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The effect of repetitive mild hyperthermia on body temperature, the autonomic nervous system, and innate and adaptive immunity

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ABSTRACT

The effect of repetitive mild hyperthermia on body temperature, the autonomic nervous system, and innate and adaptive immunity was investigated using a new hyperthermia treatment system, nanomist sauna (NMS). Six healthy volunteers participated and the concentration of catecholamines and cortisol, and the frequency and function of leukocytes in the peripheral blood were investigated before and after successive 7 days of hyperthermia treatment (20 min/day, 40°C, 100% relative humidity). After treatment, the blood level of adrenaline and cortisol on the 7th day was decreased compared with the 1st day, indicating the suppression of the sympathetic nervous system activity. Moreover, the frequency of $CD56^+$NK, $CD56^+$NKT and B cells on the 7th day tended to be increased compared with the 1st day. The frequency of HLA-DR-positive NK and NKT cells and expression of HLA-DR on B and T cells increased. The cytotoxicity of NK cells and proliferative response of B cells were also elevated. The results indicate that repetitive mild hyperthermia treatment might suppress excessive sympathetic dominance and modify immunity. Additionally, because it can provide the same effects as conventional hyperthermia treatments with minimal burden to the body, NMS may be a novel patient- and elderly-friendly hyperthermia treatment for health promotion.

Many researchers have reported that hot springs, saunas and home baths give comfortable and appropriate hyperthermic stimulation. We have also reported a series of studies where systematic mild hyperthermia treatments could activate leukocyte, enhance immunity and treat various diseases (19, 28). Thus conventional hyperthermia treatments might be effective for health promotion, including reports that hot-spring treatment is effective for the improvement of locomotive syndromes (14).

However, Pilch *et al*. reported that wet hyperthermia is more stressful than dry hyperthermia for healthy males (22). Furthermore, it is not easy for frail people (especially children, older people and patients with locomotive disorders) to regularly take conventional hyperthermic treatments. For example, the water pressure of baths and the high temperatures (80–100°C) of dry saunas are sometimes stressful and dangerous to these individuals. In addition, conventional wet sauna has the problem of high humidity. Thus aspects of conventional hyperthermia treatments, such as high temperature, water pressure and humidity might induce stress. If such stresses could be lowered, hyperthermia treatment could be useful not only for patients and older people but also for caretakers.

To pursue the further study of the effect of hyperthermia itself, we used nanomist sauna (NMS), a

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new hyperthermia treatment. NMS, a new type of sauna, is characterized by the ability to produce ultra-small fog-shaped hot water called nanomist, which barely condenses to dew. Therefore, we studied the effect of repetitive mild hyperthermia treatment with NMS on the stress response, immune defense and autonomic nervous system by analyzing body temperature, kinetic change in peripheral leukocytes, NK cell activity and the proliferation response of T and B cells.

MATERIALS AND METHODS

Subjects. Six healthy male volunteers (ages 39 to 56; average 51.0 ± 6.2 years old) participated in this study. Written informed consent was obtained from all subjects and the study was approved by the institutional review board of Niigata University.

Hyperthermic stimulation. In this study the following four kinds of hyperthermic stimulation of 20 min were given to the subjects; (i) NMS (CORONA Corporation, Niigata, Japan) (40°C, 100% relative humidity), (ii) bath (40°C), (iii) low temperature steam sauna (48°C, 80–100% relative humidity) (sauna [L]), and (iv) high temperature dry sauna (90°C, 5–10% relative humidity) (sauna [H]).

Nanomist and NMS. For the generation of nanomist, the cone rapidly rotates and centrifugally lifts water in the tank (Fig. 1A). The water then escapes from small holes in the upper part of the cone and passes through the metal mesh above the cone. Thus generated, very fine water drops (nanomist) are negatively charged and released into the air. Relatively large drops fall back into the tank (Fig. 1A). To establish the NMS, water is heated to approximately 45°C by the sheath heater, and then nanomist is generated as described above. It is then sent into the sauna room by a cross flow fan (Fig. 1B). The heated air with the nanomist comes from the air outlet on the lower side and is sucked into the upper duct. In this way air circulates in the nanomist sauna room (40°C, 100% relative humidity) (Fig. 1C). The diameter range of the water droplets of nanomist is approximately 10 to 500 nm (Fig. 1D). Additionally, it provides a non-wet feeling, different from typical steam or mist.

