Chapter

Biological Effects of Negatively Charged Particle-Dominant Indoor Air Conditions

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Abstract

To identify health-promoting indoor air conditions, we developed negatively charged particle-dominant indoor air conditions (NCPDIAC). Experiments assessing the biological effects of NCPDIAC comprised (1) 2.5-h stays in NCPDIAC or control rooms, (2) 2-week nightly stays in control followed by NCPDIAC rooms, (3) 3-month OFF to ON and ON to OFF trials in individual living homes equipped with NPCDIAC in their sleeping or living rooms, and (4) in vitro assays comparing the immune effects between negatively charged particle-dominant and control cell culture incubators. The most significant difference examined between NCPDIAC and control rooms in the 2.5-h stays was an increase in interleukin (IL)-2 with occupancy of the NCPDIAC room. For the 2-week nightly stay experiments, natural killer (NK) cell activity increased with occupancy of the NCPDIAC room. The 3-month OFF to ON trial showed an increase in NK cell activity, while the ON to OFF trial yielded a decrease in NK cell activity. Additionally, the in vitro assays also showed an increase in NK cell activity. The use of NCPDIAC resulted in increased NK cell activity, which has the effect of enhancing immune surveillance for the occurrence of cancer and improving symptoms associated with viral infections.

Keywords: indoor air, negatively charged particle, natural killer cell activity

1. Introduction

Indoor air conditions can sometimes affect human health. For example, sick building syndrome (SBS) is one of the most well-known health impairments caused by indoor air conditions [1–3]. Volatile organic compounds (VOCs) are considered to be the cause of SBS [1–3]. SBS can induce a variety of signs or symptoms such as headache; eye, nose, and throat irritation; fatigue; dizziness; and nausea. The condition of patients with SBS may worsen following exposure to certain VOCs, with individual patients revealing specific hypersensitivity to particular chemicals. It is considered that some pathophysiological alterations in the psycho-neuro-immune-endocrine network at the level of genes, molecules, proteins, cells, and

organs may occur in SBS patients, which then defines or determines their sensitivity to such low concentrations of VOCs [1–3]. However, the precise nature of these alterations is yet to be delineated. Consequently, the only advice presently available to SBS patients is to avoid exposure to VOCs for which the patient shows particular sensitivity [1–3].

Furthermore, most homes in Japan possess air-conditioning units in each room. During the winter season, the room adjacent to the bathroom which is utilized for changing clothes is narrow and particularly cold. Moreover, the lavatory is also cold. Consequently, there is a risk of changes in blood pressure and the onset of cardiovascular events caused by drastic changes in room temperature in these areas [4, 5].

Thus, decreasing the amount and use of chemicals and maintaining appropriate room temperatures at home are things that can be considered with respect to the task of establishing health-promoting indoor conditions. Additionally, although there are few reports detailing the use of indoor air under negatively charged conditions, a consideration of air electrical charges may assist with this task [6–9].

2. Development of NCPDIAC

The development of NCPDIAC has previously been reported [10].

As shown in **Figure 1**, NCPDIAC was established using extraporous charcoal paint and loading an electric voltage (approximately 72–100 V) behind the room walls [10]. The charcoal paints were mainly used for deodorization and dehumidification. As a result, the surface of the walls acquired a slightly negative charge, and small positively charged particles 20–30 nm in diameter collected on the surface of walls [10]. Thus, although negatively charged particles were not introduced into the

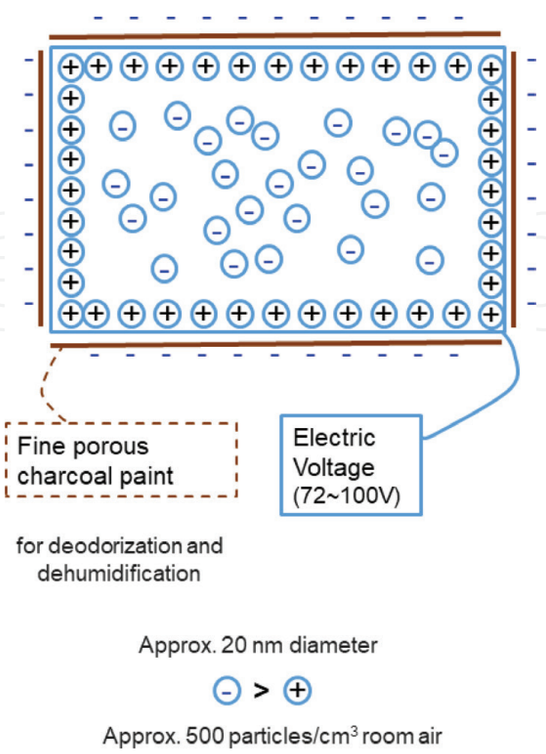

Figure 1.
NCPDIAC was established using extraporous charcoal paint and loading an electric voltage behind the walls.

indoor conditions, the balance between positively and negatively charged particles was such that negatively charged particles were predominant [10].

