

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

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Abstract

The cumulative activity of benzo(a)pyrene (BP) as one of the polycyclic aromatic hydrocarbons which is present in diesel exhaust particles on systemic immune responses was studied in mice after intranasal instillation and in vitro exposure to allergens. The main purpose was to elucidate whether BP has an adjuvant effect in this responses or not. Male Balb/cA mice were immunized with mite allergen extract (MAE) either alone or in combination with BP. Controls were given BP, or buffer alone. The animals were immunized four times. One week after the last immunization, anti-MAE IgE antibody in serum and IL-4, IL-10, IL-12p70 and IFN- γ secreted by spleen cells upon rechallenge with the same allergen, was measured by ELISA. An increased response to the antigen was observed in animals receiving MAE together with BP, compared with animals receiving MAE alone. In another attempt, bone marrow-derived dendritic cells (DCs) are obtained from naïve male Balb/c mice and were exposed to the varying doses of ovalbumin (OA) and BP. There were four groups of study: control, BP, OA and OA plus BP. An elevated response was observed in animals receiving OA together with BP, compared with animals receiving OA alone.

In conclusion, this work shows that BP has an cumulative activity for specific IgE and Th1/Th2 cytokines production after intranasal instillation and cytokine secretion by DCs after in vitro exposure, and this phenomenon is dose-dependent. Thus, it may be another evidence on immunomodulatory effect of dendritic cells.

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1. Introduction

An increased prevalence of allergic airway diseases has been observed in many parts of the world during the last decades (Strachan, 1995). Although environmental pollutants may not be the main causative factors, there is both experimental and epidemiological evidence that motor vehicle related air pollutants may play a role (Ishizaki et al., 1987). In that respect, a major focus has been on diesel exhaust particles (DEP) and their polycyclic aromatic hydrocarbon (PAH) contents which benzo(a)pyrene is one the most compounds presents in them. DEPs are ultrafine particles, highly respirable, with a mean diameter of 0.05–0.3 μm . They consist mainly of a carbon core and adsorbed organic substances, e.g. PAHs which in turn include benzo(a)pyrene (BP) (Westerholm et al., 1991). DEP have been shown to increase the production of specific IgE antibody against ovalbumin (OA) in mice after intranasal and intratracheal instillation, intraperitoneal injection or injection into the food pad (Murakana et al., 1986). Intranasal instillation of DEP and OA increased proliferative response to OA and increased IL-4 production in vitro in cervical lymph node cells, compared with mice instilled with OA alone (Fujimaki et al., 1997). Inhalation of diesel exhaust particles has been shown to enhance the production of antigen-specific IgE antibody in mice through alteration of the cytokine network (Fujimaki et al., 1994). Human volunteers exposed to nasal challenges with DEP showed increased number of IgE producing cells and increased IgE production in nasal lavage (Diaz-Sanchez et al., 1994). Human peritoneal monocytes/alveolar macrophages exposed to suspended DEP showed reduced phagocytosis and increased TNF- α release (Thomas et al., 1995). There has been some focus on the role of the adsorbed PAH (Peterson and Saxon, 1996). Organic extract from DEP and one of the major PAH adsorbed to DEP, phenanthrene, have been shown to increase in vitro production of IgE from human B cells (Takenaka et al., 1995). An increased specific IgE production was induced in mice after intraperitoneal injection of pyrene and OA compared with OA alone (Suzuki et al., 1993). Pyrene is the other major PAH adsorbed to DEP. It is, however, important to bear in mind that the particle associated PAH constitute only a small fraction (usually <1%) of the soluble organic fraction of DEP (Westerholm et al., 1991). Lovik et al.

