EFFECTS OF BENZO[a]PYRENE, 2-BROMOPROPANE, PHENOL AND 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN ON PROINFLAMMATORY CYTOKINES GENE EXPRESSION BY MICE SPLEEN CELLS

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Introduction

Since the immune system responds to variable foreign antigens, it has been presumed that environmental contaminants may affect on the immune function. Until recently, many studies showed that 2,3,7,8-tetrachlorodibenzo-p-dioxin, benzo[a]pyrene, 3-methycholanthrene cause the impairment of the immune function[1-8]. The immune response is regulated by cytokines. Because multifunctional cytokines such as interleukin-1 (IL-1), IL-6, interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) are characterized as a primary mediator of the acute inflammatory responses, they also have been known as proinflammatory cytokines[9]. In this study, we investigated the effects of pollutants on proinflammatory cytokines gene expression in mouse splenocytes.

Materials and Methods

Culture conditions: Single cell suspensions from mice spleens were washed in RPMI 1640 (Gibco BRL), and resuspended at 5 X 10^6 cells/ml in RPMI 1640 medium containing 10% FBS (Hyclone), 200mM L-glutamine (Sigma), 50mM 2-ME (Sigma), and 1mg gentamicine (Gibco BRL)/100ml medium. Cells were either left unstimulated, stimulated with anti-CD3 or treated with anti-CD3 plus benzo[a]pyrene (B[a]P, Sigma), 2-bromopropane (2-BP, Tokyo Kasei), phenol (Sigma), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, Supelco).

Quantitation of IL-2: The protocol to quantify immunoreactive murine IL-2 was according to Schumacher et al.[10] Anti-IL-2 monoclonal antibodies and murine recombinant IL-2 were obtained from PharMingen (San Diego).

Proliferation assay: Following incubation for 8 h, cell proliferation was determined using the MTT assay.

DNA agarose gel electrophoresis: The protocol to extract DNA was according to Meβmerv et al.[11] DNA was electrophoretically separated on a 1.5% agarose gel containing 1μg/ml ethidium bromide

Extraction of cellular RNA and RT-PCR: Following incubation for 18 h, total cellular RNA was extracted using TRIzol Reagent (Gibco BRL) according to the distributor's manual. Reverse-transcription reactions and PCR for IL-1, IL-6, IFNγ and TNFα were performed using the RNA PCR kit (Takara Shuzo Co.). The relative density of each band was determined by scanning with laser Computing Densitometer and ImageQuant program version 3.3 (Molecular Dynamics).

Results

Figure 1. Effect of pollutants on cell proliferation. Cells were treated for 8h with anti-CD3 (+), 10^{-6}M B[a]P plus anti-CD3, 10^{-6}M 2-BP plus anti-CD3, 10^{-6}M phenol plus anti-CD3, 10^{-9}M TCDD plus anti-CD3, or were left unstimulated (–). Although the effect of pollutants on cell proliferation was higher than the baseline level of vehicle-treated group, no significant differences detected between the pollutant-treated group and the vehicle-treated group.

Figure 1.

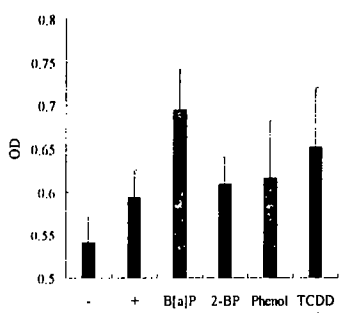

Figure 2.

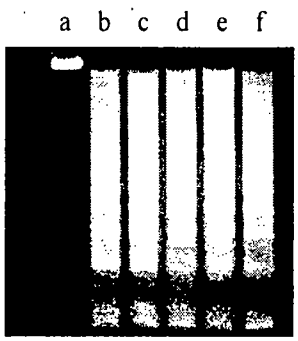

Figure 2. Effect of pollutants on apoptosis. Mouse spleen cells were exposed for 8 h to anti-CD3 (b), 10^{-6}M B[a]P plus anti-CD3 (c), 10^{-6}M 2-BP plus anti-CD3 (d), 10^{-6}M phenol plus anti-CD3 (e), 10^{-9}M TCDD plus anti-CD3 (f), or were left unstimulated (a). Exposure to B[a]P, 2-BP, phenol and TCDD resulted in a characteristic DNA ladder formation, whereas fragmentation on unstimulated cells was absent and anti-CD3 stimulated cells resulted in substantially less DNA cleavage. Variations between each pollutant-induced DNA cleavage were not significant.

