

The ex vivo study of synergistic effects of polycyclic aromatic hydrocarbon, benzo(a)pyrene with ovalbumin on systemic immune responses by oral route

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Abstract

The present study was undertaken in order to examine whether oral administration of soluble antigen together with one of polycyclic aromatic hydrocarbons (PAHs) which is present in diesel exhaust particles (DEPs) called benzo(a)pyrene (BP), induced the systemic immune response in mice or not. Mice were orally given 1 mg of ovalbumin (OA), a common food allergen, every 3 days over a period of 15 days.

The results showed that oral administration of OA plus BP produced anti-OA IgE antibodies in serum, whereas either OA or BP alone failed to show the antigen-specific IgE antibody production. Production of anti-OA IgE antibody, which is dependent on Th2 CD4⁺ T cells, was seen in mice fed with combined OA and BP was significantly higher than that of other groups. The anti-OA antibody production was associated with marked secretion of the Th1 cytokines, IFN- γ and IL-12p70 as well as the Th2 cytokines IL-4, and IL-10. These results suggest that BP may act as a mucosal adjuvant in the gut enhancing systemic Th1 and Th2 immune responses and might play a role in oral immunization and food allergy.

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1. Introduction

Oral administration of antigen with adjuvant, stimulates not only the mucosal immune system, producing antigen-specific IgA antibodies (Burgmann and Waldman, 1998; McGhee and Kiyono, 1993; Abraham and Robinson, 1991), but also activates the systemic immune system, resulting in the production of IgG and

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IgE antibodies to the fed antigen in serum (Schaffeler et al., 1997; Kubota et al., 1997; Ball et al., 1998). For instance, feeding recombinant simian immunodeficiency virus p55gag (Schaffeler et al., 1997) or hen egg lysozyme (Kubota et al., 1997) accompanied by the mucosal adjuvant cholera toxin is followed by production of serum IgG and IgE antibodies to the antigen, proliferation of mesenteric lymph node and peripheral blood mononuclear cells, and secretion of IFN- γ , IL-2, IL-5, IL-6 and IL-10 from the lymphoid cells.

Benzo(a)pyrene (BP), one of the most prominent and important compounds which is present diesel exhaust particles (DEP) generated by diesel engine-powered cars have been implicated in the incidence of allergic respiratory diseases including asthma (Takano et al., 1997; Diaz-Sanchez et al., 1994, 1997; Wade and Newman, 1993). For example, direct intratracheal administration of DEP enhances antigen-induced airway inflammation in mice (Diaz-Sanchez et al., 1997). However, a role for DEP which includes polycyclic aromatic hydrocarbons (PAH), especially BP, in the mucosal immune system in the gut has not been investigated despite the evidence that, after exposure of mice to DEP, the air-borne particulates reached not only the lung but also the gut. There are also marked deposits of DEP in the mouse intestinal tissue following exposure to them, suggesting that DEP may act as a mucosal adjuvant in the gut and play a role in oral immunization and food allergy (Takafuji et al., 1987). Finally, it has been suggested that PAHs present in DEP promote greater var-epsilon RNA transcription and translation via acting on aryl hydrocarbon receptors present on B lymphocytes (Takenaka et al., 1995).

2. Materials and methods

2.1. Animals and grouping

Male Balb/c mice, 8–9 weeks of age, were used in all experiments. The mice were bred in the animal breeding unit of Pasteur Institute of Iran, Tehran, Iran. They were maintained in a temperature- and light-controlled environment with free access to standard rodent chow and water. Mice were grouped into four separate groups containing 10 mice in each

group. Each group were divided into two groups: five mice for serum collection and five for cytokine measurement.

2.2. Administration of ovalbumin

Mice were orally given 1 mg of ovalbumin (OA) (grade VII, Sigma, St. Louis, MO) dissolved in 0.25 ml of phosphate-buffered saline (PBS) through a syringe fitted with an 18 gauge ball-point needle on day 0 and then every 3 days up to day 15 (Yoshino and Ohsawa, 1997). PBS alone (0.25 ml) was administered as a control.

2.3. Administration of benzo(a)pyrene

BP were commercially purchased (Sigma, St. Louis, MO). BP (1 mg) suspended in 0.25 ml of PBS containing 0.01% Tween 20 was orally administered immediately before each feeding of OA. PBS (0.25 ml) containing 0.01% Tween 20 alone given as a control.

