

Relationship between IgG1 levels and airway responses in guinea pigs actively and passively sensitized to hexahydrophthalic anhydride

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Organic acid anhydrides (OAAs) are industrial chemicals that may cause induction of specific IgE and airway symptoms in exposed workers. They are a good model for studies of relationships between chemical structure and the sensitizing potential of reactive low-molecular-weight compounds. Hexahydrophthalic anhydride (HHPA) is such a compound. This study aimed to evaluate the relationship between specific IgG1 levels and airway responses in a model to predict the sensitizing potential of OAAs. Guinea pigs were either actively or passively sensitized to HHPA. For active sensitization, guinea pigs were injected i.d. with 0.1 ml of olive oil (vehicle) or 0.05, 0.5, or 5% HHPA in olive oil. Passive sensitization was performed by i.p. injection of different volumes of antisera (0.75–6 ml, either unheated to keep IgE or heated to destroy IgE) taken from HHPA-sensitized guinea pigs. Specific antibody levels were evaluated with ELISA and passive cutaneous anaphylaxis. Animals were challenged 16–18 days after active sensitization, or 2 days after passive sensitization, by intratracheal instillation with HHPA conjugated to guinea pig serum albumin (HHPA-GPSA; 0.05% in saline), and the immediate effects on lung resistance (R_L), and plasma extravasation, measured as Evans blue dye extravasation, for up to 6 min were recorded. Active sensitization caused production of specific IgG1. Provocation with HHPA-GPSA caused an increase of both R_L and Evans blue dye extravasation, which was dependent upon the active sensitization dose. Challenge with HHPA-GPSA in passively sensitized guinea pigs also produced an increase in both R_L and Evans blue dye extravasation which was related to the IgG1 level. In the guinea pig model of HHPA-induced airway allergy, the airway responses are closely related to the serum levels of specific IgG1. Thus, the IgG1 levels induced by the immunization may reflect the sensitizing potential of HHPA.

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Organic acid anhydrides (OAAs) are reactive chemicals, widely used in industry as curing agents for epoxy resins, and they may cause occupational asthma in exposed workers (1). Recently, several animal models of respiratory tract allergy to OAAs have been developed (2–5). Important information on pathophysiology and exposure–response relationships may be obtained from such models (6–8). Data on the immunizing and sensitizing

properties of industrial chemicals may also be obtained (8).

The induction and monitoring of specific antibodies has been proposed as a sensitive indicator of respiratory sensitization (9). In a previous study, a guinea pig (GP) model was used to evaluate the sensitizing potential of OAAs (10). Thus, different levels of specific IgG1 responses analyzed by ELISA were found after intradermal immunization

of GPs with 13 different OAAs. The specific IgE titers analyzed by passive cutaneous anaphylaxis (PCA) were low or undetectable. However, the relationship between induced specific IgG1 titers and airway hypersensitivity has not been determined.

The airway response after allergen challenge in sensitized GPs mimics the immediate airway response in allergic asthma. This makes the GP an interesting animal for studies of allergic airway diseases and for the development of models for predicting the sensitizing potential of chemicals. In man, it is clear that IgE is the most important anaphylactic antibody, whereas IgG1 has been thought to play a similar role in the GP (11–13).

Hexahydrophthalic anhydride (HHPA) is a commonly used OAA in industry. It is a potent sensitizing compound, even at very low exposure levels, inducing specific IgE in 25–40% of exposed workers (14, 15). Thus, it is the most IgE-sensitizing OAA in man known.

This investigation aimed to evaluate the relationship between IgG1 titers and airway responses in a model to predict the sensitizing potential of low-molecular-weight organic chemicals.

Material and methods

Animals

Male, inbred, albino, Hartley strain GPs weighing 300–500 g (Sahlins AB, Malmö, Sweden) were used for the studies. All animals were acclimatized to the animal facilities for 1 week before use. The GPs were maintained in the facilities with free access to food and a cycle of 12 h of light and 12 h of darkness.

Chemicals and conjugate

HHPA (>98%) was obtained from Merck (Darmstadt, Germany), guinea pig serum albumin (GPSA) (fraction V) from Sigma Chemical Company (Stockholm, Sweden), olive oil from Apoteks-bolaget (Lund, Sweden), Evans blue dye from Janssen Chimica (Geel, Belgium), ketamine hydrochloride from Parke-Davis, SA (Barcelona, Spain), xylazine chloride from Bayer Sverige AB (Gothenburg, Sweden), and suxamethonium chloride from Kabi Vitum AB (Stockholm, Sweden).

