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Allergic reaction induced by dermal and/or respiratory exposure to low-dose phenoxyacetic acid, organophosphorus, and carbamate pesticides

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ABSTRACT

Several types of pesticides, such as organophosphates, phenoxyacetic acid, and carbamate have a high risk of affecting human health, causing allergic rhinitis and bronchial asthma-like diseases. We used our long-term sensitization method and a local lymph node assay to examine the allergic reactions caused by several types of pesticides. BALB/c mice were topically sensitized (9 times in 3 weeks), then challenged dermally or intratracheally with 2,4-D, BRP, or furathiocarb. One day post-challenge, the mice were processed to obtain biologic materials for use in assays of total IgE levels in serum and bronchoalveolar lavage fluid (BALF); differential cell counts and chemokine levels in BALF; lymphocyte counts and surface antigen expression on B-cells within regional lymph nodes (LNs); and, ex situ cytokine production by cells from these LNs. 2,4-D-induced immune responses characteristic of immediate-type respiratory reactions, as evidenced by increased total IgE levels in both serum and BALF; an influx of eosinophils, neutrophils, and chemokines (MCP-1, eotaxin, and MIP-1β) in BALF; increased surface antigen expression on B-cells IgE and MHC class II production) in both auricular and the lung-associated LNs; and increased Th2 cytokine production (IL-4, IL-5, IL-10, and IL-13) in both auricular and the lung-associated LN cells. In contrast, BRP and furathiocarb treatment yielded, at most, non-significant increases in all respiratory allergic parameters. BRP and furathiocarb induced marked proliferation of MHC Class II-positive B-cells and Th1 cytokines (IL-2, TNF-α, and IFN-γ) in only auricular LN cells. These results suggest that 2,4-D is a respiratory allergen and BRP and furathiocarb are contact allergens. As our protocol detected classified allergic responses to low-molecular-weight chemicals, it thus may be useful for detecting environmental chemical-related allergy.

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1. Introduction

Several types of pesticides, including organophosphates, phenoxyacetic acid, and carbamate, are widely used throughout the world in agriculture and/or eradicating termites around homes (Ellenhorn and Barceloux, 1988; Lopez et al., 2007; Nakadai et al., 2006; OECD, 2008; Wessels et al., 2003). These pesticides are sold in large quantities in many countries and are applied at yearly rates of 0.21 tonnes (t)/km^2 in the USA, 1.06 t/km^2 in the Netherlands, 1.29 t/km^2 in Korea, and 1.5 t/km^2 – the highest rate – in Japan (OECD, 2008). Many studies have reported deleterious effects of these pesticides on the nervous, immune, urinary, and reproductive systems (Blaylock et al., 1992; Jones and Miller, 2008; Li et al., 2007; Lopez et al., 2007; Nakadai et al., 2006; Takeuchi et al., 2002). In particular, pesticides that have diffused into ambient air have a high risk of causing allergic rhinitis and bronchial asthma-like diseases in humans (Boers et al., 2008; Hernandez et al., 2004a,b, 2006, 2008; Hoppin et al., 2002, 2007, 2008; Proskocil et al., 2008; Stejskal and Hubert, 2008). Aerial spraying is a major source of exposure (Hoppin et al., 2007, 2008; Proskocil et al., 2008), and aerial application of pesticides has been restricted in Europe and the USA in recent years (European Commission, 2006b; US EPA, 2007). However, the ease of aerial spraying has still led to its wide use on many farm products in many countries (Hoppin et al., 2002, 2007, 2008; OECD, 2008; Proskocil et al., 2008). Therefore, there is a need for protocols for the treatment and detection of respiratory allergic diseases triggered by pesticides that have diffused into the environment.

Recently, several detection methods have been developed to identify chemicals that trigger allergic diseases (Arts et al., 2003; Ban et al., 2006). However, these methods have focused on the detection of strong allergic reactions, whereas environmental chemical allergens (such as diffused pesticides) tend to have weak or minimal immunogenicity. In addition, experimental allergic

