number of lymphocytes were not observed in prolonged inactivity conditions (Fig. 5A, B). According to our recent unpublished data, β_2 -agonist, clenbuterol (dose = 1 mg/kg BW/day, for 30 days), however, decreased drastically levels of lymphocytes to about 0.5 times in the 5th to 30th day. These findings indicate that the mobilization of lymphocytes into the circulating blood was inhibited in both IMM and WBS conditions, without fractionating subsets of lymphocytes such as the B and T lymphocytes. Ronsen et al. (2001) reported that the number of lymphocytes were increased with single or repeated bouts of strenuous endurance exercise, and then normalized as soon as in rest period postexercise, indicating that responses of

lymphocytes during stresses represent stimulus specificity, or drastic changes of the number of circulating lymphocytes are induced more early in time after initiations of inactivity conditions.

Cioca et al. (2000) reported that peripheral lymphocytes were led to apoptosis by catecholamines such as dopamine and dobutamine, and these apoptotic effects were completely and partially blocked by β -receptor antagonist, propranolol, respectively, indicating that pharmacological dose of β_2 -agonist would induce large magnitude of apoptosis of lymphocytes in the circulating blood, followed by drastic reduction of their number in the present study. These facts clearly show that viabilities of lymphocytes responsible for acquired immunity are suppressed, while natural immune cells, phagocytes like neutrophil and monocyte essential for host defense are mobilized into peripheral blood and circulated through the body without activating during stresses.

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