Kinetic analysis of body temperature induced by various kinds of hyperthermia. All subjects rested for more than 20 min before they were given hyperthermic stimulation of 20 min. At that time, subjects

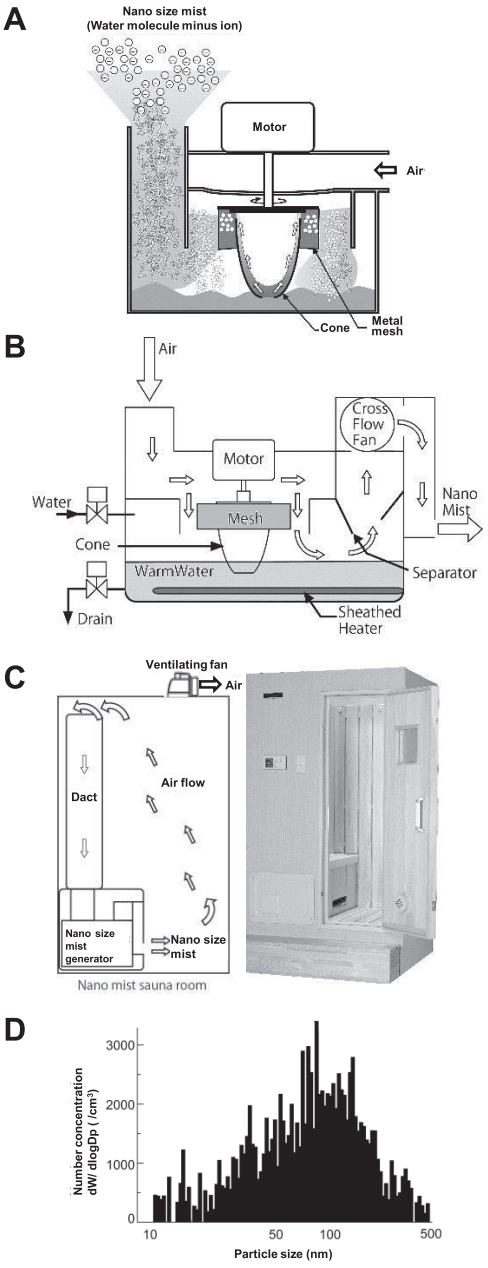

Fig. 1 Schematics of nanomist generation and the nanomist sauna (NMS). (**A**) Generation of nanomist. (**B**) Mechanism of nanomist generation. (**C**) Nanomist sauna setup. (**D**) Feature of nanomist water drop.

in the control group sat down in a work room (28.0 ± 0.5°C, 50.0 ± 5.0% relative humidity). After the hyperthermic stimulation, kinetic body temperature (hypoglossal) was measured at 0, 20, 40 and 60 min.

Experimental protocol of repetitive hyperthermic stimulation by NMS. In this study the experiment was conducted over a week to investigate the effects

of repetitive mild hyperthermia treatment using NMS. Each day, all subjects rested more than 20 min before they were given one hyperthermic stimulation of 20 min. From the second day to the last day (Day 7), subjects took the same hyperthermic stimulation as they had on the first day (Day 1). Body temperature (hypoglossal), pulse rate, the plasma concentration of adrenaline, noradrenaline and cortisol, flow cytometric analysis of leukocyte subsets, NK cell activity and the proliferation response of T cells and B cells were monitored on Day 1 and Day 7, before and after hyperthermic stimulation (Fig. 3A).

Venous blood samples and analysis. Venous blood (20 mL) was obtained from the forearm median antebrachial vein for analysis. Body temperature (hypoglossal) was measured with a mercury clinical thermometer (FAVOR; Nihon Keiryoki Kogyo Co., Ltd., Japan). Leukocyte number and subsets were determined using a hemocytometer and the May-Grünwald Giemsa staining method (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). The plasma concentration of adrenaline and noradrenaline was analyzed by HPLC and the concentration of cortisol was analyzed with the Chemiluminescent Enzyme Immunoassay (Mitsubishi Chemical Medience Corporation).

Flow cytometric analysis. Peripheral blood lymphocytes were isolated from heparinized blood by Ficoll-Paque PLUS gradient (1.077) centrifugation. The frequency of lymphocyte subsets, including T cells, B cells, NK cells and NKT cells, was examined by two-color immunofluorescence staining (17). As thermal exposure was found to induce HLA-DR (MHC class II antigen) expression on lymphocyte subsets, three-color staining was also conducted to detect the expression of this antigen on the cell surface as previously described (18). The monoclonal antibodies used included FITC-, PE-, or PerCP-conjugated anti-CD3 (UCHT-1), anti-CD56 (N901), CD19 (J3-119) (Beckman Coulter Inc., Indianapolis, IN, USA), and anti-HLA-DR (L243) (Becton Dickinson, Franklin Lakes, NJ, USA). Cells were analyzed with a FACS Calibur (Becton Dickinson). Dead cells were excluded by forward scatter and side scatter.

Functional analysis of lymphocytes. NK cell activity was determined using a ^{51}Cr-release assay (Mitsubishi Chemical Medience Corporation) (1). Proliferation of lymphocytes stimulated with pokeweed mitogen (PWM) was determined using ^{3}H-thymidine incorporation assay (Mitsubishi Chemical Medience Corporation) (7).