HHPA-GPSA conjugate was produced by dissolving 600 mg GPSA in 10 ml of 0.1 M NaHCO₃, and then adding 0.1 M NaHCO₃ to 200 ml. Solid HHPA (154 mg) was added to the stirred and chilled (4°C) GPSA solution, and the reaction was allowed to proceed overnight. The conjugate was

purified from low-molecular-weight compounds (<30 kDa) by filtration in an Amicon Ultra-filtration Cell (Model 8400, Amicon Division, W. R. Grace & Co., Danvers, MA, USA) and stored at –20°C after lyophilization. The hapten density of the conjugate was 24 mol/mol as analyzed by the TNBS method, and 16 mol/mol as determined by analysis of the formed hexahydrophthalic acid after acidic hydrolysis of the conjugate (10).

Antibody determinations

Specific IgG1 antibodies to HHPA-GPSA were analyzed by the enzyme-linked immunosorbent assay (ELISA). Wells of polystyrene microtiter plates (Maxi Sorp, Nunc, Roskilde, Denmark) were coated by adding 100 µl/well of a solution of HHPA-GPSA (150 µg/ml; 0.005 M PBS, pH 7.2) overnight at 4°C. The wells were washed six times (Titertek Microplate Washer, Flow Laboratories, Rickmansworth, UK) with a washing solution (0.1% Tween-20 in PBS) and postcoated with 200 µl/well of 4% bovine serum albumin (BSA) to block uncoated protein binding sites in the wells for 4 h at room temperature and overnight at 4°C. The plates were washed six times with the washing solution, and then 100 µl/well of the GP sera (1:50 or 1:200) was added and incubated for 1 h at room temperature. After washing six times to remove unbound antibody, 100 µl/well of 1/500 diluted (in PBS) rabbit anti-GP IgG1 (ICN Biomedicals, Inc, Costa Mesa, CA, USA) was added and incubated for 1 h at room temperature. Excess antibody was removed by washing six times and 100 µl/well of alkaline phosphatase-conjugated goat anti-rabbit IgG (H+L; ICN Biomedicals, Inc, Costa Mesa, CA, USA) was added. A volume of 100 µl (1 mg/ml in diethanolamine buffer) of substrate (*p*-nitrophenyl phosphate, Sigma Chemical Company, Stockholm Division, Sweden) was then added to each well after washing six times. The wells were read after 120 min at room temperature in a filter photometer (Titertek Multiskan LUS, Labsystems and Flow Laboratories, Finland) at 405 nm. All samples were analyzed in triplicate in HHPA-GPSA-coated wells and read against GPSA-coated wells for the nonspecific binding. The results were expressed as the absorbance corrected for the absorbance of positive controls on each microtiter plate (optical density; OD). Control sera were from eight normal GPs.

Passive cutaneous anaphylaxis (PCA)

IgE. Different dilutions of sera (0.1 ml) from HHPA-sensitized animals were injected intrader-

mally on the shaved backs of naive GPs. After 8 days, 1 ml of 0.15 M NaCl containing HHPA-GPSA (2 mg/ml) and Evans blue dye (10 mg/ml) was injected intracardially. The cutaneous responses were observed on the outer surface of the skin 30 min after the injection. A result was considered positive when the blue spot was ≥ 5 mm in diameter in at least two of four recipients tested (16).

Preparation of antisera

Twenty-one GPs were immunized with either 0.05, 0.5, or 5% HHPA in olive oil (w/v; $n=7$ for each dose) on days 0, 10, 20, and 30. The volume for each GP was 0.1 ml, which was injected intradermally in two sites on the back. Blood was collected by cardiopuncture, and sera were prepared on day 40. The sera were pooled and kept frozen until the passive transfer tests with recipient GPs. Before passive transfer, the antisera were divided into two volumes; one volume was unheated and another volume was heated at 56°C for 6 h, a procedure which destroys IgE completely (17). At a dilution of 1 : 800, the unheated antisera had an IgG1 OD for HHPA-GPSA of 1.2, and heated antisera had an OD of 1.1. Thus, IgG1 was also reduced slightly during the heat-treated procedure. In the evaluation of specific IgE by PCA, unheated antisera showed a titer of 1 : 16, and heated antisera was negative.