(1997) showed that an adjuvant effect of both DEP and of carbon black particles (CB) in mice using the popliteal lymph node assay. CB was used as a surrogate for the non-extractable carbon core. Both DEP and CB had a significant adjuvant effect on the local immune-mediated inflammatory response to OA. The results indicated that both the adsorbed organics and the non-extractable core contributed to the adjuvant effect, but DEP were shown to be slightly more potent than CB. The aim of the present study was to investigate further the additive effect of BP. This was achieved by evaluation of the effect of benzo(a)pyrene with respect to modulation of the specific IgE response towards mite allergen extract (MAE) in mice, and the assessment of IFN- γ and IL-12p70 from Th1 cytokines and IL-4,10 from Th2 cytokines, released by splenocytes in response to rechallenge with MAE using intranasal instillation as a relevant route of exposure for mite-derived allergens. We also evaluated the cytokine responses of dendritic cells (DCs) upon their exposure to BP and OA with varying doses in vitro. The reason for using OA instead of MAE in this part of our experiment was to avoid any possible interferences posed by unknown constituents in the MAE on the process of antigen loading by DCs, thus we used the OA as a highly pure allergen for loading.

2. Materials and methods

2.1. Design

Mice were immunized with the antigen MAE administered in both nostrils (intranasally), with or without simultaneous exposure to BP. Control animals were given buffer or BP only. The design was based on a pilot study with sampling times 2 days and 1, 2 and 3 weeks after last immunization and studies performed by Lovik et al. (1997), Murakana et al. (1986) and Takafuji et al. (1987). The animals were immunized four times, first with two intervals of 2 weeks and then with one interval of 1 week between the immunizations. Blood samples were collected 7 days after the last immunization. Individual serum samples were prepared for analysis of anti-MAE IgE. The study was repeated once and five animals were used per exposure group and sampling time.

2.2. Animals

Female Balb/cA mice (Pasteur Institute of Iran, Tehran, Iran) weighting 22.4 ± 0.3 g at the beginning of the experiments were used. The mice were housed on conventional sterile bedding and were given food and tap water ad libitum during the study. They were exposed to light/dark cycles of 15 h light and 9 h dark and the temperature and humidity were within recommended limits.

2.3. Benzo(a)pyrene, MAE and OA

Benzo(a)pyrene was purchased from Sigma Inc. Saint Louis, MO. It was suspended in borate buffered saline (BBS) (pH 8.0), 4 mg/ml. The suspension of BP in BBS were stirred at room temperature overnight in order to obtain a homogeneous suspension. MAE was commercially available from Allergopharma, Germany. The mixing of MAE with BP in BBS was performed by stirring at room temperature for 15 min. Ovalbumin was grade VII and purchased from Sigma, St. Louis, MO.

2.4. Immunization

Prior to immunization and exposure to BP, the mice were sedated with 0.15 ml of the mixture of Rompun (1.6 mg/ml) and Ketamin (12 mg/ml) dissolved in normal saline, intraperitoneally. The buffer or test substance solutions were shaken before administration to each animal. The volume administered intranasally was 10 μ l (5 μ l in each nostril) for all test substance solutions and buffer. The amount of MAE and BP administered to each mice is 0.05 mg.

2.5. Sampling of serum

All animals were anaesthetized with 0.15 ml of the above-mentioned solution (ip) before sampling of blood by cardiac puncture and a volume ranging from 0.5 to 1.0 ml was collected from animal. The samples were allowed to coagulate for 1 h at room temperature, centrifuged at $1000 \times g$ for 10 min and the serum samples were aliquoted and stored at -20°C .

2.6. Measurement of anti-MAE IgE

Blood was collected on day 21 after administration of MAE and BP, and sera were heat-inactivated at 56°C for 30 min to avoid any possible interference by complement components. IgE antibodies specific for MAE were measured using an ELISA. In brief, 96-well flat-bottomed microtiter plates were incubated with 100 μ l/well of MAE (100 μ l/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% Tween20, 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/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 horse-radish 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 Co., St. Louis, MO) was added per well and the plates 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). The results were expressed as absorbance units at $\text{OD}_{450} \pm \text{S.E.M.}$

2.7. Cytokine measurement

Spleens were removed 3 days after the last immunization and cell suspensions prepared. Erythrocytes in the cells lysed with Tris- NH_4Cl . The resulting cell suspension to a total concentration of five million cells/ml were cultured in 1-ml aliquots in 24-well tissue culture plates with 40 μ g/ml of MAE. 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 technique. The ELISA kits for these cytokines were commercially available from R&D Systems Inc., Minneapolis, USA.