Statistical analysis. Two-way ANOVA was performed for the analysis of the body temperature over time after hyperthermic stimulation followed by a Scheffe *post hoc* analysis as required ($P < 0.05$). In other experiments, the difference between values was determined by Student's *t*-test, Mann–Whitney's U test, Welch's *t*-test and Kruskal–Wallis test. Results are presented as the mean ± SD. *P*-values of $P < 0.05$ were considered to indicate statistical significance, and all statistical tests were two-tailed.

RESULTS

Retention effect of body temperature by various hyperthermic stimulation

The retention effect of body temperature by various hyperthermic stimulations was studied first. After hyperthermic stimulation, the kinetic change in body temperature was traced for 60 min (six subjects per group) as shown in Fig. 2 ($P < 0.05$). Soon after the hyperthermic stimuli, the bath group showed the highest body temperature (38.31°C), followed by the sauna (H), NMS and sauna (L) groups ($P < 0.05$). At that time all groups showed statistical higher body temperatures than that of the control group. However, at the later time points of 20, 40 and 60 min NMS showed the highest body temperature compared with the other groups ($P < 0.05$). Thus NMS showed the greatest retention effect of body temperature.

Effect of NMS on circulatory and endocrine system parameters

Next, the effects of repetitive NMS treatments on body temperature and pulse rate were studied, as NMS provided the greatest retention effect by single stimulation. Results of before and after treatment on both Day 1 and Day 7 were compared (Fig. 3B). Both Day 1 and Day 7 showed a marked elevation in body temperature immediately after NMS stimulation (Day 1: 36.8 ± 0.3 to 37.4 ± 0.3°C; Day 7: 36.9 ± 0.2 to 37.5 ± 0.2°C) ($P < 0.05$). However, there was not a statistically significant difference in the degree of the elevation between Day 1 and Day 7 (before stimuli: Day 1 vs Day 7, 36.8 vs 36.9°C; after stimuli: Day 1 vs Day 7, 37.4 vs 37.5°C). As for pulse after the NMS stimulation, both Day 1 and Day 7 showed an elevation compared to before NMS stimulation; however, there was no significant difference between the days.

It is well known that catecholamines and cortisol

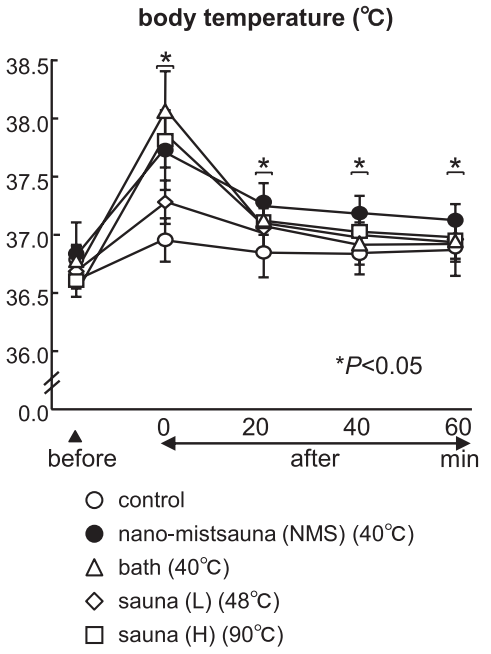

Fig. 2 Kinetic change in body temperature induced by various types of hyperthermia, tracked over 60 min (n = 6, each group).

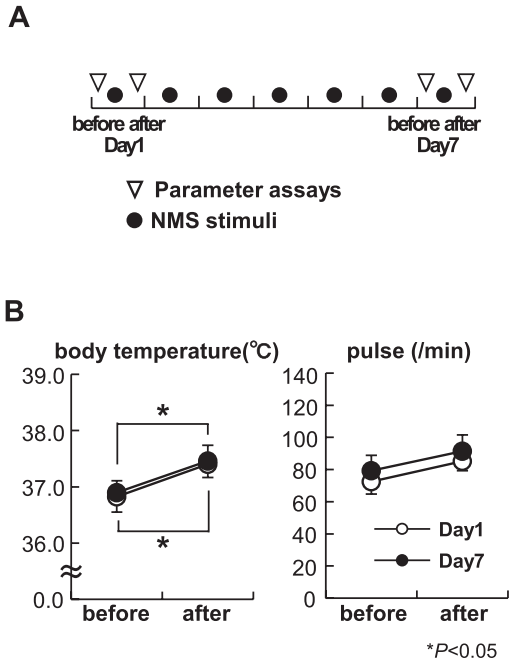

Fig. 3 Repetitive mild hyperthermia stimulation. (**A**) Treatment protocol of mild hyperthermia stimulation and (**B**) comparison between Day 1 and Day 7 of the effect of mild hyperthermia stimulation on body temperature and pulse. * $P < 0.05$.

indicate the status of autonomic nervous system and hypothalamic-pituitary-adrenal axis, especially under stress. Many investigators reported that the strong hyperthermia stimulation induced stress hormone. Therefore, to study the effect of repeated NMS on the nervous system and hypothalamic-pituitary-adrenal axis, cortisol and catecholamine (adrenaline, noradrenaline, dopamine) levels after NMS on Day 1 and Day 7 were investigated (Fig. 4A). The concentration of plasma adrenaline significantly dropped from Day 1 to Day 7 (0.043 ± 0.005 to 0.026 ± 0.005 pg/mL, $P < 0.05$), while noradrenaline showed an opposite tendency (0.36 ± 0.13 to 0.44 ± 0.13 pg/mL, $P < 0.05$). In contrast, cortisol showed no prominent differences between Day 1 and Day 7. The concentration of dopamine was below measurable limits both on Day 1 and Day 7.

