

# Public Facility Workers' Immunological Characteristics Involved with Development of Respiratory Allergic Diseases in Korea

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**Abstract:** We evaluated the immuno-pulmonary status of employees working at public facilities to determine whether they are at greater risk of developing respiratory allergies. Fifty-two employees from child daycare centers, elderly nursing homes, subway stations, and hypermarkets, and 17 office workers were recruited. All were subjected to a skin prick test (SPT) for 25 aeroallergens and the methacholine bronchial challenge test. Various immunological parameters, including plasma IgE and IgG4 levels, hematology parameters, and *in vitro* cytokine production from peripheral T cells, were assessed. Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) were also determined. Of the facility employees, 54% responded to the SPT, and house dust mite induced positive skin reactions most frequently. Compared to the SPT-negative facility employees and the office workers, the SPT-positive facility employees had upregulated plasma IgE levels and eosinophil frequency in their peripheral blood. Their peripheral T cells also showed elevated IL-4 production relative to IFN $\gamma$  production. Four public facility employees who reacted to the methacholine challenge test had elevated eosinophil frequencies, increased plasma IgE levels, and lowered FEV1/FVC values. This study suggests that workers at public facilities could show greater risk towards the development of respiratory allergic diseases.

**Key words:** Public facility workers, Respiratory allergy, Th2 hyperreactivity, Skin prick test

## Introduction

The indoor environment is an important source of exposure to various chemicals and biological contaminants, including aeroallergens and pathogenic microorganisms<sup>1,2</sup>). Occupational asthma has been reported to occur in various workplaces, including in the health care, farming, cleaning, and chemical manufacturing industries<sup>3–5</sup>). It is estimated that about 15% of adult asthma can be attributed to occupational exposure to respiratory allergens or irritants<sup>4,5</sup>). With regard to biological contami-

nants, it has been shown that exposure to these contaminants enhances the risk of workers developing asthma<sup>2,6</sup>). Other studies have also shown that the inhalation in the workplace of organic dusts containing bacteria, fungi, or animal danders may exacerbate or elevate the occurrence of respiratory allergic inflammation<sup>1,6</sup>).

Many studies have examined the impact on health of exposure to biological contaminants in non-industrial indoor environments such as children daycare centers, elderly nursing homes, department stores, public transportation terminals, hospitals, and public libraries. This has raised concern about the air quality in such public facilities and has led the Korean Ministry of Environment to enact the Air Quality Control Act in Public Facilities

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in 2005. However, most of the studies mentioned focused on the impact of exposure to biological contaminants on the users of public facilities, especially children and the elderly<sup>7-9</sup>). Much less is understood about the effects of biological contaminants on the health of the employees working at these public facilities.

In the present study, we asked whether immunological parameters that indicate respiratory allergic hyperreactivity are elevated in employees working at public facilities, namely, children daycare centers, elderly nursing homes, subway stations, and hypermarkets. We also analyzed the pulmonary functions of public facility workers. The public facility workers' immunologic and pulmonary functional parameters were compared with those of office workers.

## Subjects and Methods

### Study subjects

The subjects included 52 employees from four child daycare centers, three elderly nursing homes, three subway stations, and three hypermarkets (referred to as public facility employees or employees hereafter), and 17 age- and sex-matched office workers (Table 1). The public facility employees had a mean age of  $34.1 \pm 10.9$  yr, consisted of 37 females and 15 males, and the office workers had a mean age of  $33.7 \pm 8.8$  yr, consisted of 10 females and 7 males. The study subjects represented 40% of total workers in all public facilities and offices studied. Internal Review Board approval from the Eulji Medical Center (Research Project No. 05-09) at Seoul, Korea was obtained along with consent from all blood donors.

### Skin prick test (SPT) and pulmonary function test

The SPT was performed against 25 common aeroallergens (Allergopharma, Reinbek, Germany) according to known guidelines<sup>10</sup>). Histamine (1 mg/ml) served as a positive control and saline solution served as a negative control. The aeroallergens included two house dust mites (*Dermatophagoides farinae*, *Dermatophagoides pteronyssi-*

*nus*), one storage mite (*Tyrophagus putrescentiae*), ten fungi (*Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium notatum*, *Alternaria tenuis*, *Cladosporium herbarum*, *Mucor mucedo*, *Neurospora sitophila*, *Rhizopus nigricans*, *Fusarium* spp., *Trichophyton* spp.), five animal furs (cat fur, dog hair, rat fur, sheep wool, feathers), German cockroach, two tree mixtures, two weed pollens (mugwort, ragweed), a grass mixture, and a flower antigen (*Chrysanthemum*). Two wheal diameters were measured 15 min after initiating the test; namely, the largest wheal diameter and that perpendicular to it, and the mean of these two diameters was calculated. When the mean diameter of the wheal generated by an allergen was greater than or equal to that of the histamine positive control, a positive reaction was deemed to have occurred.