3. 2.5H stay experiments

Results of the 2.5H (2.5-h) stay experiments have previously been reported [10].

Three control rooms and three NCPDIAC rooms were built in the wide subunderground laboratory. Both types of rooms were built in a large subunderground laboratory in the Comprehensive Housing R&D Institute, SEKISUI HOUSE, Ltd., at Kizu-town, Kyoto prefecture, Japan. The area and volume of the laboratory were approximately 539 m^2 and 1564 m^3, respectively, and those of the experimental rooms were 9.1 m^2 and 22.8 m^3, respectively. The appearance of the control and NCPDIAC rooms is shown in **Figure 2A**. All of the healthy volunteers (HV) referred to in **Figure 2B** were unaware of the room type (control or NCPDIAC) they were to occupy during the experimental period. The following items were measured immediately prior to (prestay) and following (poststay) entry into the rooms as previously reported [10].

1. General conditions: blood chemistry including liver [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (γGT)] and kidney functions [creatinine, blood urea nitrogen (BUN) and uric acid], blood sugar and lactic acid levels, and peripheral blood counts (white blood cell, red blood cell, hemoglobin, hematocrit and platelet) were measured using peripheral venous blood. Blood pressure and pulse rate were also measured.

2. Stress markers: Levels of blood cortisol and salivary cortisol, chromogranin A, amylase, and secretory immunoglobulin A were measured as stress markers.

3. Parameters related to the autonomic nervous system: The autonomic nervous system was examined using the Flicker test, a stabilometer, and heart rate monitor for 3 min. The Flicker test and flicking frequencies of red, green, and yellow colors were monitored. A Gravicoder GS-7TM instrument (Anima Inc., Tokyo, Japan) was used as a stabilometer and the Romberg ratio was used as the parameter for body sway. The ratio was calculated from the whole trajectory of the body sway during a 30-s standing period with eyes closed divided by that with eyes open. The heart rate was monitored using a Heart Rate Monitor S810iTM instrument (Polar Electro, Kempele, Finland) for 3 min. During monitoring, HV sat on chairs and were kept at rest. The R wave intervals in the electrocardiogram were estimated and the standard deviation (SD) or R wave interval was considered as an index of heart rate fluctuation.

4. Immunological parameters: Serum levels of immunoglobulin (Ig) E and Ig A, and cytokines related to the Th1/Th2 balance [Interferon (IFN)-γ, tumor necrosis factor (TNF)-α, Interleukin (IL)-2, IL-4, IL-6 and IL-10] were evaluated. Individual samples for cytokine measurement were applied to the Cytometric Bead Array of Human Th1/Th2 cytokine kit II (CBA, BD Bioscience, San Jose, CA, USA) and measurements were made using FACSCalibur flow cytometry (BD Bioscience) according to the manufacturer's instructions. For samples that revealed less than the lower limit of the values from analytical methods for cytokines and immunoglobulins, the 1/10 value of the minimum values among the entire measurable samples was substituted instead of 0 or left as "unmeasurable," as previously reported [10].

5. Blood viscosity: Blood viscosity was measured using a Micro-Channel Flow Analyzer MC-FAN (MC Laboratory Inc., Tokyo, Japan) according to the manufacturer's instructions. Briefly, peripheral heparinized blood sample (100 μl) was placed into the instrument and allowed to flow through the microchannel chips, which are a model for capillary vessels, and the flowing time was recorded. The flowing blood sample was visualized using a CCD camera equipped to the microscope.

These experiments were approved by the institutional ethical committee (#114). Samples were only taken from HV who provided written informed consent.

The electrical charge in these rooms was measured using an Ion Counter EB-1000TM instrument made by Eco Holistic Inc., Suita, Japan.

Differences in the positively and negatively charged particles in control and NCPDIAC rooms are shown in **Figure 2C**. The number of positively charged particles in the rooms with NCPDIAC was reduced. However, the number of negatively charged particles in control and NCPDIAC rooms did not differ. Negatively charged air conditions were therefore formed by reducing the number of positively charged particles in NCPDIAC rooms.