Immunological parameters

It has been reported that a change in the status of the autonomic nervous system and hypothalamic-pituitary-adrenal axis affects both the number and the ratio of white blood cells (WBC) via catecholamine and cortisol. The total number of WBC, granulocytes and lymphocytes in the peripheral blood after repetitive mild hyperthermia treatment was compared between Day 1 and Day 7. The results showed that the total number of WBC and granulocytes had a trend towards being increased, while the lymphocytes did not (Fig. 4B).

Moreover, the effects of repetitive mild hyperthermia treatment on the relative frequency of NK, NKT, B and T cells were investigated (Fig. 5). The phenotype of lymphocytes was identified by two-color (CD3/CD56 and CD3/CD19) immunofluorescence staining (Fig. 5A). Compared to Day 1, on Day 7, the frequency of NKT ($CD3^+CD56^+$) cells was slightly increased (5.0 ± 1.0 to $5.9 \pm 1.8\%$), while that of T ($CD3^+CD19^-$) cells decreased (71.1 ± 6.4 to $68.6 \pm 6.0\%$). There was less change in the relative frequencies of NK ($CD3^-CD56^+$) (14.1 ± 3.9 to $14.3 \pm 3.2\%$) cells and B ($CD3^-CD19^+$) cells (11.9 ± 3.9 to $12.5 \pm 3.8\%$). There was no statistical difference (Fig. 5B).

Expression of MHC class II antigens on NKT cells and B cells after repetitive mild hyperthermia

It is well established that B cells and activated T cells express MHC class II antigens. Furthermore, we previously reported that the proportion of HLA-DR^+ NKT cells increased after mild hyperthermia treatment (28). Therefore, in this study the proportion of HLA-DR expression on B cells, T cells, NKT cells and NK cells was investigated by three-

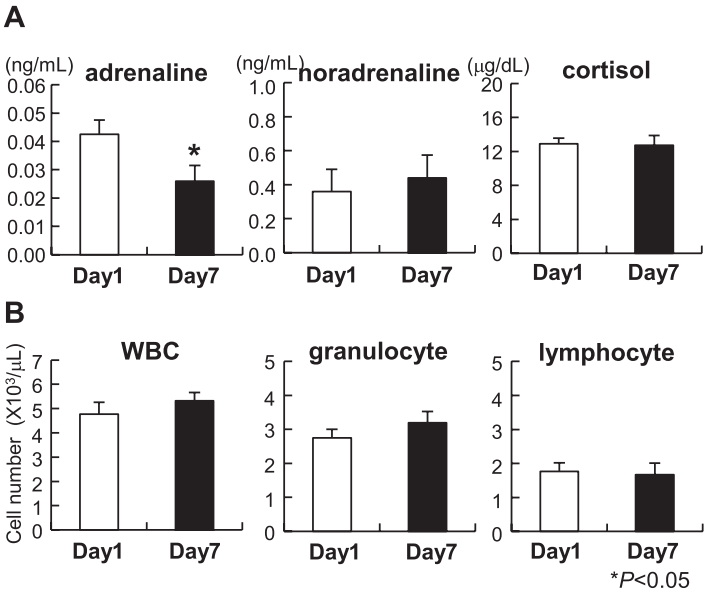

Fig. 4 Sympathetic nerve dominance was suppressed after repetitive mild hyperthermia stimulation. (**A**) Plasma level of catecholamines (adrenaline and noradrenaline) and cortisol were measured at Day 1 and Day 7 after mild hyperthermia stimulation. Data are presented as the mean ± SD, n = 6. * $P < 0.05$. (**B**) Cell number of WBCs, granulocytes and lymphocytes in blood measured at Day 1 and Day 7 after mild hyperthermia stimulation. Data are presented as the mean ± SD, n = 6.

color (CD3, CD56 [or CD19] and HLA-DR) immunofluorescence staining (Fig. 6, Table 1). NKT cells showed a marked increase in the expression of HLA-DR antigen after Day 7 of stimulation (Fig. 6, indicated by the arrow). On both Day 1 and Day 7, most B cells expressed HLA-DR antigen. However, compared with Day 1, on Day 7 the mean fluorescence intensity of HLA-DR on B cells was doubled (Fig. 6, indicated by the arrowhead). T cells increased both frequency and the mean fluorescence intensity of HLA-DR antigen after Day 7 of stimulation. Both the frequency and expression of HLA-DR on NK cells were not different between Day 1 and Day 7.