Active sensitization

For evaluation of the effects of active sensitization on the development of antibodies and airway responses induced by allergen challenge, the animals were separated into four groups, immunized with either olive oil only (control, $n=6$) or free HHPA suspended in olive oil at three different doses (0.05, 0.5, and 5%, w/v; with six, eight, and eight animals, respectively). The volume for each GP was 0.1 ml, which was injected intradermally in two sites on the back. Sera for antibody measurements were collected 13 days after injection of HHPA, and allergen challenge was performed 16–18 days after the injection.

Passive sensitization

The animals were separated into eight different groups and were given 0.75, 1.5, and 3 ml/kg (body weight) of unheated antisera intraperitoneally ($n=9$, 15, and 23) and 0.75, 1.5, 3, 4, and 6 ml/kg of heat-treated antisera ($n=9$, 15, 15, 8, and 8). The control group ($n=8$) was not given any serum. The specific IgG1 levels in the sera of animals given

heated antisera were reduced by approximately 10–25%, compared with the IgG1 levels in the sera of animals given unheated antisera.

Airway provocation

Airway provocation was performed 16–18 days after active immunization, or 2 days after passive immunization. The animals were anesthetized with 1 ml/kg i.m. of a mixture of ketamine (50 mg/ml) and xylazine (20 mg/ml), prepared in a ratio of 3 : 2. The provocation was performed by established procedures (2). In brief, the animals were placed on an electronically controlled, heated blanket (Harvard Apparatus Ltd, Edenbridge, UK) to keep the body temperature at 37°C. The right external jugular was cannulated. A polyethylene catheter was inserted into the left carotid to monitor blood pressure with a pressure transducer (model P23XL, Viggo-Spectramed, Helsingborg, Sweden). A tracheal cannula (of 10 mm length and 2.7 mm inner diameter) was inserted into the lumen of the cervical trachea through a tracheostomy, secured with a suture, and connected to a constant volume mechanical ventilator (Harvard model 50-1718, Harvard Apparatus Ltd, Edenbridge, UK). Suxamethonium chloride was administered i.v. at a dose of 10 mg/kg to stop spontaneous ventilation. The tidal volume was 10 ml/kg and the frequency was 60 breaths/min. The ventilator circuit had a total volume of 18 ml. Airflow was measured with a pneumotachograph (Model F1L; Mercury Electronics Ltd, Glasgow, UK) connected to a gas pressure transducer (Model FCO44; ± 20 mmH₂O; Furness Controls Ltd, Bexhill, UK). Transpulmonary pressure was measured with a pressure transducer (Model FCO44; ± 1000 mmH₂O; Furness Controls Ltd, Bexhill, UK), with one side attached to a catheter inserted into the right pleural cavity and the other to a side port of the intratracheal cannula. The signal from the blood-pressure transducer was amplified with an analog pre-amplifier (Kungsbacka Mät & Reglerteknik AB, Kungsbacka, Sweden). All signals were digitalized with a 12-bit analog digital board (National Instruments, Austin, TX, USA) connected to a Macintosh II computer (Apple Computer, Inc., Cupertino, CA, USA) and analyzed with specialized software (LabView, National Instruments), which was programmed to calculate R_L instantaneously by the method of Neergaard & Wirz (18, 19).

The preparation was stabilized for 10 min. Evans blue dye was slowly injected intravenously over 1 min; after another 1 min, the GPs were challenged with 0.05 ml of 0.05% (in 0.15 M NaCl) conjugate between HHPA and GP serum albumin (HHPA-GPSA). The challenge was performed by

Table 1. Serum specific IgG1 (optical density, dilution 1:50), after airway challenge peak lung resistance (R_L , cmH₂O/ml/s), and Evans blue dye extravasation (ng/mg dry tissue) in proximal and distal intrapulmonary airway (PIPA and DIPA) tissues in guinea pigs actively sensitized i.d. with 0.1 ml of different concentrations of hexahydrophthalic anhydride (HHPA). Data are expressed as median and range. * $P < 0.05$, compared to group sensitized with lower concentrations of HHPA