Differences between control and NCPDIAC room values for all items measured were determined by calculating [poststay]-[prestay]. As shown in **Figure 2D**, differences were found in IL-2 [10]. The increase in IL-2 levels (by approximately 1 pg/ml)

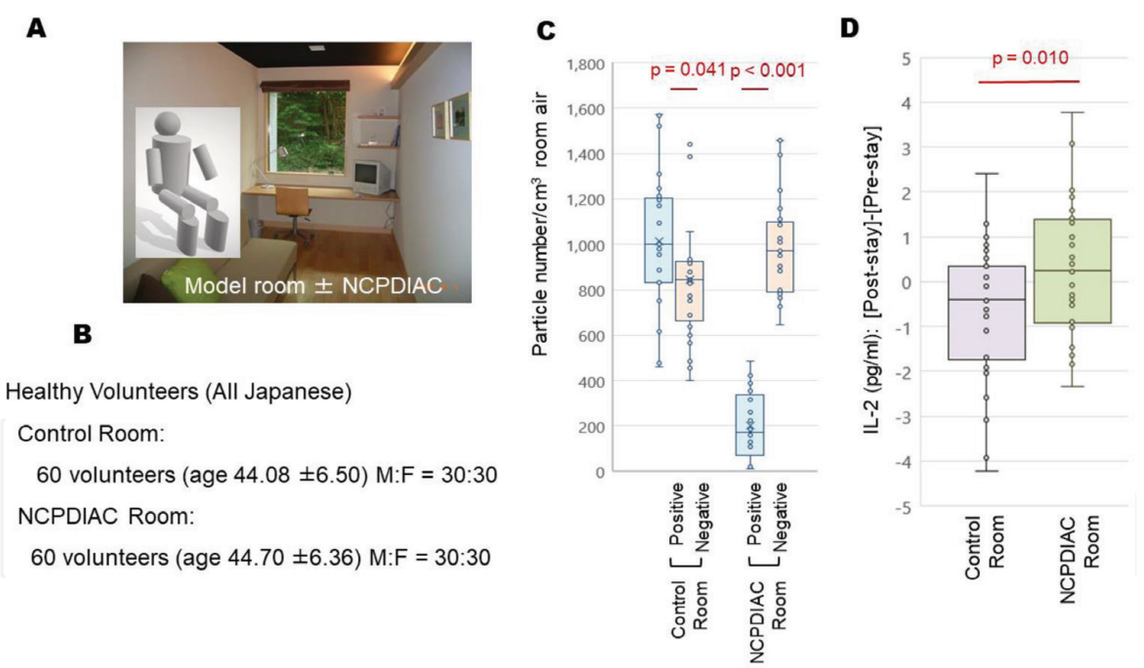

Figure 2.
(A) Control and NCPDIAC rooms were constructed as shown. Healthy volunteers occupied rooms for 2.5 h, being unaware of the room type (control or NCPDIAC). Volunteers remained within the rooms in a stable state, without sleeping or excitement. (B) A total of 60 Japanese volunteers participated in experiments for each room type. The gender ratio and average age of the volunteers were almost identical in both groups (occupants of control and NCPDIAC rooms). (C) The box-and-whisker plots show the number of positively and negatively charged particles per 1 cm³ air in control and NCPDIAC rooms. Although there was no difference in the level of positively and negatively charged particles in control rooms, NCPDIAC rooms possessed significantly lower levels of positively charged particles compared with control rooms. Furthermore, there was no difference in the level of negatively charged particles between control and NCPDIAC rooms. Statistical differences were assayed using the student T test. (D) The box-and-whisker plots show a comparison of IL-2 levels ([Post-Stay]-[Pre-Stay], pg/ml) in volunteers who occupied the control and NCPDIAC rooms during the 2.5-h stay experiments. The most significant difference found in the examined values using [poststay]-[prestay] related to IL-2 levels, which increased significantly following NCPDIAC room stays compared with control room stays. This difference was analyzed using the Mann-Whitney U test.

was considered not to be caused by any pathophysiological conditions. Additionally, it was considered that levels could return to base values in individual HV. As a result, it appears that NCPDIAC affected the immune system without any adverse effects with respect to the signs, symptoms, or measured items in the present experiments [10].

4. 2W nightly stay experiments

In the next step, 2W (2-week) nightly stay experiments were performed as previously reported [11].

Approximately 1 year after obtaining the results of the 2.5H experiments and with subsequent discussions, new participants were recruited for our 2W nightly stay experiments. This study was approved by the institutional ethical committee (#176) and samples were only taken from HV who provided written informed consent.