Effect of repetitive mild hyperthermia on immune function

The cytotoxic function of NK cells and proliferative response of T and B cells were investigated after NMS stimulation (Fig. 7). Compared with the cytotoxic activity of NK cells on Day 1, NK cell cytotoxicity on Day 7 was markedly increased (Day 1 vs Day 7: 35.7 ± 9.1 vs 61.2 ± 8.5%, $P < 0.05$). At that time, the proliferative response against mitogen (PWM) also showed a tendency to be increased compared with Day 1 (Day 1 vs Day 7: 23.4 ± 4.1 vs. 33.0 ± 4.1 $\times 10^3$ cpm).

DISCUSSION

This study revealed that the NMS mild hyperthermia treatment induced 1) a prominent elevation of body temperature with the greatest heat-retention effect, 2) a suppression of excessive dominance of sympathetic nerve, and 3) an enhancement of innate and acquired immunity.

Though the elevation of body temperature by NMS was not as high as that induced by the hot water bath, it showed a prominent difference compared with that of before the experiment (Fig. 2, 3B). Notably, the elevation of the body temperature by NMS was higher than other hyperthermia stimuli at 60 min after stimulation: the stimulus of NMS was more sustainable than the others tested. Moreover, diastolic blood pressure and level of PO_2 in venous blood tended to decrease and increase respectively, indicating that blood circulation was increased by hyperthermia induced by NMS (data not shown). Therefore, it may be considered that NMS is a mild and effective hyperthermia method. Tanaka *et al*. reported that heart rate and systolic blood pressure were elevated after bathing as well as after running (26). Therefore, bathing might be stressful to patients with cardiovascular disorders. In contrast, NMS is free from the stress of physical strain and soaking of bathing and it may be considered as a mild, effective and low-strain hyperthermia.

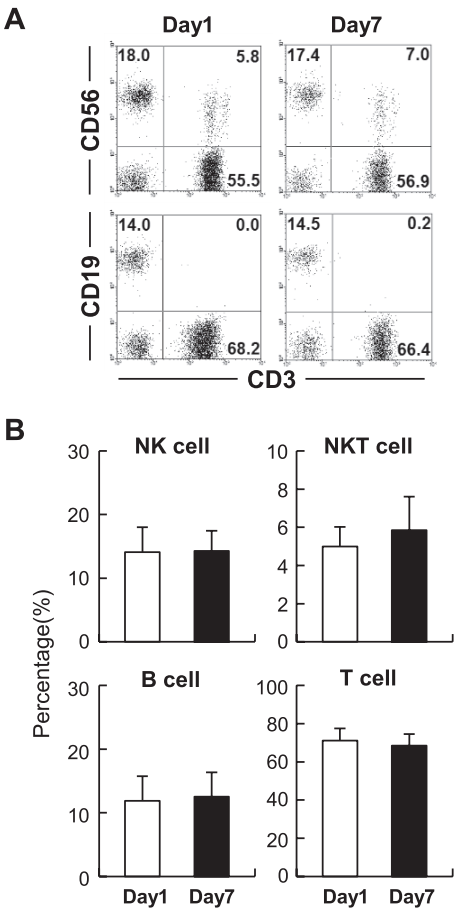

Fig. 5 Changes in lymphocyte subsets after repetitive mild hyperthermia stimulation. (**A**) Two-color staining for CD3/CD56 and for CD3/CD20. Representative results of six subjects are depicted. Numbers in the figure represent the percentages of fluorescence-positive cells. (**B**) The effect of repetitive mild hyperthermia stimulation on the percentage of lymphocyte subsets in peripheral blood. The percentage of NK cells, NKT cells, B cells and T cells in peripheral blood are shown (n = 6). Data are presented as the mean ± SD.

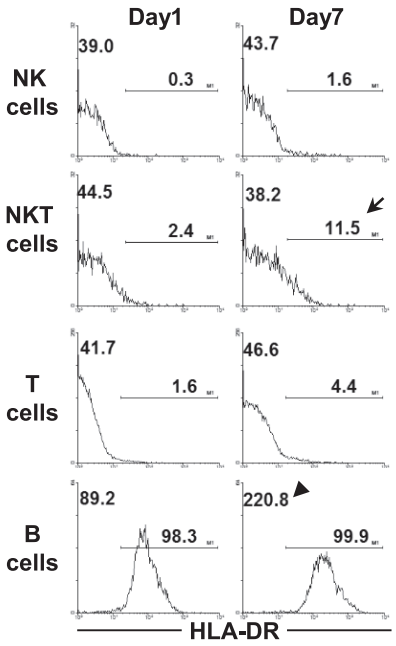

Fig. 6 Representative result of three-color staining for CD56, CD3 and HLA-DR and for CD19, CD3 and HLA-DR. By gated analysis, HLA-DR$^+$ cells were identified as NK cells (CD3$^-$CD56$^+$), NKT cells (CD3$^+$CD56$^+$), T cells (CD3$^+$CD19$^-$) and B cells (CD3$^-$CD19$^+$). In all subsets, the mean fluorescence intensity (MFI) (left top corner) and percentage (%) (right middle) are also indicated.

