VOLUME 10 ISSUE 1 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Analysis of Immunoglobulin E Antibody Production in the Human Cell Line by Polycyclic Aromatic Hydrocarbon Treatments: Considerations of Culture Conditions and Dimethyl Sulfoxide Cytotoxicity

Minato Ritsuko[1], Nishimoto Sogo[1], Honda Masato[2], Tabuchi Yoshiaki[3], Hirano Tetsushi[3], Hirayama Jun[4], Urata Makoto[5], Hong Chun-Sang[6], Srivastav Ajai K.[7] and Suzuki Nobuo[8]*

[1]Department of Food Science, Faculty of Bioresources and Environmental Sciences, Ishikawa Prefectural University, Nonoichi, Ishikawa 921-8836, Japan
[2]Botanical Garden, Institute of Nature and Environmental Technology, Kanazawa University, Kakuma, Kanazawa, Ishikawa 920-1192, Japan
[3]Life Science Research Center, University of Toyama, Sugitani, Toyama 930-0194, Japan
[4]Department of Clinical Engineering, Faculty of Health Sciences, Komatsu University, Komatsu, Ishikawa 923-0961, Japan
[5]Institute of Noto Satoumi Education and Studies, Ogi, Noto-cho, Ishikawa 927-0553, Japan
[6]Hankuk University of Foreign Studies, Yongin, Gyeonggi-do 17035, Korea
[7]Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur 273 009, India
[8]Noto Marine Laboratory, Institute of Nature and Environmental Technology, Kanazawa University, Ogi, Noto-cho, Ishikawa 927-0553, Japan

**Corresponding Author*

Received: 2nd February, 2024; **Accepted:** 28th March, 2024; **Published online:** 30th March, 2024

https://doi.org/10.33745/ijzi.2024.v10i01.055

Abstract: To determine allergic reactions of polycyclic aromatic hydrocarbons (PAHs) in humans, culture conditions of an *in vitro* bioassay system were investigated to evaluate immunoglobulin E (IgE) antibody production using the human myeloma cell line (U266 cells) by PAH treatments. In the preliminary experiment, the cell count of U266 was observed under a microscope at 12, 24, 48, and 72 h of incubation. As a result, many dead cells were observed in the 72 h culture. Accordingly, changes within 48 h were examined in detail. The cell viability reached a plateau in 24 h culture. The IgE antibody production gradually increased during the 48-h incubation period and was higher at 48 h than at 24 h. Therefore, the incubation time was determined to be 48 h. In cell culture experiments, liposoluble materials were dissolved in dimethyl sulfoxide (DMSO). Next, the DMSO cytotoxicity was examined. The results demonstrated that 0.5% and 1% of DMSO were toxic; however, 0.1% and 0.05% were nontoxic. Furthermore, benzopyrene and pyrene (each 10^{-8} M) were dissolved in 0.5% and 0.1% DMSO, respectively. We investigated the effects of cytotoxicity and IgE production on U266 cells by adding PAH dissolved in 0.5% or 0.1% of DMSO. Synergistic effects of PAHs (benzo[*a*]pyrene and pyrene) and 0.5% of DMSO were detected. Therefore, using DMSO concentrations of <0.1% was recommended to study the effects of liposoluble substances on cultured cells.

Keywords: Polycyclic aromatic hydrocarbons, Benzo[*a*]pyrene, Pyrene, Dimethyl sulfoxide, Immunoglobulin E antibody, Human myeloma cell line, Allergic reaction

Citation: Minato Ritsuko, Nishimoto Sogo, Honda Masato, Tabuchi Yoshiaki, Hirano Tetsushi, Hirayama Jun, Urata Makoto, Hong Chun-Sang, Srivastav Ajai K. and Suzuki Nobuo: Analysis of immunoglobulin e antibody production in the human cell line by polycyclic aromatic hydrocarbon treatments: Considerations of culture conditions and dimethyl sulfoxide cytotoxicity. Intern. J. Zool. Invest. 10(1): 510-519, 2024.

https://doi.org/10.33745/ijzi.2024.v10i01.055

This is an Open Access Article licensed under a Creative Commons License: Attribution 4.0 International (CC-BY). It allows unrestricted use of articles in any medium, reproduction and distribution by providing adequate credit to the author (s) and the source of publication.

Introduction

Ambient air pollution associated with human activities has been known to affect human health, particularly children (Suresh *et al.*, 2009; Jung *et al.*, 2012; Al-Daghri *et al.*, 2013). Environmental air pollution has been considered causative of the allergic response. Some studies have focused on polycyclic aromatic hydrocarbons (PAHs) as causative agents of air pollution. For example, a significant correlation has been found between PAH exposure and asthma in children (Suresh *et al.*, 2009; Jung *et al.*, 2012; Al-Daghri *et al.*, 2013). Serum PAH levels were strongly associated with biomarkers (immunoglobulin E [IgE] antibody and interleukins) of childhood asthma (Al-Daghri *et al.*, 2013).