2.8. Preparation of dendritic cells

Mice were kept under SPF conditions in our own facilities. Femurs and tibiae of male, 4–12 weeks old, Balb/c mice were removed and purified from the surrounding muscle tissue by rubbing with Kleenex tissues. Intact bones were left in 70% ethanol for 2–5 min thereafter for disinfection and washed with PBS, pH 7.4. Then both ends were cut with scissors and the marrow flushed with PBS using a Syringe with a 0.45 mm diameter needle. Clusters within the marrow suspension were disintegrated by vigorous pipetting. After one wash in PBS, about 10–15 million leukocytes were obtained per femur or tibia.

The principal method for generating bone marrow-derived dendritic cells (BM-DC) with GM-CSF and IL-4 was adapted from previous publications (Lutz et al., 1999; Son et al., 2002). 24-well plates was used for the cell culture (NUNCLON, Denmark). Cell culture medium (R10) was liquid RPMI-1640 (Sigma, St. Louise, MO, USA) supplemented with Penicillin (100 U/ml, Sigma), Streptomycin (100 µg/ml, Sigma), Gentamycin (20 µg/ml, Sigma), L-glutamin (2 mM, Sigma), 2-mercaptoethanol (50 µM, Sigma), and 10% heat-inactivated and filtered (0.22 µm, Millipore, Germany) Fetal Bovine Serum (FBS) (Euroclone). At day 0 BM leukocytes were seeded at 2 million per 24-well plate in 3 ml R10 medium containing 200 U/ml (=20 ng/ml) rmGM-CSF (Serotec, England) and 1000 U/ml rmIL-4 (Serotec, England). At day 3 another 10 ml R10 medium containing 200 U/ml rmGM-CSF were added to the plates. At days 6 and 8 half of culture supernatant was collected, centrifuged and the cell pellet resuspended in 10 ml fresh R10 containing 200 U/ml rmGM-CSF and 1000 U/ml rmIL-4, and given back into the original plate. At day 10 cells can be used already or culture can be continued to further reduce granulocyte contamination. Then plates were fed as at days 6 and 8, but the dose of rmGM-CSF and rmIL-4 in the fresh 10 ml R10 medium contained only 30–100 U/ml and 250 U/ml, respectively. For complete maturation at day 10, non-adherent cells were collected by gently pipetting, centrifuged at $300 \times g$ for 5 min at room temperature, and resuspended in 10 ml fresh R10 into a fresh 24-well plate containing 100 U/ml rmGM-CSF, 250 U/ml rmIL-4 and Tumor necrosis Factor-alpha (TNF-α) at 500 U/ml or lipopolysaccharide (LPS, Sigma) at

1 µg/ml. Cells were then cultured for one or more two days. Cultured cells were washed once and an equivalent volume mixed 1:1 in Trypan Blue (Sigma) solution. Trypan Blue negative, large leukocytes were counted as viable under the microscope in a Neubauer chamber.

2.9. FACS analysis

The expression of surface molecules on DC was analyzed under FACS (Coulter). On each step of the staining, 50,000 cells were stained with specific monoclonal antibodies for 30 min at 4 °C in 100 µl of phosphate-buffered saline (PBS) containing 5% fetal bovine serum (FBS) and 0.1% sodium azide (PBS + NaN₃). We used fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-labeled monoclonal antibodies for the staining of MHC class II (I-A/I-E, Rat IgG2a,κ,2G9) and CD11c (American Hamster IgG1, λ, HL3). FITC- or PE-labeled rat and hamster antibodies was substituted for specific antibodies to obtain negative controls. All antibodies were purchased from BD Pharmingen (San Diego, CA).

2.10. Antigen loading of DCs and cytokine assay

For experiments carried out in the presence of ovalbumin (OA), generated DCs were loaded for 6 and 24 h with 0, 10, 50, 100 µg/ml of OA. Supernatants of 1,000,000 generated DCs pulsed or not with OA were harvested 24 h following stimulation, centrifuged ($400 \times g$ for 7 min), and assayed for the presence of IL-10 and IL-12p70 by specific enzyme-linked immunosorbent assay (ELISA) using ELISA kits (R&D Systems). The sensitivity of detection of IL-10 and IL-12p70 was 4 and 2.5 pg/ml, respectively.