The changes in the levels of catecholamines and cortisol were also of interest. The plasma levels of the catecholamine adrenaline were significantly decreased. At that time, the levels of cortisol also showed a tendency to be decreased (Fig. 4A). It is well known that the levels of adrenaline and cortisol are elevated in a stress response (3, 15, 23, 24, 27). We also reported that an allostasis, such as "restrict stress", induced sympathetic nerve dominance and increase of adrenaline and cortisol in mice (24). Furthermore, it was reported that the hyperthermia stimulation increased stress hormone (11, 31). The results of this study indicated that repetitive mild hyperthermia by NMS suppressed sympathetic nerve and reduced the stress response (30). In contrast, the plasma level of noradrenaline tended to be increased. It is thought that repetitive mild hyperthermia treatment increases blood circulation and dilates peripheral vessels. Therefore it is suggested that the level of noradrenaline, which contracts peripheral vessels, is elevated to suppress excess blood vessel dilation.

Several reasons for the elevation of the number of WBC after hyperthermia have been reported, including the inflow of leukocytes from the spleen and bone marrow and condensation of plasma (2, 25). The result of our study agrees with these previous findings as the number of WBC tended to increase after hyperthermia (Fig. 4B). Furthermore, we investigated immune function, focusing on various parameters of lymphocyte subsets (Fig. 5). Our results did not show any statistically significant differences induced by repetitive mild hyperthermia treatment in the frequency of NK (CD3$^-$CD56$^+$), NKT (CD3$^+$CD56$^+$), B (CD3$^-$CD19$^+$) or T (CD3$^+$CD19$^-$) cells. In contrast, other investigators reported that hyperthermia treatment decreased the ratio of NK cells and T cells (5, 12, 29). The reason for the differences in results produced may be the magnitude of hyperthermia, as it was estimated that our protocol was only a mild hyperthermia.

Table 1 *Expression ratio and MFI of HLA-DR molecule among subpopulation*

subpopulations	percentage (%)		mean-fluorenscence intensity (MFI)	
	Day 1	Day 7	Day 1	Day 7
NK cells	0.4 ± 0.4	1.9 ± 1.2	39.6 ± 0.6	38.2 ± 3.6
NKT cells	4.2 ± 1.7	12.2 ± 1.2	41.7 ± 2.2	38.8 ± 3.2
T cells	2.9 ± 1.2	5.9 ± 1.3*	39.9 ± 3.1	47.0 ± 3.8*
B cells	99.2 ± 0.7	99.7 ± 0.2	152.5 ± 24.5	226.3 ± 21.8

*$P < 0.05$

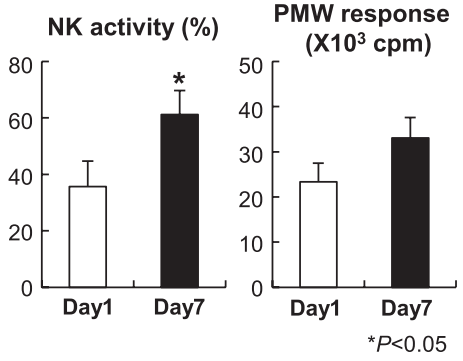

Fig. 7 Functional changes in NK cells and B cells induced by repetitive mild hyperthermia. (**left panel**) Lymphocytes were isolated from peripheral blood, and NK cell cytotoxicity against K562 cells was determined after 4-h incubation. (**right panel**) Lymphocytes were isolated from peripheral blood, and the proliferative response of B cells to PWM was determined by ^{3}H-TdR incorporation. The mean and one SD are produced from six subjects.

It is well established that hyperthermia enhances MHC antigen expression on the cell surface of leukocytes (9, 10, 16, 20). Therefore, we conducted experiments to determine if MHC II expression could be induced by repetitive mild hyperthermia stimuli. Our results showed that repetitive mild hyperthermia treatment increased the frequency of HLA-DR and MHC class II antigen expression on T cells (Fig. 6, Table 1). The mean fluorescence intensity of HLA-DR antigen (*i.e.* the expression level of HLA-DR) on B cells also tended to be increased by repetitive mild hyperthermia treatment. This change meant activation of T cell, especially $CD4^{+}$ T cells and B cells. Du G *et al*. reported that administration of 2, 4-dinitrophenol (DNP) to B6 mice induced febrile-range (39–40°C) hyperthermia and that splenic $CD4^{+}$ T cells produced increased IFN-γ and IL-2 (6). This showed that febrile-range (39–40°C) hyperthermia enhances Th1 cellular (anti-tumor) immunity. Our study did not investigate cytokine production; however, mild hyperthermia may also enhance such cytokine production.