As IgE antibody is one of the major mediators of hypersensitivity reactions such as asthma, seasonal allergy, and food allergy (Galli and Tsai, 2012), several *in vivo* studies regarding the association between IgE antibody production and PAHs have been conducted (Kanoh *et al.*, 1996; Suresh *et al.*, 2009; Kadkhoda *et al.*, 2005; Jung *et al.*, 2012; Al-Daghri *et al.*, 2013; Jo *et al.*, 2022). However, there exists little information regarding an *in vitro* study to examine IgE antibody production by PAHs. To investigate allergic reactions of PAHs in humans, a suitable *in vitro* bioassay system is considered essential to analyze IgE antibody production.

Thus, the present study developed an *in vitro* bioassay for IgE antibody production of PAHs (benzo[*a*]pyrene [BaP]; pyrene [Pyr]) using the human myeloma cell line U266 cells that are overexpressing IgE antibody (Ohno *et al.*, 2010) and examined culture conditions. In cell culture experiments, liposoluble substances were dissolved in dimethyl sulfoxide (DMSO) (Lukac *et al.*, 2013; Luo *et al.*, 2017; Qin *et al.*, 2023). DMSO is an amphiphilic molecule with a polar domain and two nonpolar methyl groups. Therefore, it is soluble in aqueous and organic media. Next, the DMSO cytotoxicity on U266 cells was investigated under determined culture conditions. Taking together, when using DMSO to dissolve liposoluble substances such as PAHs, the DMSO concentration should be considered.

Materials and Methods

Cells:

The human myeloma cell line (U266 cells) was ordered from the American-Type Culture Collection. It has been reported that U266 cells were overexpressing IgE antibodies (Ohno *et al.*, 2010). Therefore, using the U266 cells, a bioassay system for IgE antibody production by PAHs was developed.

Time course of IgE antibody production by U266 cells:

The U266 cells were precultured in the medium (RPMI 1640 Media, Thermo Fisher Scientific Inc., Grand Island, NY, USA) supplemented with 10% fetal bovine serum at 37°C in a 5% CO_2 and 95% air. After preincubation, the U266 cells were used for the time-course study of IgE antibody production. Figure 1 shows an outline of the present experiment.

U266 cells were inoculated to a polystyrene 96 well plate at 1.0×10^5 cells/ml in the RPMI 1640 medium supplemented with 10 µg/ml of insulin, 20 µg/ml of transferrin, 20 µM of ethanolamine, and 25 nM of sodium selenite (Insulin-Transferrin-Selenium, Science Cell Research Laboratories, Carlsbad, CA, USA). Before investigating IgE antibody production, preliminary cultures were performed at 12, 24, 48, and 72 h. Then, cultured cell conditions were observed under a microscope (CKX41, Olympus Corporation, Tokyo, Japan).

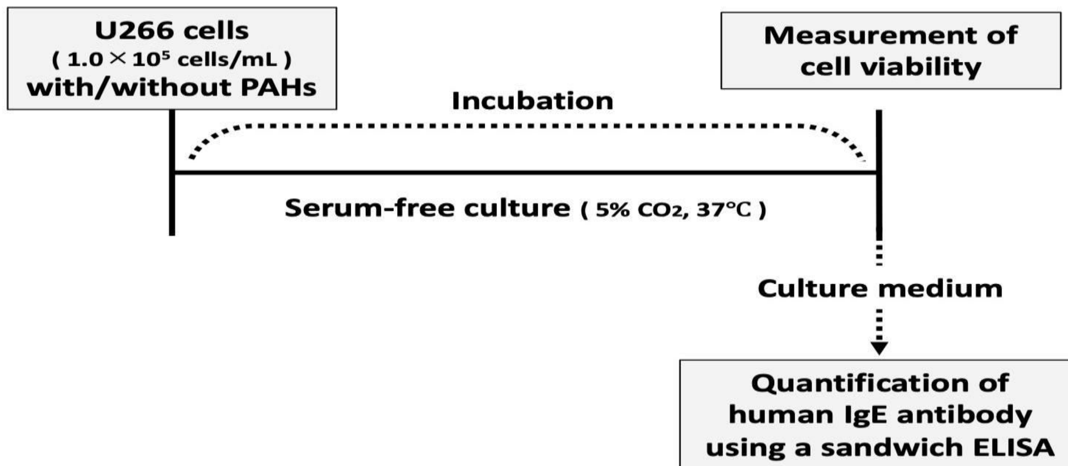

Fig. 1: Outline of time-course study for IgE antibody production in U266 cells.