Sensitization (% HHPA)	n	IgG1	Peak R_L	Evans blue dye	
				PIPA	DIPA
0	6	0.031 (0.029–0.043)	0.87 (0.72–0.93)	33 (28–46)	58 (42–79)
0.05	6	0.056 (0.017–0.440)	1.15* (1.03–1.60)	73* (56–102)	71 (50–80)
0.5	8	1.250* (1.109–1.362)	5.45* (1.99–8.96)	122* (84–163)	129* (101–212)
5	8	1.457* (1.365–1.558)	6.22 (1.71–11.53)	132 (72–417)	149 (96–401)

disconnecting the tracheal cannula from the ventilatory circuit, and the instillation was given directly and rapidly into the trachea through the tracheal cannula with a 1-ml syringe. The values of R_L and systematic blood pressure were monitored after 15, 30, 45, and 60 s, and subsequently every 30 s up to 6 min after challenge. Six minutes after the instillation of HHPA-GPSA, the animals were disconnected from the ventilator, and the thoracic cavity was opened. A catheter was inserted into the aorta through the left ventricle, and the animals were perfused with 50 ml of 0.15 M NaCl to remove the dye from the bronchial circulation. After perfusion through this route, another catheter was inserted into the pulmonary artery, and the pulmonary circulation was then perfused with 20 ml of saline to eliminate the dye in the pulmonary circulation.

The trachea and the lungs were dissected out *en bloc*, and the lung parenchyma was carefully scraped off and the extraneous tissue removed. The trachea, main bronchi, and intrapulmonary airways (IPA) were separated from each other, and the IPA were then divided into two lengthwise equal portions, arbitrarily designated the proximal IPA and the distal IPA. All airway tissues were then dried with a freeze-dryer (MicroModulyo, Edwards High Vacuum International, West Sussex, UK) for 24 h. The dry tissue samples were then weighed, and the Evans blue dye was extracted in 2 ml of formamide in a 40°C water bath for 24 h. Absorbance at 620 nm was measured in a spectrophotometer (Beckman DB, Ingeniörsfirma Hugo Tillquist, Stockholm, Sweden). The extracted Evans blue dye was quantified by interpolation on a standard curve of dye concentrations in the range of 0–20 µg/ml (linear relationship), and expressed as ng/mg dry tissue.

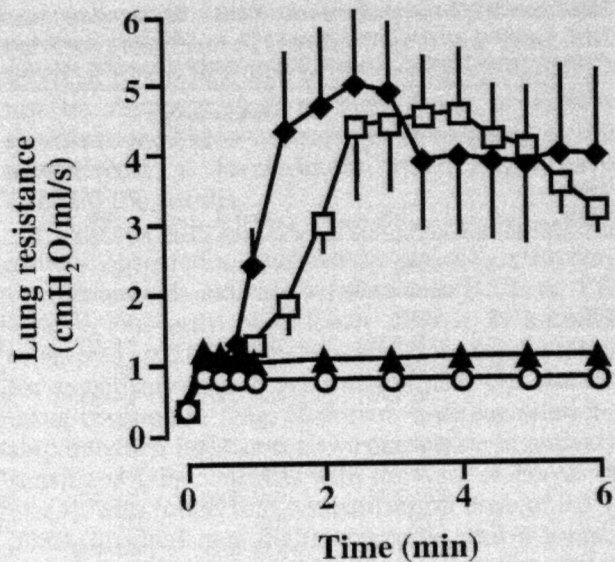


Fig. 1. Time course of lung resistance after airway instillation of conjugate between hexahydrophthalic anhydride (HHPA) and guinea pig serum albumin (HHPA-GPSA) in animals immunized i.d. with different doses of free HHPA (0, 0.05, 0.5, or 5% in oil). Data are shown as mean $\pm$ SEM.

Statistical analysis

The Mann–Whitney *U*-test (two-sample case) and the Kruskal–Wallis test (more than two-sample case) were used for comparisons between groups. A *P* value < 0.05 was considered statistically significant.

