Immunobiology 220 (2015) 1359–1368

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Exposure to negatively charged-particle dominant air-conditions on human lymphocytes *in vitro* activates immunological responses

Yasumitsu Nishimura[a], Kazuaki Takahashi[b], Akinori Mase[b], Muneo Kotani[b,c], Kazuhisa Ami[b,d], Megumi Maeda[e], Takashi Shirahama[f], Suni Lee[a], Hidenori Matsuzaki[a], Naoko Kumagai-Takei[a], Kei Yoshitome[a], Takemi Otsuki[a,*]

[a] *Department of Hygiene, Kawasaki Medical School, 577 Mastushima, Kurashiki, Okayama 701-0192 Japan*
[b] *Comprehensive Housing R&D Institute, SEKISUI HOUSE, Ltd., 6-6-4 Kabutodai, Kizugawa, Kyoto 619-0224 Japan*
[c] *Sumitomo Riko Co. Ltd., 1Higashi 3-chome, Komaki, Aichi 485-8550 Japan*
[d] *Yamada SXL Home. Ltd., 5F OAP Tower, 1-8-30 Tenma-bashi, Kita-Ku, Osaka, 530-0043, Japan*
[e] *Department of Biofunctional Chemistry, Division of Bioscience, Okayama University Graduate School of Natural Science and Technology, 1-1-1 Tsushima-Naka, Okayama 700-0082, Japan*
[f] *Artech Kohbou, Co. Ltd., 57-29, Hattandago, Higashisonogi-town, Higashisonogi, Nagasaki 859-3922, Japan*

ARTICLE INFO

Article history:
Received 9 February 2015
Received in revised form 14 March 2015
Accepted 7 July 2015
Available online 17 July 2015

Keywords:
Indoor air
T cell
NK cell
Regulatory T cell
Negatively-charged particle
Immune Stimulation

ABSTRACT

Indoor air-conditions may play an important role in human health. Investigation of house conditions that promote health revealed that negatively charged-particle dominant indoor air-conditions (NAC) induced immune stimulation. NAC was established using fine charcoal powder on walls and ceilings and utilizing forced negatively charged particles (approximate diameter: 20 nm) dominant in indoor air-conditions created by applying an electric voltage (72 V) between the backside of the walls and the ground. We reported previously that these conditions induced a slight and significant increase of interleukin-2 during 2.5 h stay, and an increase of natural killer (NK) cell cytotoxicity, when examining human subjects after a two-week night stay under these conditions. In the present study, we investigated whether exposure to NAC *in vitro* affects immune conditions. Although the concentrations of particles were different, an incubator for cell culture with NAC was set and cellular compositions and functions of various freshly isolated human lymphocytes derived from healthy donors were assayed in the NAC incubator and compared with those of cultures in a standard (STD) incubator. Results showed that NAC cultivation caused an increase of CD25 and PD-1 expressing cells in the CD4 positive fraction, enhancement of NK cell cytotoxicity, production of interferon-γ (IFNγ), and slight enhancement of regulatory T cell function. In addition, the formula designated as the "immune-index" clearly differed between STD and NAC culture conditions. Thus, NAC conditions may promote human health through slight activation of the immune system against cancer cells and virus infection as shown by this *in vitro* study and our previously reported human studies.

1. Introduction

Indoor air-conditions affect human health (Hodgson, 2002; Bardana, 2003; Mitchell et al., 2007). Typically, sick-building syndrome (SBS) is associated with various symptoms such as sensory irritation of the eyes, nose and throat, as well as neurotoxic or general health problems such as skin irritation and nonspecific hypersensitivity reactions (Hodgson, 1989; Crawford and Bolas, 1996; Menzies and Bourbeau, 1997). These symptoms are thought to appear due to dysregulation of psycho-neuro-endocrino-immune networks. In addition, indoor air aldehydes and volatile organic compounds are thought to play a causative role in SBS (Hodgson, 1989; Crawford and Bolas, 1996; Menzies and Bourbeau, 1997). Furthermore, various microorganisms such as fungus and bacteria, as well as mites, and proteins and molecules that are found in the aforementioned microorganisms, affect human health, especially in regard to allergic and hypersensitive reactions (Burrell, 1991; Husman, 1996).

In order to improve indoor air-conditions and obtain healthier conditions for home inhabitants, various trials have been

* Corresponding author. Fax: +81 86 464 1125.
E-mail address: takemi@med.kawasaki-m.ac.jp (T. Otsuki).

http://dx.doi.org/10.1016/j.imbio.2015.07.006
0171-2985/

conducted to create greater airtightness and better moisture-proof properties for individual homes (Wilkinson et al., 2007; Kim and Bernstein, 2009).

We have been creating negatively charged-particle dominant indoor air-conditions for residential rooms with special devices, and then examined the biological effects of these devices. The negatively charged-particle dominant indoor air-conditions were generated using a charcoal coating made by fine charcoal powder with which the walls and ceilings were painted. The charcoal coating designated as Health Coat^{TM} was produced by Artech Kouboh Co. Ltd. In addition, forced negatively charged-particle dominant air-conditions were created by applying an electric voltage (72 V) between the backsides of the walls of a room and the ground, and using a circuit for generating negatively charged-particle dominant indoor air-conditions (Takahashi et al., 2008, 2009; Otsuki et al., 2009). The particles measured approximately 20 nm in diameter. As we reported previously (Takahashi et al., 2008, 2009; Otsuki et al., 2009), the positively charged particles were adsorbed continuously onto the walls and ceilings following application of a small voltage. The negatively charged particles then became dominant in the rooms in which these devices were applied (Takahashi et al., 2008, 2009; Otsuki et al., 2009).