The dormitory belonging to SEKISUI HOUSE, Ltd., Kizugawa-City, Kyoto Prefecture, Japan, and ordinarily used for the training of employees was utilized for our 2W nightly stay experiments. These employees usually receive training for 3 months as shown in **Figure 3A**. Then, in the case of volunteers for our experiments, employees were moved at the second month from their original room into a control

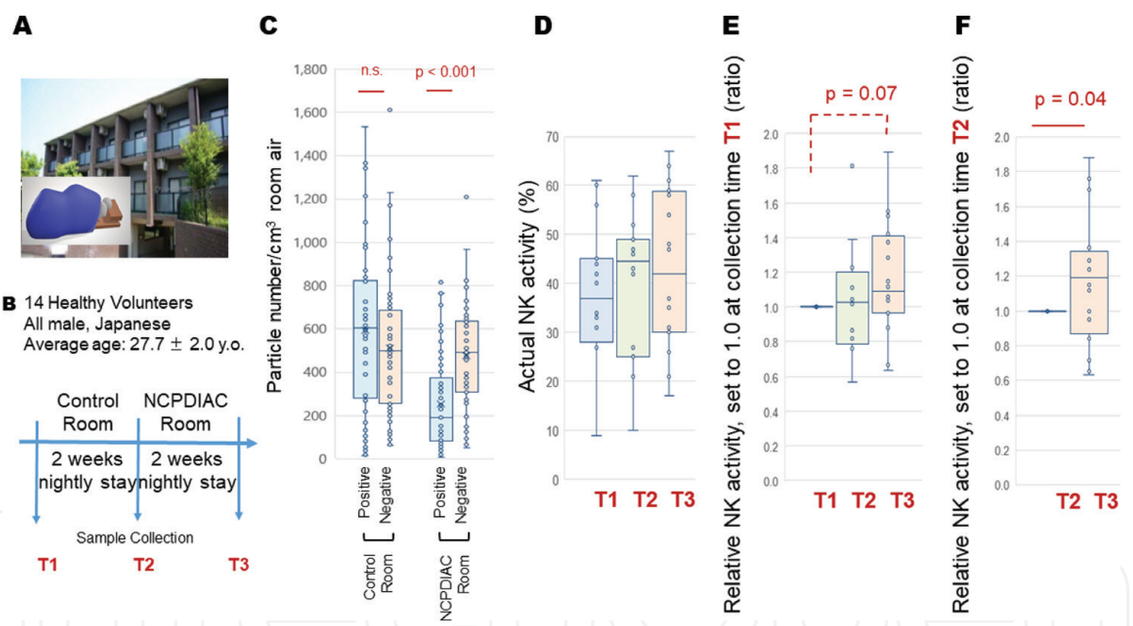

Figure 3.
(A) For the 2W (2-week) nightly stay experiments the dormitory for training employees was used. (B) All volunteers initially occupied the control room every night for 2 weeks. Thereafter, volunteers occupied NCPDIAC rooms without being aware of which room type (control or NCPDIAC) they had initially occupied. Sample collections were performed at T1, T2, and T3 time points. T1 represented the time point prior to volunteers occupying the control room. T2 represented the time point immediately following the 2W nightly stay in control rooms, and just prior to occupancy of the NCPDIAC room, while T3 represented the time point following the 2W nightly stay in the NCPDIAC room. Although HV agreed to be recruited in this study, they were unaware of the room type (control or NCPDIAC) they had initially entered. (C) The box-and-whisker plots show the number of positively and negatively charged particles per 1 cm^3 air in control and NCPDIAC rooms. Although there was no difference between the number of positively and negatively charged particles in control rooms, NCPDIAC rooms possessed significantly lower levels of positively charged particles compared with control rooms. Furthermore, there was no difference in the level of negatively charged particles between control and NCPDIAC rooms. Statistical analyses were performed using the student T test. (D) The box-and-whisker plots show the actual NK cell activity (%) at T1, T2, and T3 time points. There were no differences among the three time points. Statistical analyses were performed using the ANOVA test. (E) The box-and-whisker plots show the relative NK activity after setting T1 of individual volunteers to 1.0. Statistical analyses comparing T1 and T2 or T3 were performed using the student T test. (F) Relative NK activity after setting T2 of individual volunteers to 1.0. There was a significant increase in relative NK activity after the 2W nightly stay in the NCPDIAC rooms. Statistical analyses were performed using the student T test.

(both were the same in terms of NCPDIAC). After occupying the control room every night for 2 weeks, they were then moved into NCPDIAC rooms, without being aware of the room type (control or NCPDIAC) they had initially occupied. As shown in **Figure 3B**, sample collections were performed prior to volunteers occupying the control room (T1), after their 2W nightly stay in the control room (T2), and finally after occupying the NCPDIAC room (T3). The measured items remained unchanged during the 2.5H stay experiments. Additionally, several parameters that had not changed during the hour-based time period but were altered during the week-based time period when environmental factors or physiological conditions had changed were included as biological parameters. Those parameters comprised NK cell activity, along with urine 17 hydroxycorticosteroid (OHCS) and 8-oxo-2′-deoxyguanosine (OHdG) levels.