Results

Active sensitization

Active sensitization with different doses of HHPA in olive oil showed a dose-related increase in the OD readings of specific IgG1 antibodies to HHPA-GPSA, compared to the unsensitized control group (Table 1). PCA evaluation of IgE was negative in these three actively sensitized groups. At 16–18 days after injection with either 0.5% or 5% HHPA, a challenge with HHPA-GPSA resulted in an immediate increase in R_L , which persisted up to 6 min after challenge (Fig. 1, Table 1). In the same animals, we found significant extravasation of Evans blue dye at all airway levels (Table 1, data of trachea and main bronchi not shown). Allergen challenge in animals sensitized to 0.05% HHPA in oil resulted in a very weak, but statistically significant increase in R_L , and a significant increase of Evans blue dye extravasation in trachea, main bronchi, and proximal IPA (Table 1). The extravasation in trachea and main bronchi was not significantly different in the three sensitized groups.

Table 2. Specific IgG1 levels (optical density, dilution 1:50), after airway challenge peak lung resistance (R_L , cmH₂O/ml/s), and Evans blue dye extravasation (ng/mg dry tissue) in proximal and distal intrapulmonary (PIPA and DIPa) tissue in guinea pigs passively sensitized i.p. with different volumes of unheated or heated (IgE-destroyed) antisera to hexahydrophthalic anhydride. Data are expressed as median and range. * $P < 0.05$, compared to above group given lower volume of antisera

Volume (ml/kg)	Heated antisera					Unheated antisera				
	n	IgG1	Peak R_L	Evans blue		n	IgG1	Peak R_L	Evans blue	
				PIPA	DIPA				PIPA	DIPA
0	8	0.0065 (0.001–0.026)	1.29 (0.97–1.84)	55 (50–101)	60 (20–127)	8	0.0065 (0.001–0.026)	1.29 (0.97–1.84)	55 (50–101)	60 (20–127)
0.75	9	0.49* (0.48–0.55)	1.12 (0.79–1.94)	67 (21–82)	79 (43–114)	9	0.63* (0.56–0.73)	0.98 (0.56–50.0)	77* (65–230)	87* (51–157)
1.5	15	0.70* (0.55–0.75)	1.36 (0.34–2.27)	46 (24–90)	55 (33–84)	15	0.80* (0.67–0.89)	1.24 (0.61–50.0)	70 (31–213)	73 (24–177)
3	15	0.80* (0.64–1.02)	1.86* (0.80–20.74)	66* (39–128)	76 (39–113)	23	0.90* (0.78–1.19)	10.74* (1.15–50.0)	109 (26–383)	102* (34–224)
4	8	0.99* (0.82–1.05)	30.44* (1.41–50.0)	156 (39–397)	135 (35–309)					
6	8	1.25* (1.07–1.43)	31.01 (1.70–50.0)	120 (58–470)	116 (61–225)					

Passive sensitization

Injection of increasing volumes of unheated (IgE-positive) antisera resulted in increasing levels of IgG1 in the recipient sera, compared to the naive control animals (Table 2). Challenge with HHPA-GPSA resulted in an increase in R_L , which was stronger in animals given a higher volume of antisera than in those given a lower volume ($P < 0.01$). In the same animals, we also found significant extravasation of Evans blue dye in proximal IPA, which again was more pronounced in animals given higher volumes of antisera ($P < 0.01$). The levels of Evans blue dye in distal IPA were close to those in proximal IPA (Table 2).

The same pattern was observed in GPs after injection of heat-treated antisera (IgE destroyed). Increasing volumes of antisera resulted in a statistically significant increase in IgG1, peak R_L , and Evans blue dye extravasation in proximal IPA ($P < 0.01$). The levels of Evans blue dye in distal IPA were close to those in proximal IPA. A plateau level was reached after injection of 4 ml/kg (Table 2).

When antisera is heated, not only is IgE destroyed but also the IgG1 titer is reduced. Consequently, the IgG1 levels were lower in animals given heated antisera than in animals given the same volume of unheated antisera (Table 2). There was a tendency for injection of unheated antisera to result in higher peak R_L and Evans blue dye extravasation after challenge than in animals given the same volume of heated antisera. However, in groups of animals with corresponding IgG1 levels (no statistical differences between the groups) after injection of different volumes of heated and

unheated antisera (1.5 vs 0.75 ml, 3 vs 1.5 ml, and 4 vs 3 ml/kg), respectively, no significant differences in peak R_L after challenge were found (Table 2, Fig. 2). For the Evans blue dye extravasation, the pattern was the same except for the groups given 1.5 ml/kg heated vs 0.75 ml/kg unheated antisera.