Pulmonary function was tested in all study subjects by using a Micro Medical spirometer (Micro Medical, Kent, England). Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) were measured. The methacholine bronchial challenge test was also performed as described elsewhere<sup>11, 12</sup>). For the methacholine challenge, five different concentrations of methacholine (1.25, 2.5, 6.25, 12.5, or 25 mg/ml) were given to subjects through a nebulizer (DeVilbiss 646 nebulizer, Sunrise Medical, Somerset, PA, USA) driven by a dosimeter (Rosenthal-French dosimeter, Laboratory for Applied Immunology, Baltimore, MD, USA). The methacholine concentration inducing a 20% fall in FEV1 compared to the effect of saline inhalation (PC<sub>20</sub>) was determined by interpolating the last two FEV1 values on the y-axis versus the noncumulative log concentration of methacholine on the x-axis<sup>11</sup>).

### Peripheral blood analysis

Hematological parameters, namely, the number and frequency of lymphocytes, monocytes, and granulocytes, were determined by using a Coulter counter (Beckman coulter, Fullerton, CA, USA). To investigate T lymphocyte activity, peripheral blood mononuclear cells (PBMC, 10<sup>6</sup> cells per well) were stimulated with 5 ng phorbol 12-myristate 13-acetate (Sigma, Saint Louis, MO, USA) plus

**Table 1.** Demographic information of study subjects

Facility classification	No. facility	No. subjects (female/male)	Mean age $\pm$ SD (yr)	Mean employment duration $\pm$ SD (month)
Children daycare center	4	26 (26/0)	29.5 $\pm$ 9.4	44.3 $\pm$ 39.5
Elderly nursing home	3	9 (4/5)	37.4 $\pm$ 12.7	45.9 $\pm$ 33.4
Subway station	3	11 (5/6)	44.0 $\pm$ 7.7	214.7 $\pm$ 66.9
Hypermarket	3	6 (2/4)	30.5 $\pm$ 4.4	37.5 $\pm$ 25.4
Total employees	13	52 (37/15)	34.1 $\pm$ 10.9	79.9 $\pm$ 82.8
Office workers	4	17 (10/7)	33.7 $\pm$ 8.8	52.0 $\pm$ 39.5

500 ng ionomycin (Sigma) for 72 h. The interleukin-4 (IL-4) and interferon-gamma (IFN $\gamma$ ) levels in the culture supernatants were then assayed by a sandwich ELISA using the previously described method<sup>13, 14</sup> supplied by BD Biosciences (San Diego, CA, USA). The lower limit of detection was 15 pg/ml for IL-4 and 100 pg/ml for IFN $\gamma$ .

#### *Quantification of plasma IgE and IgG4 levels*

Plasma total IgE levels were determined by using an ELISA kit (IBL Immuno-Biological Laboratories, Hamburg, Germany). The IgG4 level in the plasma was measured by subclass-specific ELISA as previously described<sup>14</sup>. The lower detection limit for IgG4 was 1.5 ng/ml. Quality control of the IgG4 assay was performed by using WHO reference serum (National Institute for Biological Standards and Control, Hertfordshire, UK) and the Human IgG subclass profile ELISA kit (Zymed Laboratories, South San Francisco, CA, USA).

#### *Statistical analysis*

The data were initially evaluated for normal distributions. Depending on the normality of the data, statistically significant differences among groups were tested by single-factor ANOVA and Dunnett's *t*-test, or Kruskal-Wallis ANOVA and Dunn's test by using SigmaStat (Systat, Richmond, USA). The significant differences were further confirmed by Student's *t*-test or Mann-Whitney U test. Differences with  $p < 0.05$  were considered significant.

## **Results**

#### *Positive reaction to respiratory allergens*

When the 52 public facility employees and 17 office workers were subjected to SPTs using 25 aeroallergens, 53.9% and 52.9% yielded a positive result, respectively (Table 2). House dust mite triggered a positive response most frequently for both the public facility employee and office workers, followed by fungus, and animal furs (Table 3). The two groups, namely public facility employees and office workers, did not differ significantly in the SPT positivity rate and the aeroallergen distribution.

#### *Evaluation of immunological parameters that reflect the occurrence of respiratory allergic diseases*

Of the granulocytes, basophils and eosinophils are believed to play an important role in allergic airway inflammation by producing a broad spectrum of proinflammatory mediators<sup>15, 16</sup>. When the SPT-positive subjects were divided from the SPT-negative subjects, the positive subjects invariably had higher frequencies of eosinophils in their peripheral blood than the negative subjects (Table 4). Similarly marked differences between the SPT-positive and -negative subjects in peripheral blood basophil frequencies were not observed. However, the SPT negative employees did have significantly higher basophil frequencies than the SPT negative office workers (Table 4).