In the present study, cell viability was measured post-incubation using a WST-8 kit (OZ Biosciences, Marseille, France). Thereafter, the IgE antibody production in the culture medium was analyzed using an enzyme-linked immunosorbent assay (ELISA) as follows:

The goat anti-human IgE antibody (1 μg/ml) (Fortis Life Sciences, Waltham, MA, USA) in a 50 mM carbonate–bicarbonate buffer (pH 9.6) was added to a 96 well microplate (Nunc, Roskilde, Denmark) at 100 μl/well. Thereafter, the microplate was incubated for 1 h at 25°C. After washing five times with 0.05% Tween 20-PBS (T-PBS), each well was blocked with blocking buffer (Thermo Fisher Scientific Inc.) for 30 min at 25°C. After the blocking reaction, each well was washed five times with T-PBS and subsequently treated with 50 μl of the culture medium for 1 h at 25°C. After washing five times with T-PBS, 100 μl of the horseradish peroxidase-conjugated goat anti-human IgE antibody (Fortis Life Sciences) diluted 75,000 times with blocking buffer was added to each well, and the 96-well plate was incubated for 1 h at 25°C. The plate was washed with T-PBS again for five times. Then, TMB Substrate Set (BioLegend, San Diego, CA, USA) was added to each well at 100 μl/well for enzyme reaction, and the absorbance at 450 nm was measured after adding 100 μl/well of 0.2 M sulfuric acid to terminate the enzyme reaction. The IgE antibody concentration was quantitatively determined by a standard curve, using a serial dilution of human IgE antibody standard solution.

Cytotoxicity of DMSO on U266 cells:

The U266 cells were prepared using the methods described above. U266 cells were inoculated to a 96-well plate at 1.0 × 10^5 cells/ml in the RPMI 1640 medium supplemented by an insulin-transferrin-selenium supplement (Science Cell Research Laboratories) together with 0, 0.05, 0.1, 0.5, and 1% DMSO solution. Post-incubation, cell viability was measured using a WST-8 kit (OZ Biosciences). Then, IgE antibody production in the culture medium was analyzed by ELISA.

Influence of BaP and Pyr on IgE antibody production in U266 cells:

The U266 cells were inoculated to a 96-well plate at 1.0 × 10^5 cells/ml in the RPMI 1640 medium added with an insulin-transferrin-selenium supplement (Science Cell Research Laboratories) and BaP or Pyr (each 10^{-8} M) solubilized in DMSO. Post-incubation, cell viability was measured by a WST-8 kit (OZ Biosciences). Thereafter, IgE antibody production in the culture medium was analyzed by ELISA.

Statistical analysis:

All results are expressed as the means ± standard error (n = 4 or 5). The statistical s ignificance was

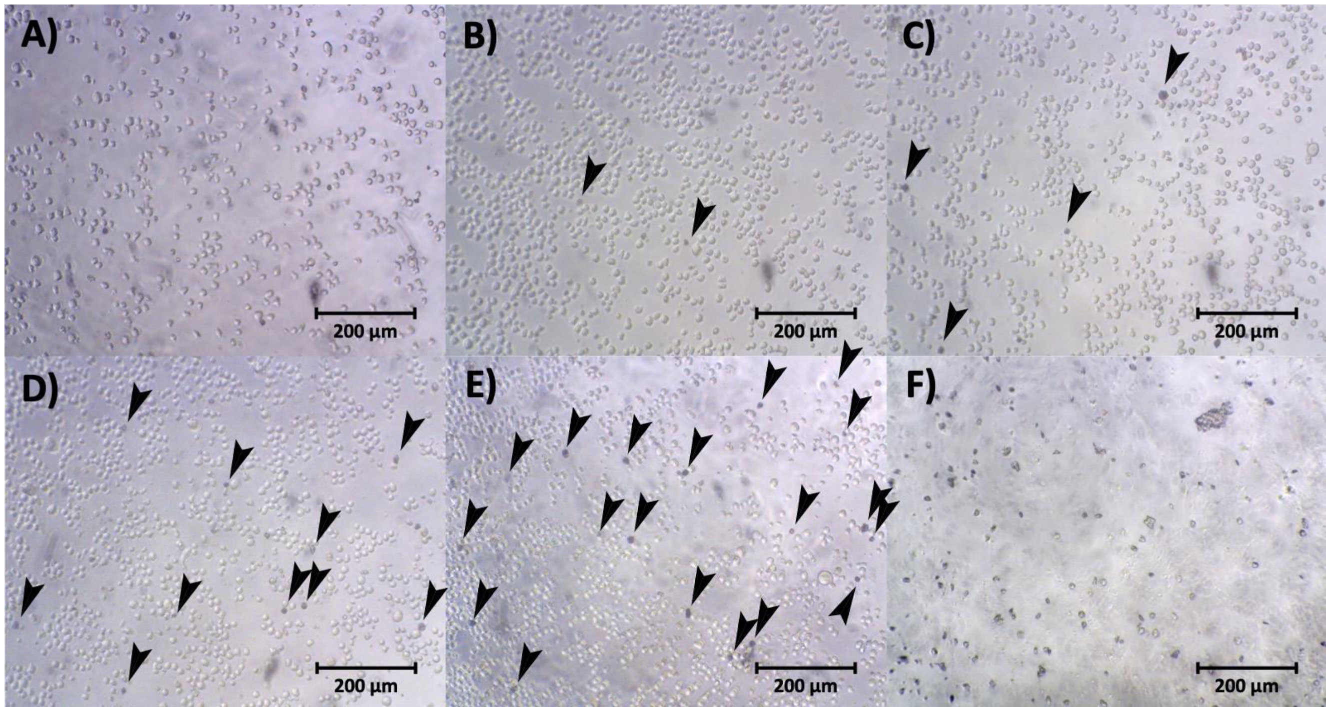

Fig. 2: Changes in cell growth of U266 during the 72-h incubation. U266 cells were incubated for (A) 0 h, (B) 12 h, (C) 24 h, (D) 48 h, and (E) 72 h of incubation. (F) Focused photograph of dead cells after 72 h of incubation. Arrow heads indicate dead cells.