2.4. Measurement of antibodies to OA

Blood was collected on day 21 after administration of OA and BP, and sera were heat inactivated at 56 °C for 30 min to avoid any possible interference of complement components. IgE antibodies specific for OA were measured using an ELISA (Yoshino, 1998). In brief, 96 well flat-bottomed microtiter plates were incubated with 100 μ l per well of OA (100 μ g/ml) at 37 °C for 3 h followed by overnight incubation at 4 °C, and washed three times with ELISA wash buffer (10 mM Tris, 0.05% Tween 20, pH 8.0). The wells were then blocked by incubation with 400 μ l of ELISA block buffer (PBS containing 0.05% Tween 20, pH 7.4) at 37 °C for 1 h. After washing, the plates were incubated with 100 μ l of a 1:5 dilution of each serum sample at 37 °C for 30 min. The plates were washed five times, and 100 μ l per well of a 1 μ g/ml of biotinylated rat anti-mouse IgE heavy chain (Serotec, England) was added and incubated at 37 °C for 1 h. After being washed, 100 μ l of a 1:5000 dilution of streptavidin-conjugated horseradish peroxidase (Sigma) was added to each well and incubated at 37 °C for 1 h. After five times washing, 100 μ l of a ready-to-use solution of tetramethylbenzidine (Sigma, St. Louis, MO) was added per well and the plates

Table 1

The effects of oral administration of BP on antibody production and cytokine secretion in terms of mean $\pm$ S.E.M.

Group	Anti-OA IgE (A 450)	IFN- γ (pg/ml)	IL-12p70 (pg/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)
Control	0.022 $\pm$ 0.0029	128 $\pm$ 3.77	28.5 $\pm$ 1.10	64.6 $\pm$ 3.26	61.3 $\pm$ 8.22
BP	0.021 $\pm$ 0.0036	138.6 $\pm$ 1.50	29.9 $\pm$ 0.58	68.3 $\pm$ 3.38	60.7 $\pm$ 7.22
OA	0.269 $\pm$ 0.0028	179.1 $\pm$ 2.21	50.4 $\pm$ 1.87	78.5 $\pm$ 3.10	130.2 $\pm$ 8.05
OA + BP	0.796 $\pm$ 0.0076*	984.4 $\pm$ 8.81*	122 $\pm$ 3.13*	396 $\pm$ 3.44*	477.3 $\pm$ 14.60*

* Values significantly higher than values from the OA, BP or buffer control groups ($P < 0.05$).

were incubated in the dark at room temperature for 30 min. Absorbance was then measured at 450 nm with a wavelength correction of 630 nm in a Titertec Multiscan spectrophotometer (Stat Fax 2100).

2.5. Cytokine measurement

Spleens were removed on day 18 and cell suspensions prepared. Erythrocytes in the cells lysed with Tris-NH₄Cl. The resulting cell suspension to a total concentration of 5 million cells per milliliter were cultured in 1 ml aliquots in 24 well tissue culture plates with 50 μ g/ml of OA (Yoshino and Ohsawa, 1997). Forty-eight hours later, supernatants were harvested and stored at -70°C until assayed. Secretion of IFN- γ , IL-12p70, IL-4, and IL-10 was quantified using sandwich ELISA techniques. The ELISA kits for these cytokines were commercially available from R&D Systems Inc., Minneapolis, USA.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) and Tukey test (as a Post-Hoc test) were used for comparing of groups. The results were expressed as OD₄₅₀ (absorbance units) $\pm$ S.E.M. and concentration (pg/ml) $\pm$ S.E.M. for IgE and cytokines, respectively.

3. Results

3.1. Effect of BP on production of anti-OA IgE antibody

To investigate the ability of BP to induce oral immunization, mice were fed 1 mg of OA with 1 mg of BP every 3 days over a period of 15 days. PBS, 1 mg of OA plus PBS, and PBS containing 0.01% Tween

20 plus 1 mg of BP were orally given as controls. Table 1 shows the results. Oral administration of OA plus 1 mg of BP was followed by significantly greater production of anti-OA IgE antibody in serum.

3.2. Effect of BP on production of anti-OA IgE antibody

To examine the effect of BP on Th1 and Th2 CD4⁺ T-cell-mediated oral immunization, anti-OA IgE antibody were measured since IgE antibody production was dependent on Th2 cells. As indicated in Table 1, there was no production of anti-OA IgE antibody in mice orally given OA without BP. Marked production of anti-OA IgE antibodies was observed in mice fed OA and 1 mg of BP.

3.3. Effect of BP on secretion of cytokines

The Th1 cytokines IFN- γ and IL-12p70 and the Th2 cytokines, IL-4 and IL-10 secreted by spleen cells from mice given OA with BP were measured. As indicated in Table 1, 5.5 and 2.5 times greater secretion of IFN- γ and IL-12p70 was observed in animals treated with OA plus 1 mg of BP, compared with those given OA plus PBS. On the other hand, OA plus 1 mg of BP increased IL-4 and IL-10 secretion up to 5.0 and 3.6 times, respectively.