Elevation of plasma IgE levels is a hallmark of immediate hypersensitivity, such as asthma, allergic rhinitis, or anaphylaxis<sup>16, 17</sup>. SPT positive employees showed significantly enhanced plasma IgE levels compared to the negative employees (Table 4). All employee subgroups showed the same trend with statistical significances for

**Table 2. Positivity of skin prick test against aeroallergens to public facility employees and their age- and sex-matched office workers**

Facility classification	No. tested	No. positive	Positive percentage
Children daycare center	26	15	57.7
Elderly nursing home	9	3	33.3
Subway station	11	6	54.5
Hypermarket	6	4	66.7
Total employees	52	28	53.9
Office workers	17	9	52.9

**Table 3. Aeroallergens yielding a positive response in the skin prick tests**

Subject group	House dust mite	Fungus	Animal fur	Cockroach	Tree pollen	Weed pollen	Grass pollen	Flower	Total <sup>a</sup>
Public facility employees (%)	32 (44.4)	9 (12.5)	8 (11.1)	5 (6.9)	3 (4.2)	7 (9.7)	2 (2.8)	6 (8.3)	72 (100)
Office workers (%)	14 (50.0)	5 (17.9)	4 (14.3)	1 (3.6)	2 (7.1)	1 (3.6)	0 (0.0)	1 (3.6)	28 (100)

<sup>a</sup>The total includes all positive SPTs (some subjects responded to more than one of the 25 allergens).

**Table 4.** Comparison of various immunological parameters that reflect respiratory allergic hyperreactivity<sup>a</sup>

Facility	Skin prick test	Eosinophils (%)	Basophils (%)	IgE (ng/ml)	IgG4 (mg/ml)	IL-4/IFN $\gamma$ ratio <sup>b</sup>
Children daycare center	positive	3.7 $\pm$ 1.9	2.2 $\pm$ 2.5	1,337 $\pm$ 1,300*	0.95 $\pm$ 0.81	8.1 $\pm$ 11.9
	negative	2.9 $\pm$ 1.9	1.0 $\pm$ 0.6 <sup>§</sup>	68 $\pm$ 148	0.62 $\pm$ 0.42	13.8 $\pm$ 29.4
Elderly nursing home	positive	2.5 $\pm$ 1.0	0.6 $\pm$ 0.5	1,655 $\pm$ 2,602	0.78 $\pm$ 0.42	10.7 $\pm$ 7.6 <sup>§</sup>
	negative	1.7 $\pm$ 0.7	0.6 $\pm$ 0.2	315 $\pm$ 463	0.79 $\pm$ 0.48	21.7 $\pm$ 32.7 <sup>§</sup>
Subway station	positive	8.4 $\pm$ 8.8*	0.9 $\pm$ 0.5	3,932 $\pm$ 4,277*, <sup>§</sup>	1.90 $\pm$ 2.03	19.1 $\pm$ 37.1
	negative	2.1 $\pm$ 1.1	0.8 $\pm$ 0.3	125 $\pm$ 126	0.93 $\pm$ 0.53	13.0 $\pm$ 18.9 <sup>§</sup>
Hypermarket	positive	4.0 $\pm$ 3.4	1.1 $\pm$ 0.7	1,032 $\pm$ 708	1.89 $\pm$ 1.45	54.7 $\pm$ 87.4 <sup>§</sup>
	negative	2.9 $\pm$ 0.6	1.4 $\pm$ 1.1	561 $\pm$ 686	0.42 $\pm$ 0.45	2.4 $\pm$ 3.4
Total employees	positive	4.6 $\pm$ 4.6*	1.6 $\pm$ 1.9	1,883 $\pm$ 2,456*	1.27 $\pm$ 1.26*	18.0 $\pm$ 39.3
	negative	2.4 $\pm$ 1.5	0.9 $\pm$ 0.5 <sup>§</sup>	183 $\pm$ 321	0.71 $\pm$ 0.46	14.4 $\pm$ 26.1 <sup>§</sup>
Office workers	positive	3.9 $\pm$ 3.1*	0.6 $\pm$ 0.3	1,336 $\pm$ 2,238	2.07 $\pm$ 2.30	2.3 $\pm$ 1.6
	negative	1.7 $\pm$ 1.0	0.6 $\pm$ 0.4	173 $\pm$ 326	2.87 $\pm$ 3.33	4.3 $\pm$ 9.6
Total subjects <sup>c</sup>	positive	4.4 $\pm$ 4.3*	1.3 $\pm$ 1.7	1,750 $\pm$ 2,386*	1.47 $\pm$ 1.58	14.0 $\pm$ 34.4
	negative	2.2 $\pm$ 1.4	0.8 $\pm$ 0.5	180 $\pm$ 318	1.25 $\pm$ 1.89	11.8 $\pm$ 23.2

