The biological effects of negatively-charged indoor air conditions

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ABSTRACT Indoor air conditions may have an impact on human health. Among the various factors affecting indoor air conditions, very few published reports deal with negatively-charged particles. In this review, we detail the biological effects of negatively-charged indoor air conditions, particularly on the psycho-neuro-endocrino-immune network. Short-term (2.5 hours) and medium-term (2 weeks) abidance in a room dominated by negatively-charged air particles resulted in activation of natural killer cell activity induced by recurrent transient activation of interleukin 2. These results indicate that negatively-charged indoor air conditions are better for immune status and may improve our daily lives.

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Key words : **indoor air, negatively-charged particle, PNEI-network, IL-2, NK activity**

INTRODUCTION

Indoor air environments are a very important consideration for human health, especially in well-developed nations, since many physical and mental stresses may be induced or modified by indoor conditions[1－3)]. In particular, sick-building syndrome (SBS)[4－6)] and multiple chemical sensitivity syndrome (MCS)[7－9)] are typical health disorders associated with indoor environments. These diseases are mainly caused by indoor air aldehydes and volatile organic compounds (VOCs) that affect human psycho-neuro-endocrino-immune (PNEI) networks[10－12)]. The concept of a PNEI network has evolved over the past few decades and is based on the close relationship between immunoregulation and brain functions[13,14)]. Particular emphasis is given to circuits involving immune cell products, the hypothalamus-pituitary-adrenal axis, and the

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sympathetic nervous system. It is thought that the various complaints and symptoms associated with SBS and MCS result from an impairment of the PNEI network and that psychological stabilization may improve symptoms in patients with SBS and MCS[10－14], although avoidance of chemicals is the most important preventive method for these disorders.

Various trials concerning the domiciliary environment have been developed to support and promote human health in indoor conditions, such as those involving the use of fewer chemicals in a building[15]. On the other hand, modification of the air electrical charge may be considerable. A few studies have demonstrated that indoor air represents a negatively charged electric condition and that this condition may be beneficial for human health[16,17]. Negatively-charged indoor air conditions are recognized in the commercial sector and many companies that are manufacturing and selling negative-ion producers and air purifiers are emphasizing that negatively-charged indoor air conditions are better for human health[18,19]. However, there is no conclusive evidence concerning the biological effects of negatively-charged indoor air conditions.

Thus, in this review, we detail the biological effects of negatively-charged indoor air conditions[20,21].

PRINCIPLE OF MAKING NEGATIVELY-CHARGED INDOOR AIR CONDITIONS

As shown schematically in the center panel of Figure 1, the generation of negatively-charged indoor air conditions involved painting a charcoal coating made by fine charcoal powder onto the walls and ceilings of a room[20,21]. A charcoal coating designated as Health Coat® was produced by Artech Kohboh Co. Ltd. In addition, forced negatively-charged air conditions were created by applying an electric voltage (72 volts) between the backside of the walls of a room and the ground, and using a circuit for generating negatively-charged air conditions. This condition was made by the selective sorption of floating positively-charged air particles on the surfaces of walls and ceilings since these surfaces were negatively charged. Thus, these conditions were recognized as involving a relative reduction of positively-charged air particles, rather than a production of negatively-charged indoor air particles[20,21].

As described below, two sets of experiments were performed to analyze the biological effects of this negatively-charged indoor air condition. These studies were approved by the Ethics Committee of Kawasaki Medical School.

The first set was designed to investigate the short-term effects[20]. Sixty healthy volunteers (HVs) were admitted to a control room (CR; there was no difference between negatively- and positively-charged air particles) or experimental room (ER; negatively-charged air being the dominant condition) for 2.5 hours and changes of various biological markers that related to the PNEI network were analyzed between CR and ER. The CR and ER were built in a wide underground laboratory of the Comprehensive Housing R&D Institute, SEKISUI HOUSE, Ltd., Kizugawa-City, Kyoto Prefecture, Japan, as shown in the top panel of Figure 1. Details of the constructed rooms were reported previously[20]. All HVs were Japanese and middle-aged individuals. The experiments were performed during 18－30 November, 2005 at the Comprehensive Housing R&D Institute, SEKISUI HOUSE, Ltd., Kizugawa City, Kyoto Prefecture, Japan.