4. Discussion

The present study demonstrates that BP may play a role in oral immunization since oral administration of OA with BP was followed by production of anti-OA IgE antibody in serum. DEP, which contains BP, have been implicated in the increased incidence of allergic airway disorders (Takano et al., 1997; Diaz-Sanchez

et al., 1994, 1997; Wade and Newman, 1993). For instance, Takano et al. (1997) reported that intratracheal administration of DEP, enhanced ovalbumin-induced airway inflammation in mice immunized with the antigen. In the studies, they also showed that intratracheally administered DEP augmented the production of serum IgE and IgG antibodies to ovalbumin. Diaz-Sanchez also demonstrated that nasal challenge with both DEP and ragweed induced markedly higher ragweed-specific IgE levels in the respiratory tissues of ragweed-sensitized subjects, compared with challenge with ragweed alone. However, the effect of DEP on the mucosal immune system in the gut has not been examined previously despite the evidence that a greater amount of DEP was observed in the gastrointestinal tract than in the lung following nasal exposure of mice to the air-borne pollutants (Takafuji et al., 1987). We also observed marked deposit of DEP in the mouse intestinal tissue after their exposure, indicating that nasally exposed DEP appeared to be trapped by pharyngeal mucosal surfaces and then carried to the stomach. More importantly, our studies demonstrated that feeding naïve mice with ovalbumin plus BP itself induced peripheral antigen-specific immune responses, suggesting that oral DEP, which highly contains BP, may act as a mucosal adjuvant in the gut. The ability of DEP to induce oral immunization also suggests that they may in part contribute to food allergy. It was previously shown that air-borne pollution might be associated with increased incidence of atopy (Rusznak et al., 1994) and that 5–6% of atopic patients were linked to food allergy (Sabbah et al., 1997). Oral administration of HEL and DEP seems to more efficiently induce Th2 CD4⁺ T-cell-mediated immune responses than Th1 helper T-cell-mediated responses, since much greater production of anti-HEL IgG1 antibodies that is dependent on Th2 cells (Estes et al., 1995; Jain et al., 1996) was observed in mice given 0.01 and 0.1 mg of DEP compared with anti-HEL IgG2a antibody production that was Th1-cell-dependent (Finkelman et al., 1988; Snapper and Paul, 1987). Furthermore, the results appear to support the previously shown data that DEP injected intratracheally or nasally enhance antigen-specific IgE production that is Th2-cell-dependent (Fujimaki et al., 1994, 1995). Oral administration of OA plus BP was also followed by secretion of both Th1 and Th2 cytokines, IFN- γ , IL-12p70, IL-4, and IL-10. In

addition, the secretion of IFN- γ and IL-12p70 along side with IL-4 and IL-10, was well correlated with production of anti-OA IgE antibody. Also increased secretion of IL-4 and IL-10 is consistent with the findings that Th2 cytokines play a role in IgE antibody production, however, we have to consider the role of BP in promoting of class switching to IgE, although these two hypotheses are not mutually exclusive. The increase in IL-4 secretion by DEP given orally was consistent with results shown by other investigators in whose studies DEP were intratracheally or nasally administered (Takano et al., 1997; Fujimaki et al., 1995). However, the increased secretion of IFN- γ was not previously demonstrated. Conversely, the nasal administration of DEP inhibited IFN- γ expression (Fujimaki et al., 1995), although Takano et al. (1997) showed that IFN- γ secretion was not affected by DEP given intratracheally. The discrepancies among these findings might have reflected differences in the routes and the frequency of DEP administration. Differences in the doses of DEP and the strains of mice used might have also in part contributed to the different results.

Digestion of HEL following its oral administration or the mucosal immune system in the gut might have contributed to the induction of Th1 responses as well as Th2 responses. Cholera toxin is a mucosal adjuvant used world-wide that induces both Th1 and Th2 responses. For instance, Kubota et al. (1997) showed that oral administration of simian immunodeficiency virus p55gag and cholera toxin was followed by production of antigen-specific IgA and IgG antibodies and secretion of IFN- γ and IL-10. However, in the studies they failed to show secretion of IL-4 following the antigen plus cholera toxin administration. Schaffeler et al. (1997) also demonstrated that oral immunization with HEL and cholera toxin resulted in proliferation of HEL-specific T cells and secretion of IL-12 but not IL-4. Furthermore, it was previously shown by Maloy et al. (1995) that feeding immune stimulating complexes containing ovalbumin produced IL-2, IL-5 and IFN- γ , but not IL-4. Therefore, the ability of DEP to stimulate IL-4 secretion may be distinct from that of other mucosal adjuvants used previously.

In summary, oral administration of OA and BP induced both Th1 and Th2 responses. These results suggest that exposure to BP might play a role in induction of oral immunization which often leads to food allergy.

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