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Immunomodulatory effects of mono-, di-, and trimethylphenols in mice

Tetsuo Yamano *, Masako Ichihara, Mitsuru Shimizu, Tsutomu Noda, Yuji Tsujimoto

Osaka City Institute of Public Health and Environmental Sciences, 8-34 Tojo-cho, Tennoji-ku, Osaka 543-0026, Japan

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Abstract

The relationship of air pollutants with the increasing prevalence of allergic diseases is a matter of concern in developed countries. In this study, the immunomodulatory effects of mono-, di-, and trimethylphenols in mice were examined as regards two aspects. First, whether or not these chemicals act as sensitizers was evaluated by local lymph node assay. Of the 13 methylphenols tested, three dimethylphenol isomers (2,4-DMP, 2,5-DMP, and 3,4-DMP) were found to induce auricular lymphocyte proliferation after dermal application on both ears of mice. Cytokine production patterns in the supernatants of cultured auricular lymphocytes from mice showed these methylphenols to be contact sensitizers. Second, the effects of methylphenols on cytokine production profiles were examined using cultured splenocytes from immunologically naive mice. Under subtoxic conditions, eight methylphenols inhibited interferon-γ (IFN-γ) production significantly, while the effect on intreluekin-4 (IL-4) production was moderate, resulting in higher IL-4/IFN-γ ratios in all of the tested chemicals, with the most prominent effect shown by 2,6-DMP. These results suggest that several methylphenols, especially dimethylphenol isomers, have potencies that affect the immune system, being immunogens themselves or modulators of the Th1/Th2 cytokine balance.

Keywords: Murine local lymph-node assay; Lymphocytes; Splenocytes; Th1/Th2 balance; Immunogens; Immunomodulatory effects

1. Introduction

Various epidemiological and laboratory-based studies have suggested that air pollutants are relevant to the increasing prevalence of allergic diseases in developed countries (see review by Gershwin, 2003). Young children are among the most susceptible targets of allergic diseases such as atopic dermatitis, asthma, and food allergy (Kim, 2004). Since infants and toddlers spend much of the day in the home, indoor pollutants would seem to be of particular importance. The major constituents of indoor pollutants are volatile organic compounds (VOCs). VOCs are generally hydrophobic and have low boiling points; hence, once absorbed they are converted to hydrophilic metabolites mainly in the liver. The immunomodulatory effects of VOCs, if any, might be brought about directly and/or via their immunologically active metabolites.

Mono-, di-, and trimethylbenzenes are widely used as solvents, and are ubiquitous VOCs found in indoor ambient air (Tanaka-Kagawa et al., 2005). The first step in the metabolism of methylbenzenes is oxidation of either a methyl-substituent or aromatic-ring; the

* Corresponding author. Tel.: +81 6 6771 3186; fax: +81 6 6772 0676.
E-mail address: tetsuo.yamano@iphes.city.osaka.jp (T. Yamano).

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former finally leading to inert, conjugated metabolites excreted in urine, while the latter is thought to involve some biological activity (Acuña-Argüelles et al., 2003; Haeseler et al., 2005). Although which parts of methylbenzenes are oxidized via one of the two steps depends on the numbers and positions of methyl substituents, our previous study revealed that a substantial portion of incorporated trimethylbenzenes are ring oxidized to form phenolic metabolites, as compared to a smaller amount of methyl-substituted benzenes, i.e., toluene and xylene isomers (Tsujimoto et al., 2005). Besides production as metabolites of incorporated methylbenzenes, several methylphenols per se have been found as air pollutants originating mainly from cigarette smoke or forest fires (Smith et al., 2002; Ward et al., 2005). Although epidemiological studies have revealed positive correlations between allergic manifestations in children and domestic exposure to VOCs including methylphenols (Lehmann et al., 2001; Rolle-Kampczyk et al., 2002; Rumchev et al., 2004), experimental studies on the effects of each VOC or its metabolites on the immune system are lacking.