The second set of experiments examined the relative medium-term effects of negatively-charged indoor air conditions[21]. The CR and ER were built in a dormitory as shown in the bottom panel of Figure 1. All subjects were company members of Sekisui House, Ltd. Ten subjects came to this

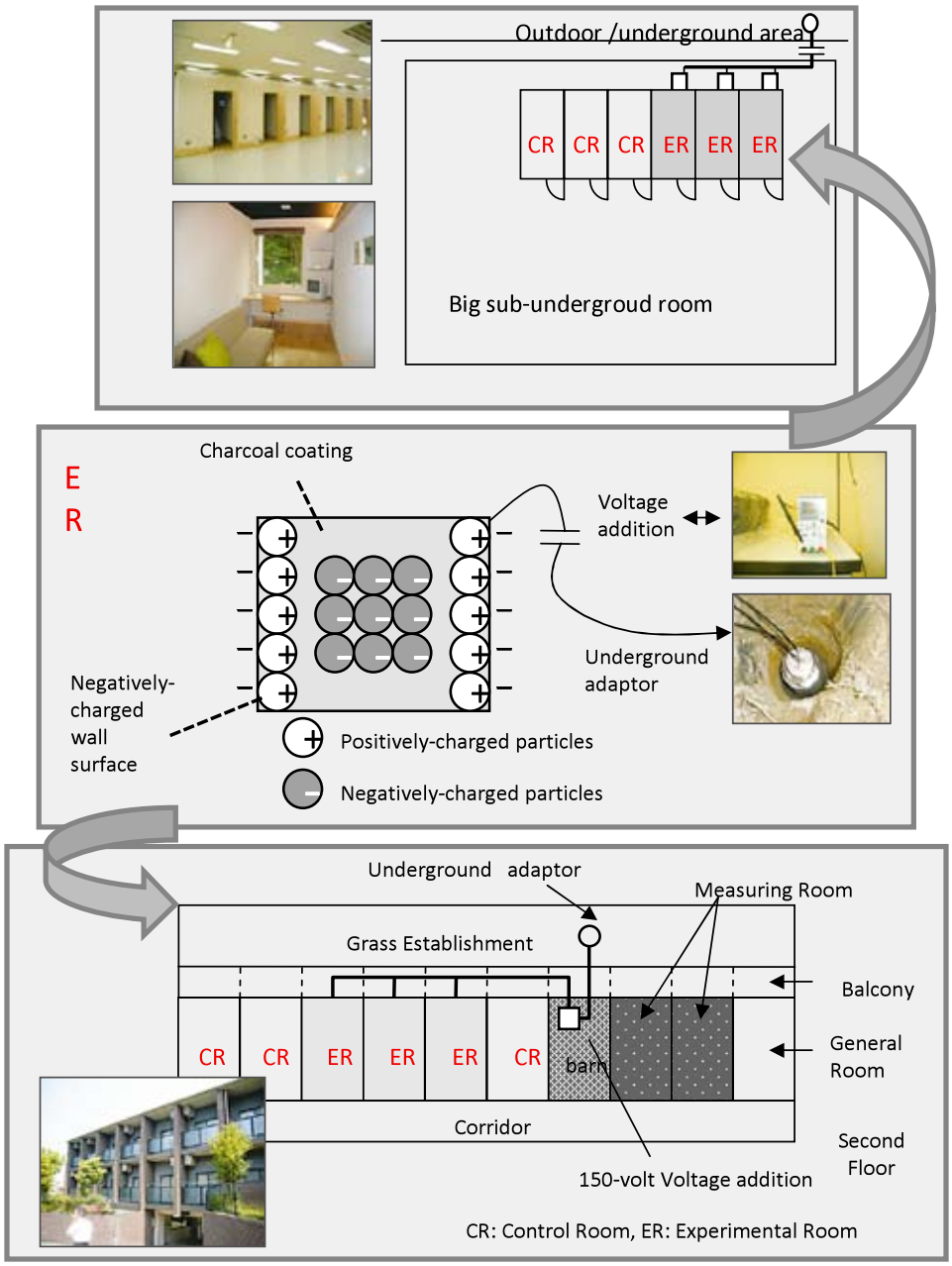

Fig. 1. [Top panel] Schematic representation and external/internal view of experimental (ER) (and also control (CR)) rooms used in the first, short-term exposure experiments. The ER and CR were constructed in a huge underground laboratory room as shown. The outer and inner appearances of both rooms were similar. However, the ER was fitted with devices comprising an underground adaptor and additional voltage to create negatively-charged indoor air conditions.
[Center panel] Schematic representation of the creation of negatively-charged indoor air conditions. Rooms were painted with a charcoal coating on the walls and ceiling, and an underground adaptor and additional voltage were then used to create a negative electrical charge on the surface of the walls. Since the positively-charged air particles were then adsorbed to the surface of the walls, the relative indoor air conditions were dominated by negatively-charged particles.
[Bottom panel] Schematic representation and external view of the dormitory used for the second, medium-term exposure experiments. Devices to create negatively-charged indoor air conditions were the same as those used for the first experiments and are shown in Figures 1 and 2.

dormitory for their company's training for three months from business offices situated in seven different prefectures in Japan, and another five were members of the Comprehensive Housing R&D Institute, Sekisui House, Ltd., situated 1 km from the dormitory. All subjects were male, and the mean age and standard deviation were 27.7 ± 2.0 years. The experiments were performed from October 6, 2007 to March 13, 2007[23]. Subjects received training or engaged in daily business practices during the daytime, and then