Finally, the effect of a repetitive mild hyperthermia on NK cells was investigated. Our results showed that the cytotoxic activity of NK cells was significantly increased by repetitive mild hyperthermia (Fig. 7). It has been reported that NK cells have enhanced cytotoxicity to the target cells depending on NKG2D (activated receptor of NK cells) expression at 39.5°C *in vitro* (21). Researchers reported that whole body hyperthermia in mice of a tumor-bearing model activated NK cells and increased their tumor infiltration (4). At that time febrile range of temperature for mild hyperthermia (39–41°C) was more important than high temperature of "heat shock"(≧42°C) (8). As the cytotoxicity of NK cells is also enhanced by hyperthermia in the physiological range, the hyperthermia method used in this study may be an effective protocol in enhancing activation of NK cells. Furthermore, in this study the proliferative response of B cells against PWM tended to increase (Fig. 7). It is well known that the response of B cells to PWM depends on T cells. Thus the results indicated not only an enhancement of MHC class II antigen expression but also an increase in B cell activation.

In summary, a mild hyperthermia induced by NMS is effective in increasing body temperature and prolonging the duration of the thermal effect. Moreover, repeated mild hyperthermia by MNS may induce suppression of the sympathetic nervous system. Acquired immunity by T and B cells as well as innate immunity by NK cells was also enhanced. However, further research, including larger scale studies, are required to support these discussions.