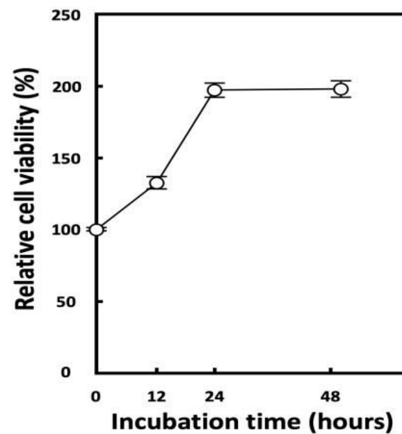

Fig. 3: Changes in cell viability of U266 cells during 48 h of incubation.

assessed using Dunnett's test. In all cases, the selected significance level was P < 0.05.

Results

Time-course study of IgE antibody production by U266 cells:

Preliminary cultures of U266 cells at 12, 24, 48, and 72 h were prepared. Then, the conditions of cultured cells were observed under a microscope. As a result, cell counts gradually increased up to 48 h of incubation (Figs. 2A-D); however, several dead cells shown by arrow heads were observed at 72 h of incubation (Figs. 2E, F). Accordingly, changes within 48 h were examined in detail.

Changes in cell viability and IgE antibody production are shown in Figures 3 and 4,

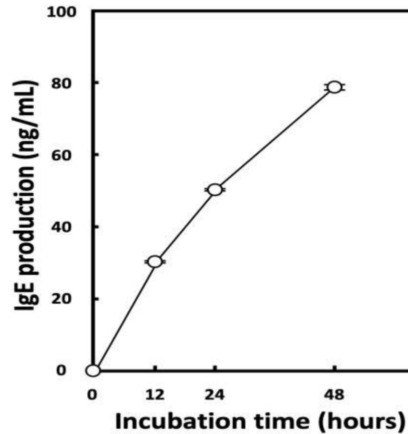

Fig. 4: Changes in IgE antibody production by U266 cells during 48 h of incubation.

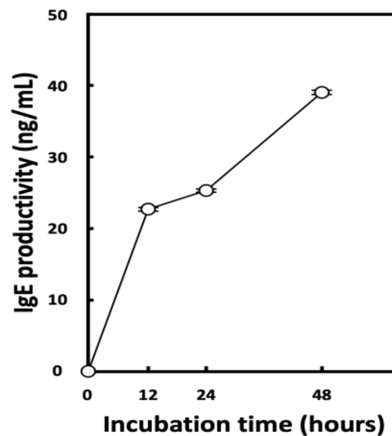

Fig. 5: Changes in IgE antibody produced per cell by U266 cells during 48 h of incubation.

respectively. The viability of U266 cells reached a plateau at 24 h of culture (Fig. 3). The production of IgE antibody gradually increased during the 48-h incubation period (Fig. 4). The IgE antibody values produced per cell were calculated (Fig. 5). As a result, IgE antibody values yielded per cell were remarkable higher at 48 h than at 24 h (Fig. 5).

DMSO cytotoxicity on U266 cells:

Based on the results of time-course study, the optimal incubation time of U266 was 48 h. At 48 h of culture, DMSO cytotoxicity on U266 cells was examined. Cell viability gradually decreased as the DMSO concentration increased, with significant differences between the experimental and control groups in the medium with 0.5% and 1% of DMSO (Fig. 6). In IgE production, as with cell viability, the addition of 0.5% and 1% of DMSO significantly decreased the IgE production (Fig. 7). Because the IgE production decreased with decreased cell viability, the IgE antibody values yielded per cell did not change by adding DMSO at 0.05%, 0.1%, 0.5%, and 1% concentrations (Fig. 8).

Influence of BaP or Pyr on IgE antibody production in U266 cells:

The influence of BaP or Pyr (each 10^{-8} M) dissolved in 0.1% or 0.5% DMSO on cell viability and IgE antibody production of U266 cells was examined.

In cell viability, no significant differences were observed between the control and experimental