2.11. Statistical analysis

One-way analysis of variance (ANOVA) and Tukey test (as a post-hoc test) were used for comparing of groups. Our results were expressed as mean of OD450 (absorbance units) ± S.E.M. and mean of concentration (pg/ml) ± S.E.M. for IgE and cytokines, respectively.

Table 1

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

Group	IgE (A450)	IL-4 (pg/ml)	IL-10 (pg/ml)	IL-12p70 (pg/ml)	IFN- γ (pg/ml)
Control	0.003 $\pm$ 0.007	30.94 $\pm$ 2.39	74.24 $\pm$ 2.28	18.88 $\pm$ 4.06	142.80 $\pm$ 2.93
BP	0.003 $\pm$ 0.0017	35.66 $\pm$ 2.43	65.38 $\pm$ 4.17	27.28 $\pm$ 3.79	137.76 $\pm$ 3.27
MAE	0.025 $\pm$ 0.0097	25.98 $\pm$ 0.60	77.26 $\pm$ 5.02	14.36 $\pm$ 1.47	123.68 $\pm$ 5.33
MAE + BP	0.264 $\pm$ 0.167 ^a	211.08 $\pm$ 7.06 ^a	400.24 $\pm$ 16.41 ^a	116.36 $\pm$ 13.65 ^a	632.78 $\pm$ 80.46 ^a

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

3. Results

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

Means of relative anti-MAE IgE values detected in serum samples from mice at one sampling time (7 days) are shown in Table 1. The means of relative anti-MAE IgE values is significantly higher than all three other groups (MAE, BP and Buffer alone). Seven days after the last exposure, the highest relative levels of specific IgE were found in mice exposed to MAE + BP. As expected, no increased serum anti-MAE IgE was observed to MAE in any of the groups that were not exposed to MAE.

3.2. Effect of BP on secretion of cytokines

The type 1 cytokines IFN- γ and IL-12-p70 and the type 2 cytokines, IL-4 and IL-10 secreted by spleen cells from mice given MAE with BP were measured. As indicated in Table 1, 5.1 and 8.1 times greater secretion of IFN- γ and IL-12p70 was observed in animals treated with MAE plus 50 μ g of BP, compared with those given MAE plus PBS. On the other hand, MAE plus 50 μ g of BP increased IL-4 and IL-10 secretion up to 8.1 and 5.1 times, respectively.

3.3. Phenotype of bone marrow-derived dendritic cells

After 9 days of culture with rmGM-CSF and rmIL-4, generated cells were analyzed for the expression of DC markers, CD11c and MHC II. Results showed that the cells displayed a high expression of CD11c (>90%) and a low expression of MHC II (Fig. 1). This profile is specific for immature murine DCs.

3.4. Production of cytokines by ovalbumin-pulsed DCs

The mean concentration of cytokines, IL-10 and IL-12p70, obtained from four groups of study, i.e., control, BP, OA and OA plus BP for three different concentrations, 10, 50 and 100 μ g/ml after 6 and 24 h