Investigation of the biological effects of these indoor air-conditions initially comprised experiments involving a short-term (2.5 h) stay in an experimental room (in which these devices were applied) and comparison with a 2.5-h stay in a control room, which was similar in appearance to the experimental room but did not involve use of the devices. This initial trial with 120 healthy volunteers revealed that various biological parameters including blood chemistry, peripheral blood counts, cytokines related to T-helper 1 and 2, stress-related enzymes and hormones, autonomic nerve functions as indicated by the Flicker test, stabilometer and heart rate monitoring, as well as blood viscosity, interleukin (IL)-2, IL-4, mean RR interval of the heart rate, and blood viscosity differed significantly between the two rooms. In particular, the slight and significant, although not pathophysiological, increase of IL-2 after a 2.5-h stay in the experimental room was the most significant. In addition, the formula designated by {Biological Response Value = 0.498 + 0.072 [IL-2] + 0.017 [pulse rate] – 0.013 [blood viscosity] – 0.009[blood sugar] + 0.003 [standard deviation of heart rate] + 0.0005 [salivary cortisol]} clearly reflected the rooms in which the volunteers stayed. Thus, we supposed that negatively charged-particle dominant indoor air-conditions may affect human immune and autonomic systems by causing slight activation of the immune system and stabilization of autonomic nerve conditions (Takahashi et al., 2008, 2009; Otsuki et al., 2009).

Night-stay experiments over a period of two weeks were then performed as reported previously (Takahashi et al., 2008, 2009; Otsuki et al., 2009). Fifteen volunteers stayed in rooms with negatively charged-particle dominant air-conditions for two weeks during the night and various biological parameters of the subjects were analyzed. Although individual biological reactions differed from subject to subject, natural killer (NK) cell activity increased significantly following a stay in rooms with these devices at night for two weeks. This result complements our previous investigation which revealed a slight increase of IL-2 during short-stay experiments (IL-2 seemed to return to the basic level immediately after leaving the experimental room), and indicates that negatively charged-particle dominant air-conditions cause chronic and recurrent stimulation of NK cells and result in the steady activation of NK cells. These indoor air-conditions may therefore, contribute to the improvement of human health under these living conditions. We are presently conducting a long-term monitoring experiment to determine whether occupation of living or sleeping rooms with the experimental devices induces an increase of NK cell activity.

On the other hand, *in vitro* experiments to determine the effects of negatively charged-particle dominant air-conditions on lymphocyte characteristics have not been performed. In this report, we present results which indicate that these air-conditions stimulate the immune response during short-stay and two-week stay experiments.

2. Material and methods

2.1. Experimental and control incubator settings

The standard (STD) and experimental (negatively charged-particle dominant; NC) incubators comprised a general cell culture incubator (MCO-5ACUV/5AC, Panasonic Co. Ltd., Osaka, Japan). All cell cultures were performed at 37 °C with 5% CO_2 in a humidified atmosphere. All cells were cultured in RPMI1640 medium supplemented with 10% FBS, streptomycin and penicillin. 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 as shown in Fig. 1. 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/cc.

2.2. Lymphocytes

Human peripheral blood samples from eight healthy volunteers were collected by drowning venous blood from three healthy volunteers using a heparinized syringe. Peripheral blood mononuclear cells (PBMCs) were collected using the Ficoll-Hypaque gradient method, which were then incubated in the experimental (with negatively charged-particle circulation) or control (as standard) incubators without addition of cytokines. These experiments were approved by the ethical committee of Kawasaki Medical School (No. 883). Written informed consent was obtained from all volunteers.

2.3. Composition of PBMCs and alteration of activating marker expressions

PBMCs in the standard or experimental incubators were analyzed for changes in cellular composition using cell surface markers, *i.e.*, CD4 for helper T cells, CD8 for cytotoxic T cells, CD3- CD56 (neural cell adhesion molecule-1: NCAM-1)+ NK cells which possess higher cytokine producing capacity, CD3- CD16+ NK cells which express FcγRIII that binds to the Fc portion of immunoglobulin, and CD19 for B cells. Furthermore, for all fractions mentioned above, the expression of CD25 (IL2 receptor α) was examined after one or two weeks of incubation in the experimental or standard incubator. In addition, PD-1 (programmed cell death 1) molecules on their surface as the activation marker and surface CD44 expression were analyzed for CD4+ and CD8+ fractions using flow cytometry (BD FACSCalibur^{TM} Cell Analyzer, BD Biosciences, San Jose, CA). All monoclonal antibodies for staining markers were purchased from MBL, Co., Ltd, Nagoya, Japan, as summarized in Fig. 2.

The examinations of cell surface markers were performed at one and two weeks culture period. Since it was considered that the effects of experimental air-condition may be weaker than standard stimulation procedure for various human lymphocytes, the whole medium changes were just performed at one week time point. In addition, the results obtained at one week were mainly analyzed, because it was considered that two weeks cultivation with one-time medium change may not suitable for these cultures.