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REFERENCES

1. Abo T and Balch CM (1981) A differentiation antigen of human NK and K cells identified by a monoclonal antibody

(HNK-1). *J Immunol* **123**, 1024–1029.
2. Athens JW, Raab SO, Haab OP, Mauer AM, Ashenbrucker H, Cartwright GE and Wintrobe MM (1961) Leukokinetic studies. III. The distribution of granulocytes in the blood of normal subjects. *J Clin Invest* **40**, 159–164.
3. Brazaitis M, Eimantas N, Daniuseviciute L, Mickeviciene D, Steponaviciute R and Skurvydas A (2014) Two stragies for response to 14°C cold-water immersion: Is there a difference in the response of motor, cognitive, immne and stress markers? *PLoS One* **9**, e109020.
4. Burd R, Dziedzic TS, Xu Y, Caligiuri MA, Subjeck JR and Repaskey EA (1998) Tumor cell apoptosis, lymphocyte recruitment and tumor vascular changes are induced by low temperature, long duration (fever-like) whole body hyperthermia. *J Cell Physiol* **177**, 137–147.
5. Ciavarra RP, Silvester S and Brody T (1987) Analysis of T-cell subset proliferation at afebrile and febrile temperatures: differential response of Lyt-$1^{+}23^{-}$ lymphocytes to hyperthermia following mitogen and antigen stimulation and its functional consequence on development of cytotoxic lymphocytes. *Cell Immunol* **107**, 293–306.
6. Du G, Liu Y, Li J, Wang Y and Li H (2013) Hypothermic microenvromnet plays a key role in tumor immune subversion. *Int Immunopharmacol* **17**, 245–253.
7. Farnes P, Barker BE, Brownhill LE and Fanger H (1964) Mitogenic activity in *Phytolacca americana* (Pokeweed). *Lancet* **2**, 1100–1101.
8. Hanson DF (1997) Fever, temperature, and the immune response. *Ann NY Acad Sci* **813**, 453–464.
9. Huang YH, Haegerstrand A and Frostegrad J (1996) Effects of in vitro hyperthermia on proliferative responses and lymphocyte activity. *Clin Exp Immunol* **103**, 61–66.
10. Ito A, Shinkai M, Honda H, Wakabayashi T, Yoshida J and Kobayashi T (2001) Augmentation of MHC class I antigen presentation via heat shock protein expression by hyperthermia. *Cancer Immunol Immunother* **50**, 512–522.
11. Jimenez C, Melin B, Savourey G, Launay JC, Alonso A and Mathieu J (2007) Effects of passive hyperthermia versus exercise-induced hyperthermia on immune responses: hormonal implications. *Eur Cytokine Netw* **18**, 154–161.
12. Koga S, Izumi A, Maeda M, Shimizu N, Osaki Y and Kanayama H (1983) The effects of total body hyperthermia combined with anticancer drugs on immunity in advanced cancer patients. *Cancer* **52**, 1173–1177.
13. Kukkonen-Harjula K and Kauppinen K (2006) Health effects and risk of sauna bathing. *Int J Circumpolar Health* **65**, 195–205.
14. Maeda M (2006) Hot spring water bathing and the thermology. *Biomed Thermol* **26**, 58–61.
15. Maruyama S, Minagawa M, Shimizu T, Oya H, Yamamoto S, Musha N, Abo W, Weerasinghe A, Hatakeyama K and Abo T (1999) Administration of glucocorticoid markedly increases the numbers of granulocytes and extrathymic T cells in the bone marrow. *Cell Immunol* **194**, 28–35.
16. McCormick JK, Tripp TJ, LleraAS, Sundberg EJ, Dinges MM, Mariuzza RA and Schlievert PM (2003) Functional analysis of the TCR binding domain of toxic shock syndrome toxin-1 predicts further diversity in MHC class II/superantigen/TCR ternary complexs. *J Immunol* **171**, 1385–1392.
17. Miyaji C, Watanabe H, Minagawa M, Toma H, Kawamura T, Nohara Y, Nozaki H, Sato Y and Abo T (1997) Numerical and Functional characteristics of lymphocyte subsets in centenarians. *J Clin Immunol* **17**, 420–429.
18. Miyaji C, Watanabe H, Toma H, Akisaka M, Tomiyama K, Sato Y and Abo T (2000) Functional alteration of granulocytes, NK cells, and natural killer T cells in centenarians. *Human Immunol* **61**, 908–916.
19. Ohishi T, Nukuzuma C, Seki A, Watanabe M, Tomiyama-Miyaji C, Kainuma E, Inoue M, Kuwano Y and Abo T (2009) Alkalization of blood pH is responsible for survival of cancer patients by mild hyperthermia. *Biomed Res (Tokyo)* **30**, 95–100.
20. Ostberg JR, Patel R and Repasky EA (2000) Regulation of immune activity by mild (fever-range) whole body hyperthermia: effects on epidermal Langerhans cells. *Cell Stress Chaperons* **5**, 458–461.
21. Ostberg JR, Dayanc BE, Yuan M, Oflazoglu E and Repasky EA (2007) Enhancement of natural killer (NK) cell cytotoxicity by fever-range thermal stress is dependent on NKG2D function and is associated with plasma membrane NKG2D clustering and increased expression of MICA on target cells. *J Leukoc Biol* **82**, 1322–1331.
22. Pilch W, Szygula Z, Palka T, Pilch P, Cison T, Wiecha S and Tota Ł (2014) Comparison of physiological reactions and physiological strain in healthy men under heat stress in dry and steam heat saunas. *Biol Sport* **31**, 145–149.
23. Sagiyama K, Tsuchida M, Kawamura H, Wang S, Li C, Bai X, Nagura T, Nozoe S and Abo T (2004) Age-related bias in function of natural killer T cells and granulocytes after stress: reciprocal association of steroid hormones and sympathetic nerves. *Clin Exp Immunol* **135**, 56–63.
24. Shimizu T, Kawamura T, Miyaji C, Oya H, Bannai M, Yamamoto S, Weerasinghe A, Halder RC, Watanabe H, Hatakeyama K and Abo T (2000) Resistance of extrathymic T cells to stress and role of endogenous glucocorticoids in stress associated immunosuppression. *Scand J Immunol* **51**, 285–292.
25. Statland BE, Winkel P and Bokelund H (1973) Factors contributing to intra-individual variation of serum constituents: 2. Effects of exercise and diet on variation of serum constituents in healthy subjects. *Clin Chem* **19**, 1380–1383.
26. Tanaka N, Miyata M, Shimodozono M, Deguchi A, Kokushou M, Hayasaka S and Goto Y (2011) Comparative study ov the effects of health promotion of single bathing and 200m running. *J Jpn Soc Balneol Climatol Phys Med* **74**, 263–272.
27. Thomaa MV, Kirschbaumb C, Wolfa JM and Rohledera N (2012) Acute stress responses in salivary alpha-amylase predict increases of plasma norepinephrine. *Biol Psychol* **91**, 342–348.
28. Tomiyama-Miyaji C, Watanabe M, Ohishi T, Kanda Y, Kainuma E, Bakir HY, Shen JW, Ren HW, Inoue M, Tajima K, Bai XF and Abo T (2007) Modulation of the endcrine and immunesystems by well-controlled hyperthermia equipment. *Biomed Res (Tokyo)* **28**, 119–125.
29. Wang XX, Kitada Y, Matsui K, Ohkawa S, Sugiyama T, Kohno H, Shimizu S, Lai JE, Matsuno H, Yamaguchi M and Yamaguchi N (1999) Variation of cell populations taking charge of immunity in human peripheral blood following hot spring bathing. —Quantitative discussion. *J Jpn Soc Balneol Climatol Phys Med* **62**, 129–134.
30. Watanabe M, Tomiyama C, Honma T, Inada A, Hayakawa T and Abo T (2011) Effects of a new hyperthermia treatment, Nano-mist Sauna, on our body temperature, energy production and immune and autonomic nerve system. *J Jpn Soc Balneol Climatol Phys Med* **74**, 96–102.
31. Won SJ and Lin MT (1985) Thermal stresses reduce natural killer cell cytotoxicity. *J Appl Physiol* **79**, 732–737.