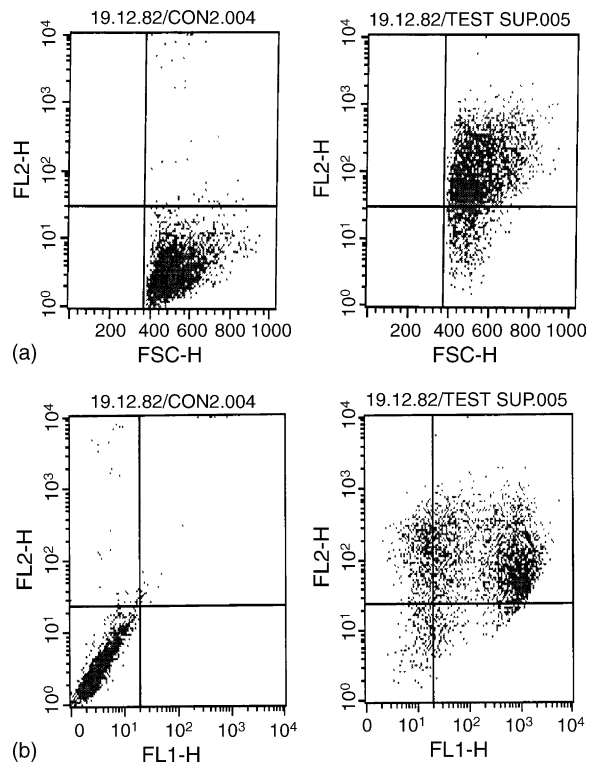


Fig. 1. The flowcytometric analysis of Balb/c mouse bone marrow-derived DCs. (a) At day 9 of in vitro DC culture: forward scatter (FSC-H) against CD11c positive cells indicates immature DCs. (b) At day 10th antigen-loaded non-adherent mature DCs were analyzed: mouse surface MHC class II molecules (I-A/I-E)(FL1-H) plotted against CD11c (FL2-H). The charts for the isotype controls are shown at the left side of the figure.

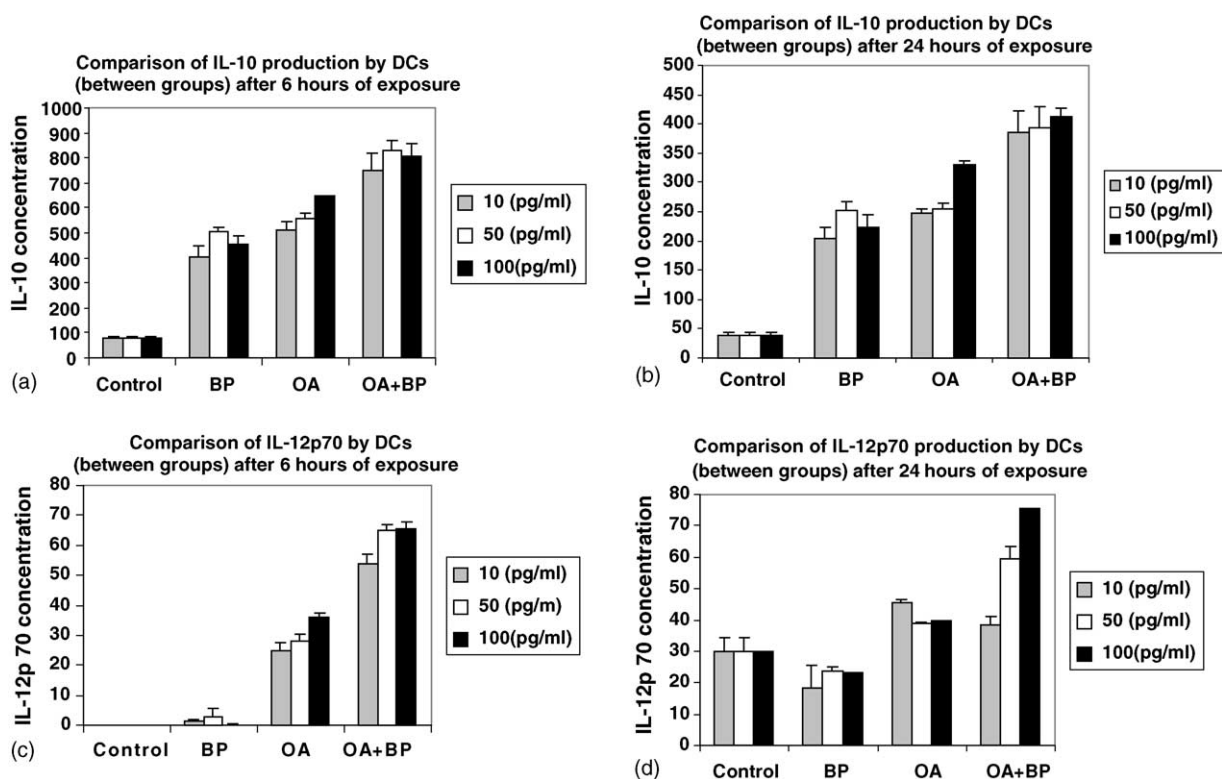


Fig. 2. The effects of in vitro exposure of BP together with ovalbumin with varying doses on cytokine secretion by mouse bone marrow-derived dendritic cells in terms of mean $\pm$ S.E.M. (a–d). In all cases, the amounts of the group OA + BP are significantly higher than those of other groups.