Discussion

These studies show that active sensitization of GPs to HHPA produces a pronounced increase of specific IgG1 antibodies. Airway challenge of animals actively as well as passively sensitized to HHPA-GPSA caused severe airflow obstruction and concomitant plasma extravasation into the airway wall. The levels of IgG1 antibodies in the recipient sera were related to the increases of immediate-onset airway responses to allergen challenge. Destroying IgE by heating the antisera did not affect these responses.

Intradermal injection of free anhydrides is a simple and efficient way to produce specific antibodies in GPs (2–4, 10). In the present study, the specific IgG1 increased in a dose-dependent way over a dose range of 0.05–5% HHPA. This is in accordance with earlier findings for HHPA (Zhang & Welinder, unpublished data), trimellitic anhydride (TMA) (2, 4), and phthalic anhydride (PA) (5). Previous experiments have also shown a close relationship between IgG1 and total specific IgG (Zhang & Welinder, unpublished data). In the sera of GPs actively immunized with a single dose of HHPA, we could not find specific IgE antibodies by the PCA test. In contrast, specific IgE was found in the sera of GPs immunized with booster doses of HHPA, suggesting that repeated exposures to

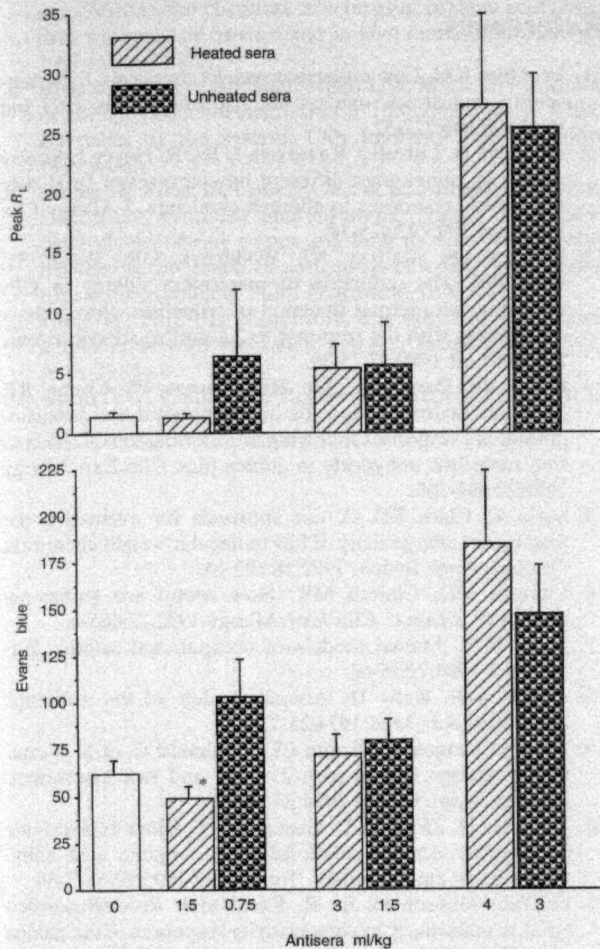


Fig. 2. Peak lung resistance (R_L ; cmH₂O/ml/s) (upper panel) and airway microvascular extravasation of Evans blue dye (lower panel) in proximal intrapulmonary airways after antigen challenge of guinea pigs passively sensitized i.p. with different volumes of heated (IgE-destroyed) and unheated antisera to hexahydrophthalic anhydride. Paired groups (heated vs unheated) showed statistically parallel levels of serum specific IgG1. * $P < 0.05$.

HHPA are required to produce this antibody in the GP. The IgG1 induced by immunization with HHPA is hapten specific, as proven by ELISA inhibition (20). This was also demonstrated by a low airway response in GPs actively sensitized to HHPA and challenged with a conjugate of succinic anhydride and GPSA (20).

Airway responses at challenge were evaluated by the recording of peak R_L and extravasation of Evans blue dye. The airway responses peaked in 2–4 min with a slight variation from animal to animal. The animals were ventilated with a constant-volume ventilator, leading to an increased airway intraluminal pressure when the airflow obstruction was increased. High-pressure ventilation may increase the pressure of the airway wall

and reduce the local blood flow and thus reduce the extravasation of Evans blue dye. Thus, the levels of Evans blue dye in the central airways may not be representative at high doses of HHPA sensitization. There was no sign of bleeding of the parenchyma, a development which might have affected the results.