Figure 3A.

a b c d e f

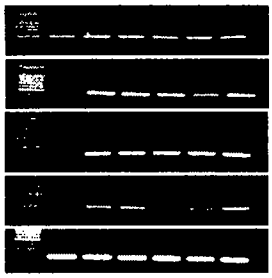

Figure 3B

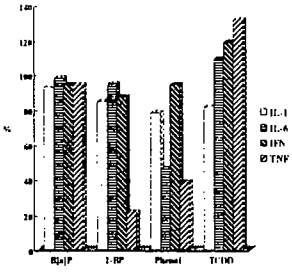

Figure 3. Cytokines gene expressions in response to pollutants. Cells were left unstimulated (a), stimulated with anti-CD3 (b), exposed to anti-CD3 plus 10^{-6}M B[a]P (c), 10^{-6}M 2-BP (d), 10^{-6}6M phenol (e), or 10^{-9}M TCDD (f) for 18 h. Relative induction of cytokines gene is expressed in comparison to an anti-CD3 stimulated control (B). A 1st panel; IL-1β, A 2nd panel; IL-6, A 3rd panel; IFNγ, A 4th panel; TNFα, A 5th panel: β-actin.

As shown in Figure 3 and 4, B[a]P plus anti-CD3 caused no significant change in cytokines gene expression. In contrast with the effect of B[a]P, 2-BP plus anti-CD3 decreased TNFα gene expression, and phenol plus anti-CD3 diminished IL-1, IL-6 and TNFα gene expression when compared to those of the vehicle-treated cells. TCDD plus anti-CD3 showed a tendency to enhance the IL-6, IFNγ and TNFα gene induction, whereas it inhibited IL-1 gene induction.

Discussion

Previous investigations have showed that B[a]P decreased IL-1 and IL-2 production[4,5], and increase IL-6 production[2]. However, we observed that B[a]P did not alter the proinflammatory cytokines gene expression by spleen cells at 10^{-6}M concentration. We think that this discrepancy may due to different of the used cells and T cell stimulant such as anti-CD3.

Effects of TCDD on cytokines production appears to be very complex and sensitive to experimental differences and stimulation of cells investigated by different groups expression[1,3,6,7]. The present study has extended some of these previous findings and showed that TCDD plus anti-CD3 upregulate IL-6, IFNγ and TNFα gene expression, and downregulate IL-1 gene expression. To our knowledge, little is known about the effects of phenol and 2-BP on cytokines production by mouse spleen cells. Here, we have demonstrated that phenol downregulate IL-1, IL-6 and TNFα gene expression, and 2-BP suppress TNFα gene expression. We currently have no explanation for these results, although they were repeatable findings.

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References

1. Fan,F., Yan,B.F., Wood,G., Viluksela,M.anf Rozman,K.K. (1997) Cytokines (IL-1 beta, and TNF alpha) in relation to biochemical and immunological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rats. Toxicology, 116,9-16.

2. Fujimaki,H., Shiraishi,F., Aoki,Y. and Saneyoshi,K.(1997) Modulated cytokine production from cervical lymph node cells treated with B[a]P and PCB. Chemosphere, 34,1487-1493.

3. Kerkvliet,N.I., Baecher-Steppan,L., Shepherd,D.M., Oughton,J., Vorderstrasse,B.and DeKrey,G. (1996) Inhibition of TC-1 cytokine production, effector cytotoxic T lymphocyte development and alloantibody production by 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Immunol. 157,2310-2319.

4. Lyte,M. and Bick,P.H. (1986) Modulation of interleukin-1 production by macrophages following benzo[a]pyrene exposure. Int J Immunopharmacol. 8,377-381.

5. Lyte,M., Blanton,R.H., Myers,M.J. and Bick,P.H. (1987) Effect of in vivo administration of the carcinogen benzo[a]pyrene on interleukin-2 and interleukin-3 production. Int J Immunopharmacol. 9,307-312.

6. Moos,A.B., Oughton,J.A. and Kerkvliet,N.I. (1997) The effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) on tumor necrosis factor (TNF) production by peritoneal cells. Toxicol Lett. 90,145-153.

7. Sutter,T., Guzman,K., Dold,K. and Greenlee,W. (1991) Targets of dioxin, genes for plasminogen activator inhibitor-2 and interleukin-1β. Science, 254,415-418.

8. Talcott,P.A., Exon,J.H., Mather,G.G. and Koller,L.D. (1990) Alternations of rat natural killer (NK) cell cytotoxicity and cytokine production by 3-methycholanthrene (3-MC). Int J Immunopharmacol. 12,917-926.

9. Abbas,A.K., Lichtman,A.H.and Pober,J.S. (2000) Chapter 11. Cytokines. In: Cellular and molecular immunology. W.B.Saunders Company, Philadelphia, London, New York, St. Louis, Sydney, Toronto, pp.235-269.

10. Schumacher,J.H., O'garra, A., Shrader,B., van Kimmenade,A., Bond,M.W., Mosmann,T.R. and Coffnab,R.L. (1988) The characterization of four monoclonal antibodies specific for mouse IL-5 and development of mouse and human IL-5 enzyme-linked immunosorbent. J Immunol. 141,1576-1581.

11. Meβmer,U.K., Reed,J.C. and Brune,B. (1996) Bacl-2 protects macrophages from nitric oxide-induced apoptosis. J Biol Chem. 271,20192-20197.