are indicated in Fig. 2. As indicated in Fig. 2, 1.8 and 1.2 times greater secretion of IL-12p70 and IL-10 was observed in cultures treated with 100 μ g/ml OA plus BP, compared with those treated with OA alone after 6 h of culture. Likewise, the amounts are 1.8 and 1.2 times greater than those of the above-mentioned cultures treated with OA alone after 24 h, for IL-12p70 and IL-10, respectively. The amount of both cytokines in cultures treated with OA plus BP were significantly greater than that of other treatments ($P < 0.05$).

4. Discussion

This study elucidates that with intranasal exposure there is an elevated response to the mite allergen extract in the groups that received MAE together with BP, compared with groups receiving MAE alone. The increased response is seen as both increased serum anti-MAE IgE

antibody and IL-4, IL-10, IL-12p70 and IFN- γ concentrations in splenocyte culture supernatants. However, there was no consistent difference in the concentration of serum anti-MAE IgE antibody (relative absorbance values) and cytokine concentrations between the animals responding in the MAE + BP group. The specific IgE response in the animals measured as absorbance units in the ELISA was relatively low. This may be due to low doses of both MAE and BP. However, despite the small doses, it was possible to differentiate between the responses in the different exposure groups. The model used in the present study may be relevant for allergic rhinitis ('hay fever') which is the most common disease due to pollen allergy. Allergic rhinitis is the result of inhalation of pollen to the upper respiratory tract. An important question is whether administration of dissolved MAE or BP suspended in buffer will mimic this type of exposure. Unpublished experiments revealed with two times 5 μ l trypan blue administered in the

nostrils of Balb/c mice showed uniform distribution of the blue color over the nasal cavity mucosa within 10 min. Takafuji et al. (1987) showed that substances administered in the nostrils were also distributed to the lungs in addition to the gastro-intestinal tract; the distribution to the lungs was not secondary to gastro-intestinal absorption. Thus, administration of solutes or suspended particles in the nostrils may produce an exposure of the same areas of the respiratory tract as inhalation of large particles as e.g. pollen and this route of exposure may be relevant for exposure to aeroallergens and air pollution.

The mechanisms of the cumulative effect of BP on the IgE response are not yet fully understood. Previous studies have shown that DEP, organics eluted from DEP, pyrene and phenanthrene may increase the IgE production in vivo and in vitro (Murakana et al., 1986; Fujimaki et al., 1997; Takenaka et al., 1995; Suzuki et al., 1993; Takafuji et al., 1987; Tsien et al., 1997). In addition, Lovik et al. (1997) demonstrated that DEP augmented the intensity and prolonged the duration of the local inflammation after allergen inoculation. It is, however, demonstrated that DEP after airway exposure may modulate the immune response by inducing inflammation or the release of pro-inflammatory mediators (Diaz-Sanchez et al., 1994; Kobayashi and Ito, 1995; Nikula et al., 1995). DEP challenges in humans were shown to increase the production of several cytokines mRNA in nasal lavage cells (Diaz-Sanchez et al., 1994, 1996) and intranasal and intratracheal instillation of DEP in mice showed an increased IL-4 production in cervical and mediastinal lymph node cells (Diaz-Sanchez et al., 1994; Fujimaki et al., 1995). However, the organics such as BP may exert an additive effect by mechanisms different from those suggested for the particles such as DEP. It is demonstrated that organic extracts of DEP and phenanthrene may increase IgE production by enhancing differentiation of B cells already committed to IgE synthesis and not by inducing class switching by IL-4 production (Takenaka et al., 1995; Tsien et al., 1997). Studies with benzo(a)pyrene and phenanthrene have led to focus on the PAH as responsible for the cumulative activity of DEP (Peterson and Saxon, 1996; Suzuki et al., 1993; Tsien et al., 1997). It is, however, important to emphasize that the PAH only constitute a small part of the soluble organic fraction of DEP (Westerholm et al., 1991). In conclusion, the present study has demonstrated that BP has an

additive effect on systemic IgE to MAE and cytokine secretion by spleen cell cultures after intranasal instillation which is considered relevant for human exposure. In summary, intranasal administration of MAE and BP induced both type 1 and type 2 cytokine responses. These results suggest that exposure to BP might play a role in induction of mucosal immunization which often leads to allergy or may be used in the arena of mucosal vaccination.