Challenge with HHPA-GPSA gave rise to severe airflow obstruction and airway plasma extravasation in animals actively sensitized to 0.5% or 5% HHPA, but only to a lesser degree in animals sensitized to 0.05% HHPA. This dose-relationship for sensitization is in accordance with GP studies using ovalbumin (21). However, a direct relationship between IgG1 and airway response in actively sensitized GPs contrasts with the data of Hayes et al. (4), who found that a sensitization dose of 0.3% TMA resulted in a higher response and a longer duration of pulmonary inflation pressure after intratracheal allergen exposure, compared with a sensitization dose of 30% TMA, even though the higher dose produced higher titers of IgG1 antibodies. The explanation of this discrepancy is not obvious. Arakawa et al. (2) followed specific IgG1, R_L , and Evans blue dye extravasation in groups of GPs for up to 8 weeks after active immunization with TMA. IgG1 and Evans blue dye extravasation increased with time, while R_L peaked after 2–3 weeks. Thus, in this study, IgG1 levels and change in lung resistance were compared in animals of different age and body weight.

Severe airflow obstruction and concomitant plasma extravasation were also observed after challenge of passively sensitized animals. Inactivation of IgE antibodies by heating did not eliminate the response. Moreover, if we compare animals given unheated or heated sera, the airway responses are similar when the serum IgG1 levels are similar. Thus, it seems likely that IgG1 is an important antibody for the immediate-onset airway response in this animal model of anhydride allergy, as has been found in this species previously (11, 22).

The major mechanism inducing airflow obstruction in the airways is contraction of the bronchial smooth muscle. However, swelling of the airway wall, which may be induced by plasma extravasation, may cause airflow obstruction to a minor degree and may potentiate the reduction in inner airway diameter induced by a mild bronchial smooth-muscle contraction (23). Therefore, it is of interest to evaluate both changes in airflow obstruction and plasma extravasation in the same reactions provoked by allergen. It is likely that the major mechanism inducing airflow obstruction in the present experiments is contraction of the bronchial smooth muscle, because, in a similar experimental model, we found that provocation with

platelet-activating factor (PAF) produced much more pronounced Evans blue dye extravasation, but only small changes in lung resistance (24, 25). Moreover, we can conclude that the allergen challenge in HHPA-sensitized GPs causes not only airflow obstruction, but also plasma extravasation, further indicating that the model mimics asthma in man (26).

In a previous work, specific IgG1 levels after immunization of GPs were recorded for different OAAs, and reasonable agreement was obtained with findings in exposed workers (10). The relationship between specific IgG1 levels and airway responses at challenge demonstrated in the present work may indicate that the IgG1 levels induced by immunization reflect the sensitizing potential of the OAAs. Thus, a very weak increase in lung resistance was obtained in GPs sensitized to succinic anhydride (SA) and challenged with an SA-GPSA conjugate, a result which agrees with the low IgG1 titers obtained after immunization (Zhang et al. unpublished). GPs with high IgG1 levels after immunization (methyltetrahydrophthalic anhydride) accordingly showed severe airflow obstructions (27). Methyltetrahydrophthalic anhydride induces allergic rhinitis and asthma in man at low exposure levels (28), while such data have not been reported for SA.

The induction and monitoring of specific antibodies as a simple and sensitive indicator of respiratory sensitization was suggested by the ECETOC Task Force on Respiratory Allergy (9). This suggestion is supported by the present findings. However, more data are needed before firm conclusions can be drawn.

The present experiments show that allergen challenge in HHPA-sensitized GPs results in both airflow obstruction and plasma extravasation, and that these responses are related to the serum levels of specific IgG1. Overall, the GP model of HHPA-induced airway allergy mimics several features of anhydride-induced acute asthma. Thus, immunization of GPs to different OAAs and analysis of the induced antibody levels may, after further validation, prove to be a valuable approach for studies of allergenicity and structure-activity relationships. The model may also be used to predict the development of allergies in an occupational environment where the use of structurally new anhydrides is initiated.

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