All volunteers initially occupied the control room every night for 2 weeks. Thereafter, volunteers occupied NCPDIAC rooms without being aware of which room type (control or NCPDIAC) they had initially occupied. Sample collection was performed at T1, T2, and T3 time points. T1 represented the time point prior to volunteers occupying the control room. T2 represented the time point immediately following the 2W nightly stay in control rooms, and just prior to occupancy of the NCPDIAC room, while T3 represented the time point following the 2W nightly stay in the NCPDIAC room.

Among all the items measured, significant change was only found in the NK activity. The NK cell activity was determined using a ^{51}Cr-release assay according to a method outlined in previous reports [12, 13]. The effector cell (mononuclear cell)-to-target cell (K562 cell) ratio was 10:1. However, there was a wide range of individual actual NK cell activity in the vicinity of approximately 10% to greater than 50%. In the 2W nightly stay experiments, the NK cell activity among HV ranged from less than 30% to near 60% at T1 (**Figure 3B**). Although actual NK activity measurements did not reveal any statistical significance (**Figure 3D**) due to the wide variation in individual volunteers, when the relative NK activity was set to 1.0 at T1, there was a tendency toward increased NK cell activity at T3 (**Figure 3E**) [11]. Since the room conditions prior to T1 and T2 collections were basically the same, the relative NK cell activity between T2 and T3 was compared, with T2 being set to 1.0 in individual HV [11]. As shown in **Figure 3F**, there was a significant increase in relative NK activity at T3, after the 2W nightly stay with NCPDIAC [11].

5. In vitro experiments

The in vitro experiments were performed to examine cellular alterations under negative particle dominant conditions. Results have previously been reported [14].

Freshly isolated peripheral blood mononuclear cells derived from seven healthy volunteers were cultured in a standard CO_2 incubator at 37°C with 5% CO_2 under humidified conditions (standard cell culture conditions comprised a humidity of 95%). For these experiments, it was impossible to use charcoal paint or to load an electric voltage. Thus, negatively charged particles were forced in and circulated. The difference between positively and negatively charged particles in 1 cm^3 of incubator air was approximately 3000 (**Figure 4A**) [14].

This study was approved by the institutional ethical committee (#883) and samples were only taken from the HV who provided written informed consent. Peripheral blood mononuclear cells were derived from samples obtained from HV.

For the NK cell activity, K562 cells, a human immortalized myelogenous leukemia cell line, were stained with Vybrant™ Dio Cell-Labeling Solution by incubation for 20 min at room temperature [14]. Dio-stained cells were then washed with phosphate buffered slain (PBS), and peripheral blood mononuclear cells (PBMC)