Very recently, Langenkamp et al. (2000) showed that monocyte-derived DCs stimulated with LPS, rapidly produce IL-12p70, leading to a Th1 response, and that after 24 h they lose their capacity to produce this pro-Th1 cytokine and acquire the capacity to favor a Th2 response associated with an increase in CD86:CD80 ratio. In our study upon loading of bone marrow-derived DCs with ovalbumin, IL-12p70 began to produce by DCs and increased by 24 h but this is reverse for another cytokine, i.e. IL-10.

In the present study we showed that the two cytokines IL-12p70 and IL-10 are elaborated under the influence of BP compared with control group, but its underlying mechanism is not clear. Interestingly the above-mentioned phenomenon is dose dependently. Furthermore, DCs loaded with ovalbumin, as a potential allergen, matured and produced greater amounts of the two cytokines than those of control group and more interestingly this one is dose dependently either. When the two substances, i.e., BP and ovalbumin, were combined, the cytokine secretion was significantly increased in a dose dependent manner in comparison with the control group.

Many cytokines are very efficient in focusing the immune response towards a Th1 or a Th2 profile. IL-10 is known to be a cytokine that inhibits type 1 responses, thus it is a type 2 cytokine (Romagnani, 1995). In contrast, IL-12p70 is an inducer of IFN- γ production by T lymphocytes and so might favor Th1 responses (Romagnani, 1995). Both IL-10 and IL-12p70 are produced by DCs (Lore et al., 1998), suggesting that DCs could have a key role in the establishment of an immune response. IL-10 is produced by DCs in response to different antigens such as bacteria (Probst et al., 1997) and their derived products. DCs cultured in the presence of recombinant IL-10 showed a decreased expression of CD86 responsible for an inhibitory effect on alloreactive reactions (Marriott et al., 1999; Cella et al., 1996). However, another study indicated that

DCs that have undergone maturation in the presence of IL-10 down-regulated their own production of IL-12 and were unable to induce the synthesis of IFN- γ by T cells. Finally, in IL-10 knockout mice, DCs rapidly and efficiently promote Th1 responses (Igietseme et al., 2000). These data support the idea that IL-10 is essential for mounting Th2 responses. IL-12 is constitutively produced by DCs (Macatonia et al., 1995) and is enhanced in response to a broad spectrum of stimuli such as microbial stimulation, parasites and CD40 ligation through CD40L on activated T cells. IL-12 production is inhibited by IL-4 and IL-10, or after exposure with agents inducing type 2 responses (Braun et al., 1999).

Taken together, these data suggest that DCs upon their exposure to the ovalbumin as a potential food allergen, load them and secrete cytokines which they in turn make a microenvironment. This microenvironment itself may induce type 1 and/or type 2 responses. Regarding the immediate IL-10 response and its peak during 6 h, it can probably trigger the secretion of IL-4 by antigen-specific T cells which in turn make their counterparts in B cell population to class switch towards IgE antibody and then we have specific IgE, but as it turned out this scenario does not take a long time and by 24 h IL-12p70 secreted from the original DCs alleviate the latter response, i.e. IL-10 secretion and down-regulation of the downstream IL-4, but instead augments the production and secretion of IFN- γ by T cells and therefore modulate the response. The more important finding was the additive effect of BP together with OA in producing the IL-10 from DCs in a dose-dependent manner.

Finally, we can conclude that DCs are immunoinitiators which begin, promote and modulate the immune responses but the other factors such as the nature and dose of the allergen, route of exposure and genetic basis of host, determine the fate of immune response, if it leads to a typical immune response, tolerance or allergy.

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