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responses to allergens are dependent on many factors, including sensitizing route, application method, and animal species. Therefore, new protocols are needed to detect (as well as eventually treat) and treat respiratory allergies caused by weakly immunogenic and low-dose allergens. Previously, in our first stage of studies, we developed a new method of detecting environmental chemical-related dermal or respiratory allergic diseases. We used typical chemical sensitizers (2,4-dinitrochlorobenzene [DNCB], trimellitic anhydride [TMA], and toluene diisocyanate [TDI]) in a long-term dermal sensitization protocol followed by dermal or respiratory challenge (Fukuyama et al., 2008a,b). DNCB is a contact allergen, whereas the others (TMA and TDI) are respiratory allergens. Indeed, TMA-induced prominent increases in several parameters indicative of induced allergic responses, including IgE levels (antigen-specific IgE level and IgE-positive B-cell population), eosinophil proliferation, local (lung airway) chemokine levels (MCP-1, MIP-1β, and eotaxin) and cytokine levels (interleukin [IL]-4, -10, and -13). TDI induced significant increases in IgE-positive B-cell numbers and local (lung airway) cytokine production. In contrast, DNCB sensitization only yielded non-significant increases in each of these parameters. These results demonstrated that our method is able to detect and classify allergic reactions caused by chemicals present in the environment at weakly immunogenic and low doses.

In this second stage study, we examined the allergic reactions caused by several types of pesticides using our long-term sensitization method (Fukuyama et al., 2008a,b) in conjunction with a local lymph node assay (LLNA) (National Institute of Environmental Health Sciences, 1999; Basketter et al., 2002). The LLNA was developed initially for hazard identification (Basketter et al., 1999; Dearman et al., 1999) and has now been evaluated extensively and validated formally (Dearman et al., 1999; Gerberick et al., 2000; Basketter et al., 2002). The chemicals used in the study reported here were the phenoxyacetic acid pesticide 2,4-D, the organophosphorus pesticide BRP, and the carbamate pesticide furathiocarb. These pesticides were specifically selected for study in that: Cushman and Street (1982) demonstrated the ability of 2,4-D to elicit respiratory allergy-specific IgE antibodies in Balb/c mice (although Hoppin et al. (2002) showed that 2,4-D was not associated with wheezing.); and, a material data sheet stated that furathiocarb and BRP induced a positive response in a guinea pig skin sensitization study (ACGIH, 2002).

2. Materials and methods

2.1. Chemicals

Standard 2,4-D ($C_{12}C_6H_3OCH_2COOH$), BRP ($C_4H_7Br_2C_{12}O_4P$), and furathiocarb ($C_{18}H_{26}N_2O_5S$), acetone, and olive oil were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). For the dermal sensitization and challenge, each test chemical was dissolved in 4:1 acetone:olive oil (AOO). For the intratracheal challenge, each test chemical was dissolved in acetone and diluted in sterile physiological saline solution. The concentrations of 2,4-D, BRP, and furathiocarb used are presented in Table 1. These concentrations were selected to avoid systemic toxicity and/or excessive local sensitization (particularly in the preliminary test) while still permitting comparisons of the sensitizing potencies of the chemicals. In the LLNA we focused on determining the EC3 value (the concentration of a chemical required to elicit a threshold positive response), which was used to select suitable concentrations of 2,4-D, BRP, and furathiocarb for the main study. To confirm the allergenicity of 2,4-D, BRP, and furathiocarb, we adopted a sensitization dose that was less than the EC3 values and a challenge dose that was 10 or 100 times lower than the sensitization dose (see Table 1).

Table 1
Chemical concentrations used.

Chemical	Local lymph node assay (LLNA)	Main study	
		Sensitization	Challenge
2,4-D	0%/1%/3%/10%/30%	5%	Low: 0.05%, high: 0.5%
BRP	0%/0.1%/0.3%/1%/3%	0.3%	Low: 0.003%, high: 0.03%
Furathiocarb	0%/0.1%/0.3%/1%/3%	1%	Low: 0.01%, high: 0.1%

2.2. Animals

Female CBA/Jn mice (age, 7 weeks) (for the LLNA study) and female BALB/c mice (age, 7 weeks) (for the main study) were purchased from Charles River Japan Laboratories (Atsugi, Kanagawa, Japan) and housed individually under controlled lighting (lights on from 07:00 to 19:00 h), temperature (22 ± 3 °C), humidity (55 ± 15%), and ventilation (at least ten 100% fresh-air changes hourly). Food (Certified Pellet Diet MF, Oriental Yeast Co., Tokyo, Japan) and water were available ad libitum.

This study was conducted in accordance with the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science (Japanese Association for Laboratory Animal Science, 1987).