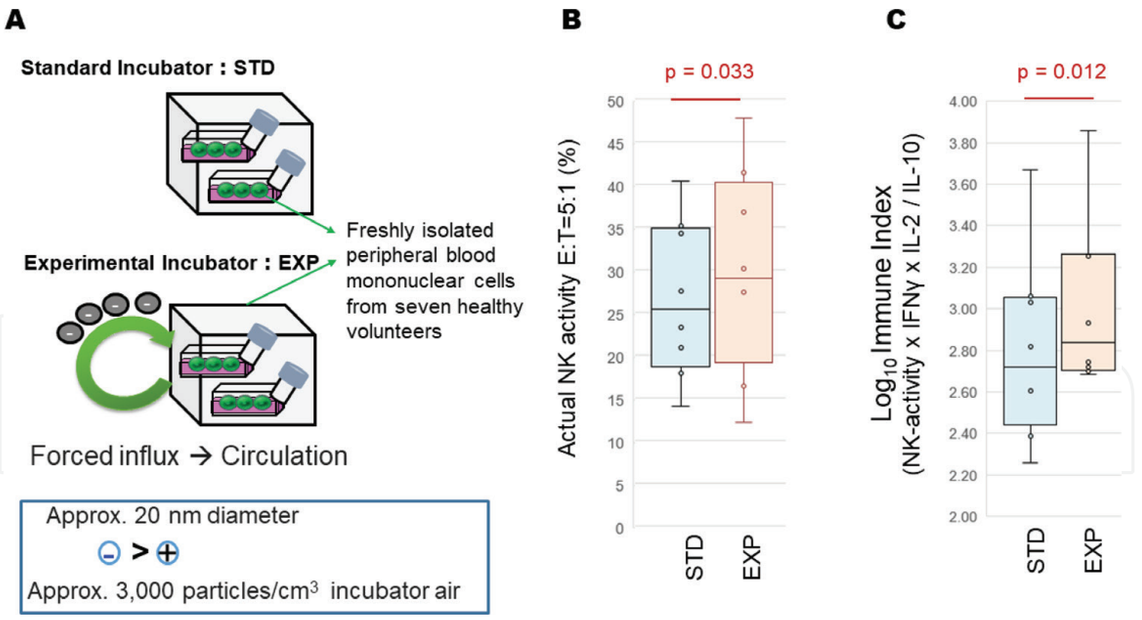

Figure 4.
(A) Peripheral blood mononuclear cells from HV were incubated in standard (STD) or experimental (EXP) incubators. Negatively charged particles (20–30 nm in diameter) were forced into incubators and then circulated. The experimental incubator was set to generate negatively charged particles using a neutralizing instrument that created negatively charged particles (SJ-M200, Keyence Co. Ltd., Osaka, Japan). This instrument yielded negatively charged particles that were set to directly enter the inside of the incubator (by making a hole). Since the interior volume of the incubator was 49 l, the negatively charged particles entered and passed out at a rate of approximately 3000 particles/cm^3. (B) The box-and-whisker plots show the increase in actual NK cell activity, represented by the E/T (effector vs. target cells) ratio, which, being 5:1, showed greater increase with experimental incubators and negatively charged particle-dominant culture conditions compared with standard incubators. (C) The LOG$_{10}$ value of the "immune index" as [NC-activisty X IFN-γ concentration X IL-2 concentration]/[IL-10 concentration] was compared between standard and experimental incubator conditions. Experimental incubator conditions showed a significant increase in this index.

were incubated with 5000 Dio-labeled K562 cells in 96-well round bottom plates at an effector cell-to-target cell (E/T) ratio of 2.5:1, 5:1, or 10:1 for 5 h in experimental or standard incubators. Following incubation, cells were collected and stained with propidium iodide (PI) at 5 μg/ml and the percentage of PI+ Dio-labeled cells among the total Dio-labeled cells, representing the percentage of lysed cells, was examined using FACSCalibur flow cytometry. The percentage of specific lysis induced by effector cells was calculated after analyzing the substrate and spontaneous dead cell numbers expressed in wells without effector [14].

Additionally, peripheral blood mononuclear cells were cultured in RPMI1640 culture medium with antibiotics just as for standard cell cultures for 1 or 2 weeks without any stimulants such as cytokines. The concentrations of IFN-γ, IL-2, and IL-10 (as well as IL-6, TNF-α, and IL-4) in supernatants were measured. Additionally, other items such as surface CD25, CD69, programmed death-1 (PD1), and CD44 expression in CD4+ T helper cells, CD8+ T cells, and NK cells were measured [14].

It is noteworthy that NK cell activity was significantly higher when incubations were performed in the experimental incubator compared with those performed in the standard incubator (**Figure 4B**). Additionally, if we calculate the "immune index" as [NC-activity X IFN-γ concentration X IL-2 concentration]/[IL-10 concentration] and compare this index from standard and experimental incubators using log$_{10}$ titer (**Figure 4C**), there is a significantly greater increase in this "Log$_{10}$ Immune Index" associated with the use of experimental incubators compared with the use of standard incubators. The higher value of this item was assumed to reflect stimulation of immune status [14].

These results indicated that a predominance of negatively charged particles induces immune stimulation at non-pathophysiological levels [14].

6. 3M ON and OFF trials

Finally, long-term (3-month, or 3M) stay experiments were performed and the results have previously been reported [15].

Following the aforementioned experiments (2.5H and 2W nightly stay experiments and evaluations), it seemed that NCPDIAC stimulated NK activity with no adverse effects on HV [15]. The increase in NK activity could be accounted for by the short-term, albeit slight, increase in IL-2, which may activate NK cells during the 2W period. The following experiments were then applied in living homes. The homes of seven volunteers were modified for NCPDIAC, targeting mainly sleeping rooms and living rooms. A switch panel approximately 4 × 22 × 29 cm in size had been fitted. Volunteers would then switch this panel ON and OFF every 3 months. Then, prior to and following every 3-month ON or OFF living period, clinical measurements including NK cell activity and others (as measured in the 2W nightly stay experiments) were performed. A total of 16 OFF to ON (3M ON) and 13 ON to OFF (3M OFF) trials were performed as shown in **Figure 5A** [15].

Blood samples were taken just before switching ON or OFF. Thus, during the OFF to ON (3M ON) period, HV stayed at home with NCPDIAC (sleeping room and living room). During the ON to OFF (3M OFF) period, HV occupied rooms in their homes without NCPDIAC [15].