returned to the dormitory for dinner, leisure time, bathing, and sleeping. Subjects joined the experiments only after their informed consent was obtained. There were no advantages or disadvantages associated with whether company members agreed or declined to join the experiments. For these second experiments, subjects were initially admitted to the pre-living room, which was the same as the CR, for two weeks. They then moved to the CR, and although air conditions were the same, subjects were not notified that they were admitted to the CR or ER. After a stay of two weeks, they were again moved to the next room. This final room was the ER, and subjects were not told that they were in the experimental room. The reason why all the subjects were sequentially admitted to the CR and ER is that there was a limited number of HVs. Thus, a comparative study was not carried out. We checked the biological responses in each subject before and after admission to the ER. The CR was the first step in the experimental protocol because it was better to avoid any long-term (more than two weeks) effects of the ER[21].

INDOOR AIR CONDITIONS IN THE CR AND ER

Indoor air conditions such as temperature, humidity, air pollutants and electrical charge were measured for all experimental rooms in the two sets of experiments[20,21]. Temperature and humidity were continuously monitored during the entire experimental periods. For the entire duration of the two sets of experiments, there was no difference between CR and ER regarding temperature and humidity[20,21].

Air pollutants designated by the guideline concerning indoor room concentrations for indoor air pollutants, released by the Japanese Ministry of Health, Labor and Welfare and covering levels of formaldehyde, acetaldehyde, toluene, xylene, styrene, ethyl benzene, paradichlorobenzene and total VOC, were measured using an active sampling method. All concentrations of air pollutants showed levels below the legal range and there were no differences between CR and ER for both experiments[20,21].