There exist at least two subsets of T helper (Th) cells: Th1 and Th2. These subsets are differentiated by their corresponding cytokine production profiles. Representative Th1 cytokines such as interferon-γ (IFN-γ) induce differentiation of immature Th into Th1 cells, while inhibiting the activity of Th2 cells. Th2 cytokines such as intreluekin-4 (IL-4), on the other hand, act in a manner reciprocal to that of Th1 cytokines. The balance between Th1 and Th2 differentiation is critically related to the status of allergic disorders. In this study, the effects of mono-, di-, and trimethylphenols on cytokine production profiles were evaluated using cultured mouse splenocytes. Whether these chemicals per se act as immediate or delayed type immunogens was also evaluated using the murine local lymph node assay (LLNA).

2. Materials and methods

2.1. Chemicals

Thirteen isomers of mono-, di-, and trimethylphenols were used in the study. 2-Methylphenol (2-MP), 3-methylphenol (3-MP), 4-methylphenol (4-MP), 2,4-dimethylphenol (2,4-DMP), 2,4,6-trimethylphenol (2,4,6-TMP), and 3,4,5-trimethylphenol (3,4,5-TMP) were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). 2,5-Dimethylphenol (2,5-DMP), 3,4-dimethylphenol (3,4-DMP), 2,3,5-trimethylphenol (2,3,5-TMP) and 2,3,6-trimethylphenol (2,3,6-TMP) were from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and 2,3-dimethylphenol (2,3-DMP), 2,6-dimethylphenol (2,6-DMP), and 3,5-dimethylphenol (3,5-DMP) from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). 2,4-Dinitrochlorobenzene (DNCB) was obtained from Katayama Chemical Inc. (Osaka, Japan), and trimellitic anhydride (TMA) from Nacalai Tesque Inc. (Kyoto, Japan). All other chemicals used were of superior grade.

2.2. Laboratory animals

Six- to eight-week-old male BALB/cA mice were obtained from CLEA Japan (Tokyo, Japan) and were used throughout the study.

2.3. Auricular lymphocyte sensitization

Groups of three mice were exposed to 25 μl of 1 M methylphenols, 0.3% DNCB, 3% TMA, or vehicle (acetone/olive oil, 4:1, AOO) through application to the dorsum of both ears for 3 consecutive days. Three or five days after the last application, a pair of auricular lymph nodes (LNs) from each mouse was excised for the LLNA and cell culture for cytokine analysis, respectively.

2.4. Non-radioactive murine LLNA

One day before isolation of LNs, 5-bromo-2′-deoxyuridine (BrdU, 150 mg/kg/15 ml saline) was administered intraperitoneally to each mouse. The next day, single-cell suspensions were prepared, and the enzyme-linked immunosorbent assay (ELISA) was used to measure amounts of BrdU incorporated in LNs from each mouse (Yamano et al., 2003). A stimulation index was calculated as the ratio of the BrdU amount in LNs of chemical-treated mice to that of vehicle-treated mice.

2.5. Lymphocyte culture

Five days after the last application of the chemicals, the auricular LNs were excised and suspended in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (standard medium). Single-cell suspensions were prepared by passing the LNs through a 70 μm nylon cell strainer. The cells were washed twice in standard medium (10 min, 400 × *g*, 4 °C) and resuspended in standard medium supplemented with 10% heat-inactivated fetal bovine serum (Wako Pure Chemical Industries). The total cell count in each suspension was measured using a CDA-500 automatic cell counter (Sysmex Corp., Kobe, Japan). Cell suspensions of 2×10^6 cells/ml in 200 μl culture medium were seeded in duplicate in 96-well plates with 5 μg/ml concanavalin A (ConA) used as a mitogen. The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 48 h. After incubation, the supernatants were obtained by centrifugation (400 × *g*, 5 min) for the analysis of IFN-γ and IL-4 concentrations.