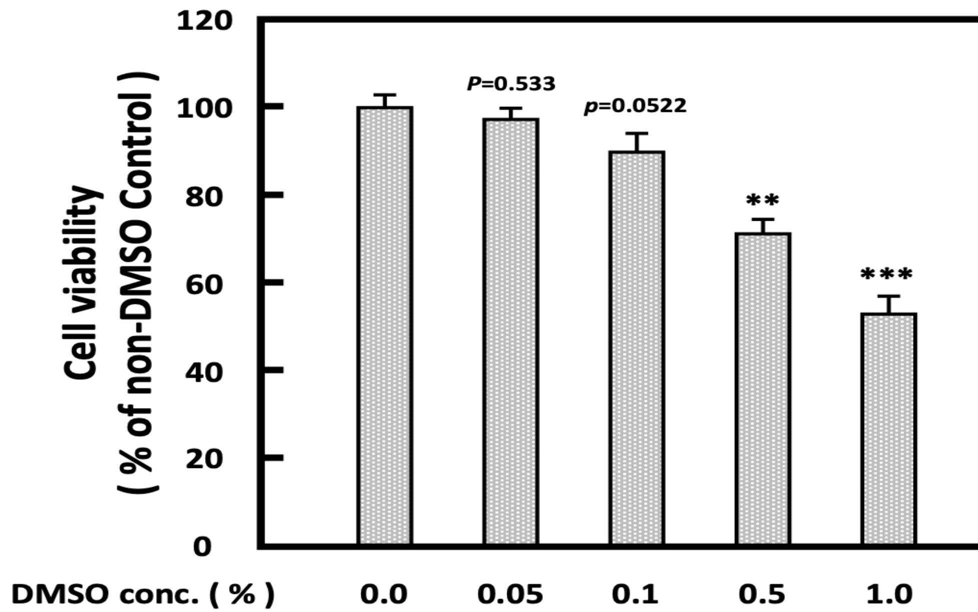

Fig. 6: Effects of DMSO on cell viability of U266 cells at 48 h of incubation. Statistically significant differences at **P < 0.01 and ***P < 0.001 compared with the values in the control cells. DMSO, dimethyl sulfoxide.

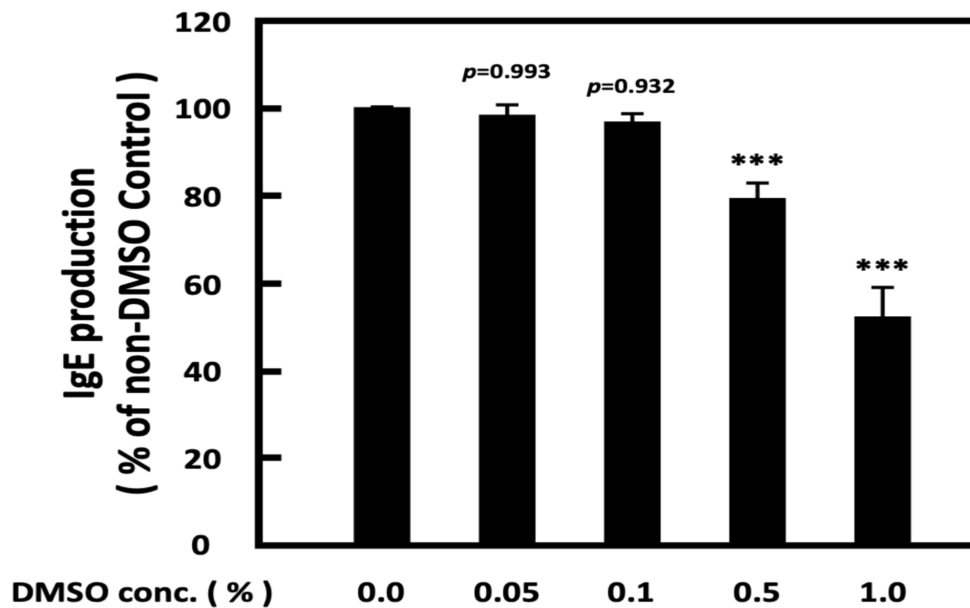

Fig. 7: Effects of DMSO on IgE production of U266 cells at 48 h of incubation. A statistically significant difference at ***P < 0.001 compared with the values in the control cells. DMSO, dimethyl sulfoxide.

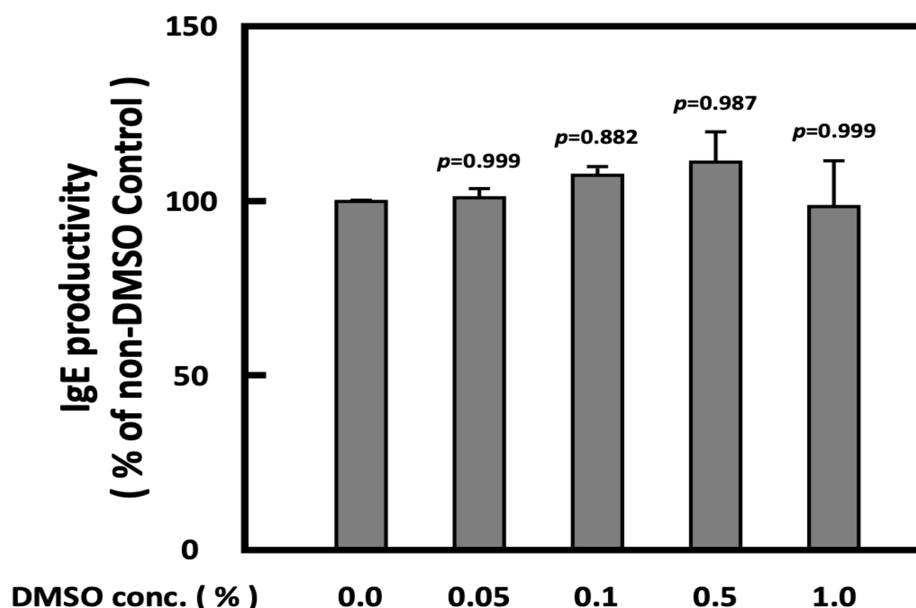

Fig. 8: Effects of dimethyl sulfoxide (DMSO) on IgE produced per cell by U266 cells at 48 h of incubation.