Electrical charge in these rooms was measured using an Ion Counter (EB-1000TM, Eco Holistic Inc., Suita, Japan). In these studies, particles sized less than 2 nm were monitored. Electrical charge in two representative rooms (Room C for the ER and Room D for the CR) was monitored every morning, and all rooms were monitored twice a week during the entire experimental period in the first experiment[20]. In the second set of experiments, the electrical charges in the two groups of rooms (all six rooms) were monitored once a week for the entire experimental period[21]. The results showed that there was an approximate 500-particle (/1,000 mm^3) higher concentration of negatively charged than positively charged air particles during the entire experimental periods of both experiments. There were no differences in negatively charged air particles between CR and ER, but the numbers of positively charged air particles in the ER were reduced in both experiments[20,21]. This difference resulted in negatively-charged indoor air conditions.

BIOLOGICAL MONITORING

The following parameters were monitored before and after admission to the ER or CR[20,21].

1) General conditions: Blood chemistry including liver and kidney functions, blood sugar and lactic acid, and peripheral blood count were measured using peripheral blood samples. In addition, blood pressure and pulse rate were measured at pre- and post-admission to the CR and ER.

2) Stress markers: Blood cortisol and salivary cortisol, chromogranin A, amylase, and secretory immunoglobulin (Ig) A were measured as stress markers. Saliva was collected by SalivetteTM (Sarstedt Ag & Co., Nümbrecht, Germany) according to the manufacturer's instructions. Briefly, each subject kept the cotton part of the Salivette in his/her mouth for 3 min. The cotton was then frozen at -20℃ until centrifugal collection of the saliva. The values of each item were corrected by the total protein of the individual sample.

3) Parameters related to the autonomic nervous system: The autonomic nervous system was examined through a Flicker test, stabilometer, and heart rate monitoring for 3 min. The Flicker test was performed using a Handy Flicker HFTM (Neitz Instruments Co. Ltd., Tokyo, Japan), and flicking frequencies of red, green and yellow colors were monitored. A Gravicoder GS-7TM (Anima Inc., Tokyo, Japan) was used as a stabilometer and the Romberg ratio was used as a measure of body sway. The ratio was calculated using the whole trajectory of body sway during 30 sec while standing with eyes closed divided by that recorded with eyes open. Heart rate was monitored using a Heart Rate Monitor S810iTM (Polar Electro, Kempele, Finland) for 3 min. During monitoring, subjects were lying on a bed and remained at rest. The frequency-domain parameters of heart rate variability such as low-frequency power [LF], high-frequency power [HF], and low-/high-frequency ratio [LF/HF] were analyzed using MemCalc/Tarawa software (G.M.S. Co. Ltd., Tokyo, Japan).

4) Immunological parameters: Serum levels of Ig E and Ig A, 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. The 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. Samples that showed less than the lower limitation of analytical methods for cytokines and Igs, 1/10 the value of minimum values among whole measurable samples, were substituted instead of using "0" or the designation "unmeasurable".

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[22,23]. Briefly, a peripheral heparinized blood sample (100 μ l) was put into the instrument and flowed through the micro-channel chips, which are a model for capillary vessels, and the flowing time was recorded. The flowing blood sample could also be visualized using the CCD camera attached to the microscope's objective.

In addition to the above-mentioned parameters measured after 2.5 hours of abidance, natural killer (NK) cell killing activity, urine 8-hydroxydeoxyguanosine (8-OHdG) concentration, urine 17-hydroxycorticosteroids (17OHCS) and specific Ig E classes for 26 kinds of typical allergens (MAST26 test, SRL Inc., Tachikawa, Tokyo, Japan) were also added to the 2-week study because it was thought that these newer parameters might be altered following a week-based experimental period, in contrast to one that is hour-based[21]. The 26 allergens in MAST26 were as followings: *Dermatophagoides farinae*, house dust, epithelium of cats and dogs, *Phleum pretense*, *Anthoxanthum odorathum*, *Ambrosia artemisiifolia*, mugwort, *Cryptomeria japonica*, *Penicillium viridicatum*,

Cladosporium, *Candida*, *Alternaria*, *Aspergillus*, wheat, soybean, rice, *Thunnus*, salmon, shrimp, crab, cheddar cheese, milk, beef, chicken meat, and egg albumin.