All seven HV comprised Japanese living in Japan and were asked to join this project by first-class registered architects who are colleagues of the authors. The average age of the volunteers was 54.86 ± 9.15 years and included five males and two females.

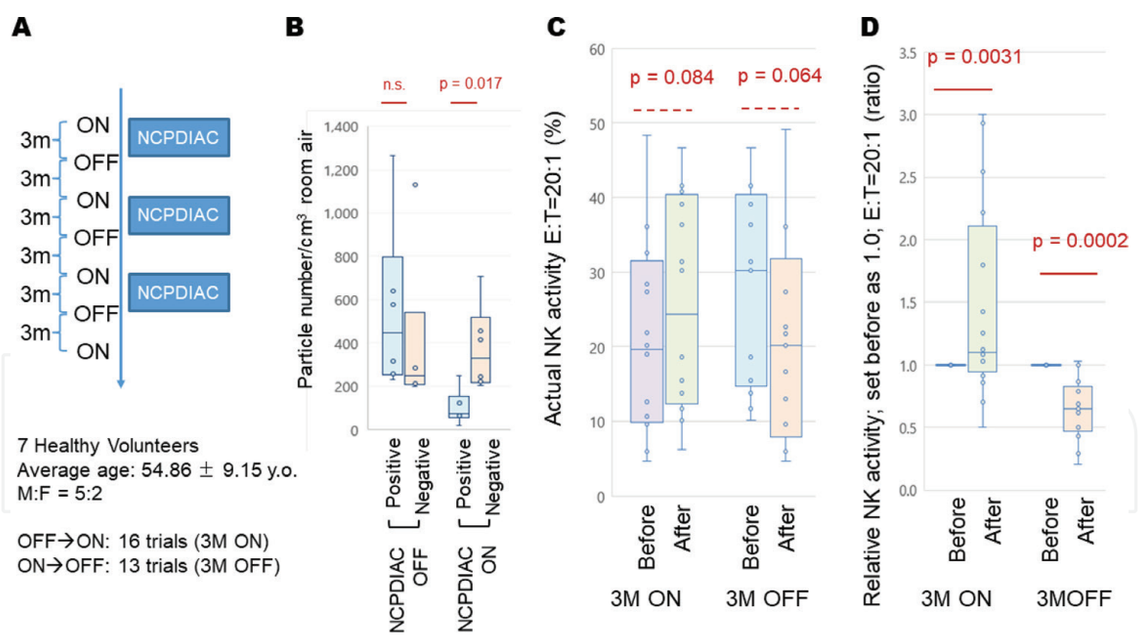

Figure 5.
(A) Seven healthy volunteers utilized sleeping and living rooms with NCPDIAC in their homes. During this period, the occupants themselves would switch NCPDIAC ON and OFF. Sixteen trials of OFF to ON (3M ON) and thirteen trials of ON to OFF (3M OFF) were executed. (B) The levels of positively and negatively charged particles in the representative six rooms including the homes of HV where the NCPDIAC apparatus was set were measured using as ion counter (EM-1000, Eco Holistic Inc., Suita, Japan) during the OFF and ON periods. During the OFF period, there was no difference between positively and negatively charged particles; however, during the ON period, the level of positively charged particles was significantly reduced, thereby establishing a difference between the number of positively and negatively charged particles. Statistical analyses were performed using the student T test. (C) Changes in actual NK cell activity prior to and following the 3M ON and 3M OFF periods. Values tended to increase during the 3M ON and decrease during the 3M OFF periods. (D) Relative NK cell activities with NK cell activity previously set to 1.0. There was a significant increase in relative NK cell activity during the 3M ON period and a significant decrease during the 3M OFF period. Statistical analyses were performed using the student T test.

All volunteers had built or renovated their residential homes prior to being recruited to this project and agreed to set up an NCPDIAC device for the experiments [15].

This study was approved by the institutional ethical committee (#854) and samples were only taken from HV who provided written informed consent.

The clinical parameters measured were similar to those determined for the 2W nightly stay experiments with additional cytokines being measured. Twenty-nine cytokines were measured using the Luminex 26 Cytokine Plex Kit Human Cytokine/Chemokine Panel (MPXHCYTO60KPMX26, Merck Millipore, Billerica, MA) [15]. Additionally, adipokines and cytokines related to oxidative stress in serum from 16 ON and 13 OFF trials were measured using the Human Adipokine Magnetic 14-Plex Panel with Luminex instruments (Bio-rad, Hercules, CA, USA). Fourteen of the cytokines examined comprised IL-1β, IL-10, IL-6, monocyte chemotactic protein (MCP)-1, leptin, SAA (serum amyloid A), hepatocyte growth factor (HGF), insulin, lipocalin-2, TNF-α, B cell activating factor (BAFF: belonging to the tumor necrosis factor family), resistin, plasminogen activator inhibitor (PAI)-1, and IL-8 [16].