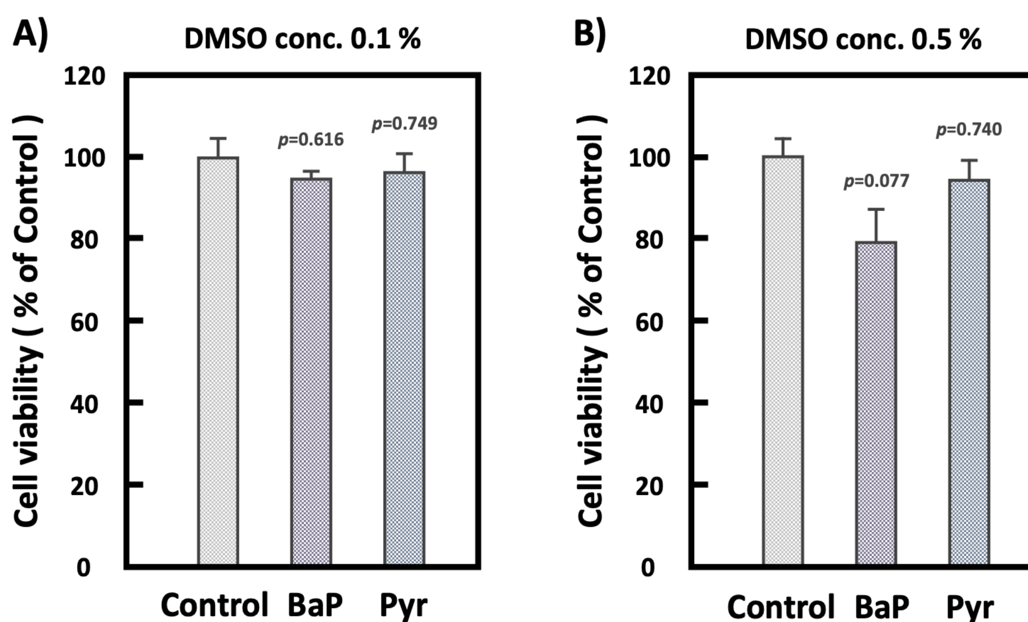

Fig. 9: Effects of BaP and Pyr dissolved in 0.1% (A) or 0.5% (B) DMSO on cell viability of U266 cells at 48 h of incubation. BaP, benzo[*a*]pyrene; Pyr, pyrene; DMSO, dimethyl sulfoxide.

cells treated with BaP or Pyr dissolved in 0.1% or 0.5% DMSO (Fig. 9). By BaP dissolved in 0.5% DMSO treatments, however, cell viability tended to decrease (P = 0.077)(Fig. 9).

In IgE production as well as cell viability, a significant difference was not obtained between the control and experimental cells treated with BaP or Pyr dissolved in 0.1% or 0.5% DMSO although IgE production tended to increase by BaP or Pyr treatments (Fig. 10).

Conversely, IgE antibody values yielded per cell significantly increased by BaP dissolved in 0.5% DMSO treatments (Fig. 11). Furthermore, other treatments have not shown significant