MODIFICATION OF BIOLOGICAL PARAMETERS

In the first set of experiments involving short-term abidance, pre-abidance and post-abidance values of the various parameters were compared between CR and ER using the Mann-Whitney *U* test of StatFlex version 5.0 software for Windows (Artech Co. Ltd., Osaka, Japan), which yields results that are compatible with SPSS software. Changes of IL-2, IL-4, blood viscosity and the RR-interval of heart rate showed statistical significance. Among these parameters, the change of IL-2 levels was the most significant. Subjects that entered ER showed increased IL-2 levels compared with those that entered CR, as shown in panel A of Figure 2 and reported previously[22].

In addition, a multiple/stepwise regression test was used to derive the following formula to evaluate subjects that entered the ER:

Biological response values = 0.498+ 0.0005[salivary cortisol] + 0.072 [IL-2] + 0.003 [standard deviation of heart rate (fluctuation of heart rates)] − 0.013 [blood viscosity] − 0.0009 [blood sugar] + 0.017 [pulse rate]

Using the above formula, the values of variables in individual subjects that entered the CR and ER were re-calculated. Thereafter, as shown in panel B of Figure 2, values from subjects that entered the ER were significantly higher than those of CR subjects. In the above formula, as the constant numbers for IL-2 and blood viscosity were higher than other parameters, these two parameters were considered to be related significantly to ER conditions. In other words, these two parameters were particularly affected by negatively-charged indoor air conditions[20].

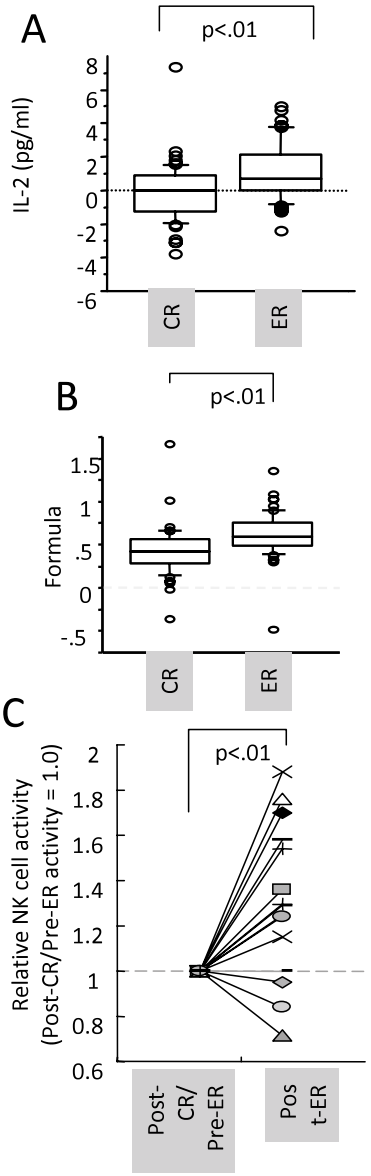

Fig. 2. [A] Statistically significant differences for changes (pre-abidance to a CR or ER room compared with post-abidance) in the serum level of IL-2. Changes in the level of IL-2 in subjects that entered the ER were higher than those of the CR. [B] The formula constructed from the multiple/stepwise regression test using data obtained from the first set of experiments showed that the subjects who entered ER and CR were clearly divided. On the basis of variables used in this formula, serum levels of IL-2 and blood viscosity were considered the most important parameters that were affected by negatively-charged indoor air conditions. [C] The relative NK activities of individual subjects are shown such that the post-CR/pre-ER period is given the value of 1.0. The Wilcoxon Signed Ranks test indicated there is a significant increase of NK activity following ER abidance.

In the second set of experiments, statistical significance was analyzed after taking into consideration the limited number of subjects. This statistical analysis involved the use of the Wilcoxon Signed Ranks test to compare values of pre- and post-admission to the ER for individual parameters[21].