After analyzing all of the results, it was determined that all items measured except for NK activity revealed no significant difference between 3M ON and 3M OFF conditions. NK activity was measured as the E/T ratio and was calculated to be 10:1 and 20:1. With an actual NK activity of 20:1, there was a tendency of NK activity to increase in the 3M ON period and decrease in the 3M OFF period [15]. Additionally, the relative changes in NK activity set before as 1.0 for individual values of 3M ON and 3M OFF periods revealed a significant increase during the 3M ON periods and a decrease during the 3M OFF periods in the 20:1 E/T ratio as shown in **Figure 5C** [with an E/T ratio of 10:1, similar significant results were obtained in the 3M ON ($p = 0.017$) and 3M OFF ($p = 0.012$) periods] [15].

Taken together, it was shown that NCPDIAC cause enhancement of NK cell activity even in living homes. The apparatus utilized for establishing NCPDIAC may possess advantages in reducing the occurrence of cancers, as well as reducing signs or symptoms associated with virus-infected diseases such as influenza [15].

For adipokines and cytokines related to oxidative stress, there were no significant changes observed [16]. However, with the exception of one case, serum amyloid A (SAA) levels decreased significantly during the ON trials (data not shown) [16]. Considering that SAA is an acute phase-reactive protein like C-reactive protein (CRP), this observed decrease may indicate a prevention of cardiovascular and atherosclerotic changes, since an increase in high-sensitive CRP is associated with subsequent detection of these events [16].

7. Discussion

Initial assessment of the biological effects of NCPDIAC began with evaluations of 2.5H stay experiments since similar experiments had yet to be reported and investigations should involve collaboration with HV. Thus, our initial 2.5H stay experiments demonstrated a small but significant increase in IL-2. Additionally, it was assumed that there were no adverse effects during the 2.5H period. These experiments were then followed with 2W nightly stay experiments. However, to set up NCPDIAC in the homes of HV was very difficult since sleeping and/or living rooms required alterations. Thus, we used a dormitory that belonged to the collaborating house company where many trainees (company employees) would be staying during the 3M period. With these experiments, we found an enhancement of NK cell activity during the 2W nightly stay. Additionally, there were no adverse effects as determined by the various parameters examined.

During these studies employing HV, in vitro assays were performed. From the in vitro experiments, we confirmed the increased NK cell activity and slight immune-stimulatory effects of negatively charged particles. Again, there were no adverse effects to the human body as determined by the various parameters examined.

These results encouraged us to apply NCPDIAC in the actual homes (sleeping and living rooms) of volunteers. Following approval by the institutional ethical committee and obtaining written informed consent, the homes of 7 HV were set up with NCPDIAC. Results showed that the 3M ON period enhanced and the 3M OFF period reduced NK cell activity.

It was important to proceed with these experiments in a methodical, step-by-step fashion as careful consideration and confirmation of the results were required to preserve the health and well-being of the volunteers.

8. Conclusions

In this chapter, the establishment of NCPDIAC and results of the experiments for short-term (2.5H, 2.5-h), mid-term (2W, 2-week), and relatively long-term (3M, 3-month in actual living homes) stays were shown.

Long-term monitoring in actual living homes comprising 6-month, 12-month, and 3-year duration periods has commenced, and the results will be reported in due course.

It is extremely important that appropriate living environments are investigated and created that mitigate or prevent the onset of many diseases such as cancers and virus-infected diseases. In addition to NCPDIAC, other devices that maintain stable temperature as well as increase air tightness may prevent acute accidents related to cardiovascular events. Our recent long-term trial monitoring experiments have included these devices. We hope that these health-promoting indoor environments enable people to live healthier and happier lives.

Notes and Acknowledgements

All experiments described in this chapter were approved by the Ethics Committee of the Kawasaki Medical School, Kurashiki, Japan.

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Conflicts of interest

For the short-term (2.5H) and mid-term (2W) stay experiments and the in vitro experiments, the Department of Hygiene, Kawasaki Medical School, obtained research funding from SEKISUI HOUSE Ltd., Osaka, Japan. For the long-term (3M) stay experiments, the Department of Hygiene obtained research funding from Yamada SXL Hone Co. Ltd., Takasaki, Japan. Additionally, the extraporous charcoal paints were provided by Artech Kohboh, Co. Ltd., Omura, Nagasaki, Japan.

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