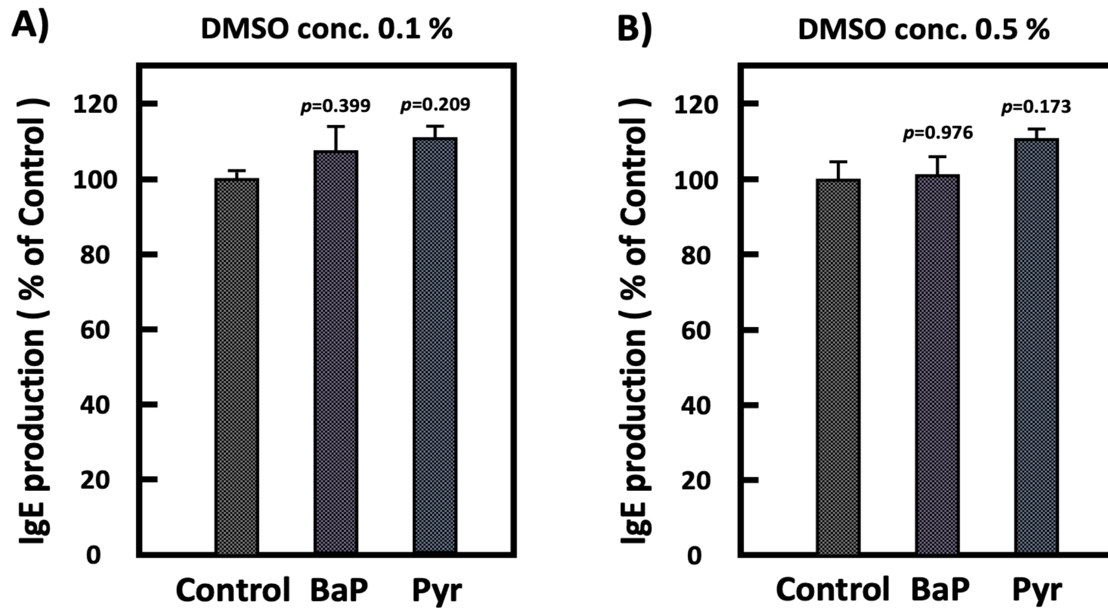

Fig. 10: Effects of BaP and Pyr dissolved in 0.1% (A) or 0.5% (B) DMSO on IgE production of U266 cells at 48 h of incubation. BaP, benzo[*a*]pyrene; Pyr, pyrene; DMSO, dimethyl sulfoxide.

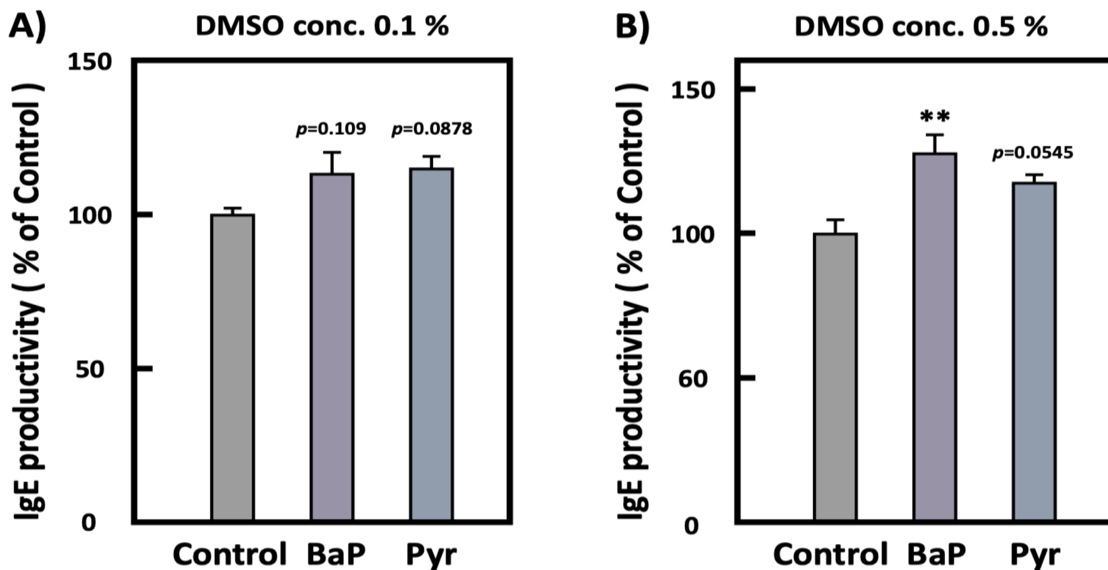

Fig. 11: Effects of BaP and Pyr dissolved in 0.1% (A) or 0.5% (B) DMSO on IgE produced per cell by U266 cell at 48 h of incubation. A statistically significant difference at $^{**}P < 0.01$ compared with values in the control cells. BaP, benzo[*a*]pyrene; Pyr, pyrene; DMSO, dimethyl sulfoxide.

difference although IgE antibody values yielded per cell tended to increase by PAH treatments (Fig. 11).

Discussion

To determine the direct influence of PAHs on IgE antibody production, an *in vitro* bioassay system was developed using U266 cells. First, we investigated the culture conditions of U266 cells. As a result, cell counts gradually increased up to 48 h of incubation; however, several dead cells shown by arrow heads were observed at 72 h of incubation. Furthermore, we found that IgE antibody values yielded per cell were remarkably

higher at 48 h than at 24 h. Accordingly, we decided that incubation time for U266 cells is 48 h. Thereafter, we investigated the DMSO cytotoxicity on U266 cells. At 48 h of culture, cell viability gradually decreased as the DMSO concentration increased, with significant differences between the experimental and control groups in the medium with 0.5% and 1% DMSO. In IgE production, as with cell viability, the addition of 0.5% and 1% DMSO significantly decreased the IgE production. Thus, DMSO cytotoxicity on U266 cells could be clearly demonstrated. Moreover, the IgE antibody yielded per cell significantly increased by BaP dissolved in 0.5% DMSO treatments. This significant increase in IgE production by BaP is most likely because of the additive and synergistic effects of BaP and DMSO.

Cytotoxic effects of DMSO on cochlear organotypic cultures have been reported (Qi *et al.*, 2008). In the cochlear organotypic cultures as well as U266 cell culture, toxic effects were observed at DMSO concentrations higher than 0.5% although 0.1% and 0.25% DMSO concentrations have little or no damage. Our present data show that IgE antibody values yielded per cell did not change by adding 0.05%, 0.1%, 0.5%, and 1% DMSO. This result caused by the IgE production decreased with decreased cell viability. Data on IgE production per cell may obscure the DMSO toxicity. Overall, we emphasize that when DMSO is used as a solvent, DMSO toxicity should be properly evaluated.