The results revealed that only NK activity showed statistically significant differences, as shown in panel C of Figure 2. NK activity varied from subject to subject and changed during the pre-CR time, post-CR/pre-ER time, and post-ER time. However, when we considered the post-CR/pre-ER time as 1.0 in each subject and relative changes of post-ER were calculated, there was a significant increase of NK activity, although four subjects showed decreased activities. NK activity is very important for tumor immunity and in combating other pathogens and viruses. Negatively-charged air conditions may be helpful in preventing cancers and infections when people are continuously living in a house with negatively-charged air conditions[21].

DISCUSSION AND FUTURE ANALYSES

The investigations outlined in these studies produced important results. First of all, it should be noted that the experimental rooms utilized in these studies were used to estimate only the effects of negatively-charged air conditions on parameters indicative of human health. Temperature, humidity and air pollutants were controlled, and there was no difference between the CR and ER groups other than air charge. In addition, these experimental rooms contained no air pollutants. Thus, it is important to appreciate that investigations using these rooms do present the biological effects of negatively-charged indoor air conditions. Negatively-charged indoor air conditions are recognized in the commercial sector, and many companies that manufacture and sell negative-ion producers and air purifiers emphasize that negatively-charged indoor air conditions are better for human health[18,19]. However, there is no scientifically conclusive evidence concerning the biological effects of negatively-charged indoor air conditions. As a first step in obtaining data concerning the biological effects of negatively-charged indoor air conditions, the NCEI network was analyzed in the first set of experiments by placing subjects in negatively-charged indoor air conditions for a short-time and monitoring biological markers of human health[22]. The investigation could then move to the relatively medium-term experiments used as the second set of experiments of this study. Devices to make experimental negatively-charged indoor air conditions were applied to the dormitory and subjects spent the night-time in these conditions for two weeks[23].

The results showed that the serum IL-2 level was the most sensitive and variable parameter that reflected the effects of negatively-charged indoor air conditions for short-term abidance. The slight increase of IL-2 can be translated as a slight activation of general immune status. However, this change will return to the pre-abidance level because changes of cytokine at this level are not expected to persist for periods that last from several hours to days[24–26]. However, a very important finding was detected by the second set of experiments. In these experiments, subjects that had stayed two weeks in negatively-charged indoor air conditions during the night-time showed significant enhancement of NK cell activity. It is well known that NK cells can be activated by IL-2. At this point, the two sets of experiments were connected. As shown in Figure 3, short-term exposure to negatively-charged indoor air conditions led to IL-2 activation and elevation during the short exposure period. However, IL-2 is returned to normal or pre-exposure levels if subjects are exposed repeatedly as with individuals of experiment 2 (during the night-time), and NK cells are additionally activated by repeated exposure

