ENHANCEMENT OF CYTOKINE PRODUCTIONS FROM CERVICAL LYMPH NODE CELLS TREATED WITH BaP AND PCB

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1. INTRODUCTION

The immune system responds not only to airborne antigens such as bacteria, viruses and pollen but also air pollutants. Epidemiologically, the positive relationship between air pollution and allergic diseases has been suggested [1, 2]. Ishizaki et al. indicated that the prevalence of Japanese cedar pollinosis may be related to an increase of diesel exhaust inhalation in Japan [3]. Recently, we studied the effects of intratracheal instillation with diesel exhaust particulates (DEP) and ovalbumin (OA) as an antigen in mice, and showed that enhanced in vitro interleukin 4(IL-4) production from mediastinal lymph node cells was observed [4]. These results suggest that an administration with DEP may enhance antigen specific immune response via local cytokine production by T lymphocyte activation.

DEP contain polycyclic aromatic hydrocarbons such as benzo[a]pyrene (BaP). When DEP affects T lymphocytes to include cytokine production, it appears that DEP may act through the Ah receptor because specific binding to the Ah receptor was detected with BaP[5].

Considerable interest has centered on the comparison of the effects of BaP and polychlorinated biphenyls (PCB) which bind to the Ah receptor. In this study, the effects of BaP as a component of DEP on cytokine production were compared with those of PCBs.

2. MATERIALS AND METHODS

Animals

Balb/c mice, 7-week-old, were obtained from Charles River Japan Inc., Kanagawa, Japan. During the experiments, food and water were given ad libitum.

Immunization and Isolation of lymph node cells

Balb/c mice were intranasally instilled with OA three times at an interval of 3 weeks. One week after the last instillation, cervical lymph node cells were isolated. Cell suspensions passed through stainless-steel mesh were washed by centrifugation. Lymph node cells were cultured with antigen presenting cells (APC) obtained by 30Gy irradiation of spleen cells in the

presence of OA.

Cytokine productions

After stimulation with OA, (3 times) OA specific lymph node cells cultured with APC were treated with various concentrations of BaP or 3,3'- 4,4',5-pentaCB (PCB) in the presence or absence of OA. After 24 and 48 hours, culture supernatants were collected and stored at -80°C until assayed for cytokines.

Assay for cytokines

IL-4 and Interleukin-6 (IL-6) levels in culture supernatants were measured by ELISA (Endogen INC. MA). Color development was measured at 450 nm and 550 nm using a Corona Microplate Photometer (Ibaraki, Japan). Values are mean ±SE in triplicate wells.

3. RESULTS

To examine spontaneous release of IL-4 and IL-6 from lymph node cells, BaP and PCB were added to the culture of lymph node cells and APC without OA. However, IL-4 and IL-6 were not induced by BaP and PCB.

In Figure 1, the secretion of IL-4 after 24(a) and 48(b) hours in culture are shown in Figure 1. Addition of 10 μM BaP to the culture significantly increased OA-stimulated IL-4 production in culture supernatants after 24 hours. Although treatment with 100 μM BaP for 24 hours did not increase IL-4 production, treatment for 48 hours markedly increased secretion of this cytokine. No increase of IL-4 production was observed in the cells cultured with 1 μM BaP.

On the other hand, low concentrations of PCB markedly increased IL-4 production from lymph node cells incubated for 24 hours. After 48 hours, no significant increase of IL-4 production was shown after treatment with PCB.

Treatment with 10 μM BaP and 5 nM PCB for 48 hours significantly increased IL-6 production (Figure 2).

4. CONCLUSION

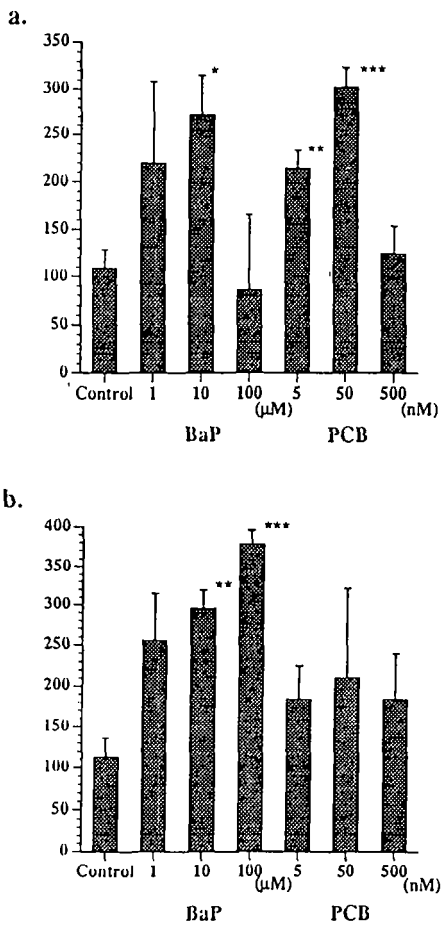
In vitro treatment with BaP and PCB increased levels of IL-4 and IL-6 in lymph node cells. However, the results suggest that induced cytokine production by BaP and PCB may be due to different mechanisms.

5. REFERENCES

1) Kaneko, S., K. Shimada, H. Horiuchi, A. Endo, M. Kodama, N.Shinoda, T. Imai, and K. Takiguchi (1980): Nasal allergy and air pollution.Oto.Rhino.Laryngol. Tokyo 23,270-281.
2) Robertoson,G.(1984): Analysis of relationships between symptoms and environmental factor over time. Environ. Res. 33-130-143.

3) Ishizaki, T., K.Koizumi, R. Ikemori, Y. Ishiyama, and E. Kushibiki (1987): Studies of prevalence of Japanese cedar pollinosis among the residents in a densely cultivated area. Ann.Allergy 58,265-270.
4) Fujimaki, H.,O.Nohara, T.Ichinose, N.Watanabe, and S.Saito (1994): IL-4 production in mediastinal lymph node cells in mice intratracheally instilled with diesel exhaust particulates and antigen. Toxicology 92,261-268.
5) Waithe,W.I,. M.Michaud, P.A.Harper, A.B.Okey, and A. Anderson (1991): The Ah receptor, cytochrome P450IA1 mRNA induction, and aryl hydrocarbon hydroxylase in a human lymphoblastoid cell line. Biochem.Pharmacol. 41,85-92.

Fig.1 IL-4 production by LNC treated with BaP and PCB



* P<0.05
** P<0.01
*** P<0.001

Fig.2 IL-6 production by LNC treated with BaP and PCB