Phenanthrene, one of the toxic PAHs and BaP, enhanced the IgE antibody production in the IgE-producing human B cell line (Tsien *et al.*, 1997). Therefore, PAHs have an ability to induce IgE production. The US Environmental Protection Agency listed 16 PAHs as harmful PAHs (Honda and Suzuki, 2020). Thus, a comprehensive analysis of PAHs should be conducted to identify PAHs with a strong ability to produce the IgE antibody. Using our developed *in vitro* bioassay system, we are planning to analyze the influence of PAHs on IgE antibody production comprehensively.

Conclusion

After considering culture conditions and DMSO cytotoxicity, an *in vitro* bioassay system for IgE antibody production was developed using the human myeloma cell line (U266 cells) to determine the allergic reactions of PAHs in humans. PAHs (BaP or Pyr) (each 10^{-8} M) increased the IgE production on U266 cells. Thus, our developed bioassay system will be utilized for a comprehensive investigation of several PAHs to elucidate the allergic reactions of PAHs in humans.

Acknowledgements

This work was supported by the cooperative research program of the Institute of Nature and Environmental Technology, Kanazawa University, Kanazawa, Japan (Accept Nos. 23015, 23021, 23028, 22038, and 23055).

References

Al-Daghri NM, Alokail MS, Abd-Alrahman SH, Draz HM, Yakout SM and Clerici M. (2013) Polycyclic aromatic hydrocarbon exposure and pediatric asthma in children: A case–control study. Environ Health 12: 1.

Galli SJ and Tsai M. (2012) IgE and mast cells in allergic disease. Nat Med. 18: 693-704.

Honda M and Suzuki N. (2020) Toxicities of polycyclic aromatic hydrocarbons for aquatic animals. Int J Environ Res Public Health 17: 1363.

Jo JH, Kim DH and Ryu JY. (2022) Exposure to polycyclic aromatic hydrocarbons and serum total IgE in the Korean adults: the Third Korean National Environmental Health Survey (2015-2017). Ann Occup Environ Med. 34: e43.

Jung KH, Yan B, Moors K, Chillrud SN, Perzanowski MS, Whyatt RM, Hoepner L, Goldstein I, Zhang B, Camann D, Kinney PL, Perera FP and Miller RL. (2012) Repeated exposure to polycyclic aromatic hydrocarbons and asthma: Effect of seroatopy. Ann Allergy Asthma Immunol. 109: 249-254.

Kadkhoda K, Pourfathollah AA, Pourpak Z and Kazemnejad A. (2005) The cumulative activity of benzo(*a*)pyrene on systemic immune responses with mite allergen extract after intranasal instillation and ex vivo response to ovalbumin in mice. Toxicol Lett. 157: 31-39.

Kanoh T, Suzuki T, Ishimori M, Ikeda S, Ohasawa M, Ohkuni H and Tunetoshi Y. (1996) Adjuvant activities of pyrene, anthracene, fluoranthene and

benzo(*a*)pyrene in production of anti-IgE antibody to Japanese cedar pollen allergen in mice. J Clin Lab Immunol. 48: 133-147.

Lukac N, Lukacova J, Pinto B, Knazicka Z, Tvrda E and Massanyi P. (2013) The effect of nonylphenol on the motility and viability of bovine spermatozoa *in vitro*. J Environ Sci Health Part A. 48: 973-979.

Luo F, Huang WY, Guo Y, Ruan GY, Peng R and Li XP. (2017) 17β-estradiol lowers triglycerides in adipocytes *via* estrogen receptor α and it may be attenuated by inflammation. Lipids Health Dis. 16: 182.

Ohno F, Sugahara T, Kanda K and Nishimoto S. (2010) Proteose peptone fraction of bovine milk depressed IgE production *in vitro* and *in vivo*. Biosci Biotechnol Biochem. 74: 1332-1337.

Qi W, Ding D and Salvi RJ. (2008) Cytotoxic effects of dimethyl sulphoxide (DMSO) on cochlear organotypic cultures. Hear Res. 236: 52-60.

Qin X, Zhang J, Wang M, Feng Y, Zhao Y, Zhang H and Guo W. (2023) The effect of thalidomide on the invasive ability of gastric cancer cells by regulating miR-524-5p/FSTL1. Cell Mol Biol (Noisy-le-grand). 69: 137-142.

Suresh R, Shally A, Mahdi AA, Patel DK, Singh VK and Rita M. (2009) Assessment of association of exposure to polycyclic aromatic hydrocarbons with bronchial asthma and oxidative stress in children: A case control study. Indian J Occup Environ Med. 13: 33-37.

Tsien A, Diaz-Sanchez D, Ma J and Saxon A. (1997) The organic component of diesel exhaust particles and phenanthrene, a major polyaromatic hydrocarbon constituent, enhances IgE production by IgE-secreting EBV-transformed human B cells *in vitro*. Toxicol Appl Pharmacol. 142: 256-263.