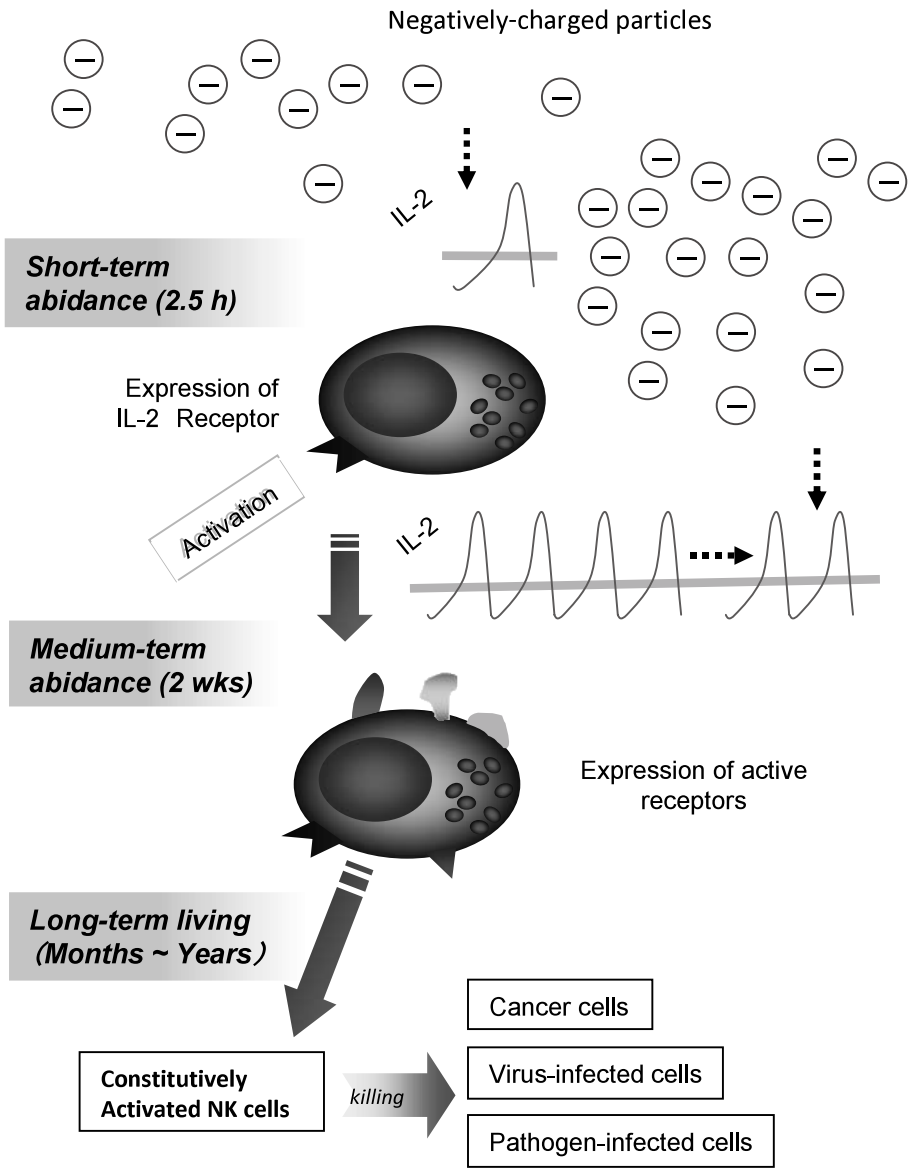

Fig. 3. Schematic representation of the speculative effects of negatively-charged air conditions on the human immune system, including the short-term activation of IL-2 and repeated and chronic stimulation of IL-2 to NK cells. NK cells are stimulated recurrently and maintain a higher activity that prevents cancerous and infectious diseases.

to slightly elevated IL-2 levels before ceasing their activation[27–29]. NK cells then show a constitutive activated status after (at least) a period of one week. This hypothesis is supported by the combined results of the two sets of experiments presented in this study.

What directions should be taken by future investigations? One direction would be to examine the biological effects of negatively-charged indoor air conditions for longer time periods such as months or years. Thus, this direction may involve monitoring people actually living in their own house with a fitted device to make negatively-charged indoor air conditions. This can be achieved because

there were no significant worse biological effects from the two sets of experiments performed over a period of hours or days.

Another direction for future studies would be to investigate cellular and molecular settings. Some effects on immunocompetent cells by exposure to negatively-charged air conditions were detected by the two sets of experiments, namely, enhancement of IL-2, which is produced by various immunocompetent cells such as T cells and antigen presenting cells, and NK cell activation. Thus, *in vitro* experiments could be performed using freshly isolated immunocompetent cells from healthy or pathological subjects, including patients with cancers or allergies, or immunological cell lines can be utilized, once an *in vitro* exposure device is developed to suit these experiments. Actually, the preliminary results show activation of NK cell function and T cell activation within the non-pathological manner (data not shown).

These forthcoming experiments may result in a better understanding of negatively-charged indoor air conditions and might help spread the use of this device in practical situations involving social environments.

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