2.3. Local lymph node assay

We conducted an LLNA as a preliminary test to select the concentrations of 2,4-D, BRP, and furathiocarb for use in the main studies. The assay was performed as described by Kimber and Weisenberger (1989), with minor modifications. After a 1-week acclimatization period, CBA/Jn mice were allocated randomly to dose and control groups ($n = 5$ per group). A 25-μl aliquot of test solution or solvent only was applied daily to the dorsum of each ear of each mouse for 3 consecutive days (days 1–3). On day 6, ^{3}H-methyl thymidine (^{3}H-TdR, 20 μCi/animal; GE Healthcare Bioscience, Tokyo, Japan) was injected via the tail vein into all test and control mice; 5 h after injection, the mice were euthanized, and the auricular LNs on both sides of each mouse were removed, weighed, and pooled by mouse in phosphate-buffered saline (PBS, Gibco, Tokyo, Japan). Single-cell suspensions of LNs in 5 ml PBS were prepared by passage through sterile 70-μm nylon cell strainers (Falcon, Tokyo, Japan). The LN cell suspension was washed twice with an excess of PBS, and the cell pellet was incubated in 3 ml 5% trichloroacetic acid (TCA, Wako Pure Chemical Industries, Ltd.) at 4 °C for approximately 18 h. Each cell pellet was resuspended in 1 ml TCA and transferred to 9 ml of scintillation fluid (AtomLight, PerkinElmer Japan, Tokyo, Japan). Incorporation of ^{3}H-TdR was measured with a β-scintillation counter (LC-5100, Aloka, Tokyo, Japan) as disintegrations per minute (DPM) for each mouse.

Stimulation indexes (SIs) and EC3 values were calculated from the ^{3}H-TdR incorporation data. The SI was calculated by dividing the mean ^{3}H-TdR incorporation value for each treatment group by that of the solvent control group. The EC3 value is an estimate of the amount of test solution required to induce an SI of 3 (Basketter et al., 1999). In the standard LLNA, the criterion for a positive response is an SI of 3 or greater (Dearman et al., 1999).

2.4. Experimental design (main study: long-term sensitization)

For the main study, two different protocols (dermal challenge and respiratory challenge) were used. In both the dermal and respiratory challenge protocols, the BALB/c mice were divided into five groups for each chemical (2,4-D, BRP, and furathiocarb): Group−/− (both sensitized and challenged with solvent only), Group−/+ (sensitized with solvent only and challenged with high-dose test solution), Group+/− (sensitized with test solution and challenged with solvent only), Group+/+^{low} (sensitized with test solution and challenged with low-dose test solution), and Group+/+^{high} (sensitized with test solution and challenged with high-dose test solution). In this study, we define −/−, −/+ and +/− groups as the control groups.

After a 1-week acclimatization period, mice were allocated randomly to dose and control groups ($n = 6$ or 7 per group). In both the dermal and respiratory challenge protocols, a 25-μl aliquot of the test solution or solvent alone was applied to the dorsum of each ear of each mouse on days 1–3, 8–10, and 15–17 for dermal sensitization. In the dermal challenge protocol, 2 weeks after the last sensitization (day 31), a 25-μl aliquot of test solution or solvent only was applied as previously for the challenge. In the respiratory challenge protocol, 2 weeks after the last sensitization (day 31), mice were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg), and then challenged with a 50-μl aliquot of test solution or solvent alone injected intratracheally via a 29 G needle. On the day after challenge (day 32), all mice were anesthetized with pentobarbital sodium (75 mg/kg) and sacrificed. Blood samples were taken from the inferior vena cava, and serum samples were assayed for total IgE. Each animal's auricular (dermal challenge protocol) or lung-associated (respiratory challenge protocol) LNs were removed and pooled by mouse in RPMI 1640 medium (Gibco, Tokyo, Japan). In the respiratory challenge protocol, bronchoalveolar lavage fluid (BALF) was collected by cannulating the trachea and lavaging the lungs three times with 1 ml phosphate-buffered saline (PBS, Gibco, Tokyo, Japan) supplemented with 1% heat-inactivated fetal calf serum (FCS, Gibco) warmed to 37 °C. The first BALF fraction from each animal was then centrifuged at 350 × *g* for 5 min, and then all the supernatants from all mice in a group were pooled. The supernatant was assayed for total IgE, chemokine (eotaxin, MCP-1, MIP-1β, and RANTES), and cytokine (IL-6 and TNF-α) levels. The cell pellets of the first fraction and the other two fractions were pooled and centrifuged at 350 × *g* for 5 min. The supernatant was removed, and the cell pellet was used for cell counts and differentials.

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