\*Significantly different from the aeroallergen test negatives in each group. <sup>§</sup>Significantly different from the aeroallergen test negative office workers. <sup>§</sup>Significantly different from the aeroallergen test positive office workers. <sup>a</sup>The results are expressed as means  $\pm$  SD. <sup>b</sup>The ratio was calculated by dividing the IL-4 levels (pg/ml) in each culture supernatant by the IFN $\gamma$  levels (pg/ml) and then multiplying by 10<sup>4</sup>. <sup>c</sup>The total subjects include both all public facility employees and office workers.

the children daycare center and subway station employees. When we examined the plasma IgG4 levels, we found that the SPT positive employees had significantly higher levels than the negative employees and that most of the employee subgroups showed a similar trend, although these differences did not achieve statistical significance.

To test whether the PBMCs of the subjects show skewing toward type-2 helper T cell (Th2)-mediated immune responses, we stimulated them with phorbol 12-myristate 13-acetate plus ionomycin and then measured the IL-4 and IFN $\gamma$  levels in the supernatants. These values were expressed as a ratio by dividing the IL-4 levels (pg/ml) by the IFN $\gamma$  (pg/ml) levels and then multiplying by 10<sup>4</sup> (Table 4). The SPT-positive and negative employees did not significantly differ in their mean ratios, regardless of their workplace. There was also no difference between the SPT positive and negative office workers. However, the negative employees had significantly higher ratios than the negative office workers, which suggests the public facility employees show a potential skewing toward Th2 reactivity.

Thus, in general, many of the immunological parameters that reflect respiratory allergic hyperreactivity were upregulated (except IgG4) in the aeroallergen-positive public facility employees compared to the allergen-negative employees and the office workers.

#### Assessment of pulmonary function

To identify the participants who suffered from respira-

tory allergic diseases, such as asthma, at the time of our investigation, we performed a pulmonary function test combined with a methacholine bronchial challenge test. The FEV1 values were similar for the SPT positive (3.04  $\pm$  0.57 liter) and negative employees (3.07  $\pm$  0.42 liter). The mean FEV1s of the facility employees also did not differ significantly from those of the office workers (3.36  $\pm$  0.55 and 3.09  $\pm$  0.81 liter for the SPT-positive and -negative office workers, respectively). However, the mean FEV1 value of the children daycare center employees irrespective of their aeroallergen positivity (2.88  $\pm$  0.34 and 2.88  $\pm$  0.26 liter for the SPT-positive and -negative employees, respectively) was lower than the other workers (elderly nursing homes: 3.48  $\pm$  1.28, 3.11  $\pm$  0.42; subway stations: 3.16  $\pm$  0.64, 3.13  $\pm$  0.46; hypermarkets: 3.15  $\pm$  0.52, 3.87  $\pm$  0.16; office: 3.36  $\pm$  0.55, 3.09  $\pm$  0.81 liters for the SPT-positive and negative workers, respectively) with statistical significance ( $p < 0.05$ ) from the SPT positive office workers. It has been suggested that FEV1/FVC less than 70% can be defined as having an airflow obstruction problem<sup>18</sup>). The FEV1/FVC values of all of the study subjects except for one aeroallergen test-positive employee (55.7%) exceeded 70% (86.7  $\pm$  5.6%).

When the methacholine bronchial challenge test was performed, four employees exhibited bronchial hyperreactivity since their average PC<sub>20</sub> was 7.3  $\pm$  6.2 mg/ml accompanied with a 31.3  $\pm$  3.9% reduction in FEV1 (Table 5). All four subjects were positive for the aeroallergen SPT and worked at children daycare center or

**Table 5. Immunological and pulmonary functional characteristics of study subjects showing a 20% or more reduction in FEV1**

	Subject 1	Subject 2	Subject 3	Subject 4
Workplace	Children daycare center	Children daycare center	Children daycare center	Elderly nursing home
Positive allergen	<i>D. pteronyssinus</i> , <i>D. farinae</i>	<i>D. pteronyssinus</i> , <i>D. farinae</i> , <i>T. putrescentiae</i> , cat fur, dog hair, mugwort, grass pollen	<i>D. pteronyssinus</i> , <i>D. farinae</i> , dog hair	<i>Fusarium</i> spp
Eosinophils (%)	3.5	7.1	3.7	2.6
Basophils (%)	2.9	8.7	1.1	1.0
IgE (ng/ml)	1,444	353	3,898	307
IgG4 (mg/ml)	2.9	0.3	1.2	0.5
IL-4/IFN $\gamma$ ratio	1.1	0.4	3.9	8.6
FEV1 (liter)	3.26	2.57	2.94	2.14
FEV1/FVC (%)	82	80	90	56

**Table 6. Comparison of immuno-pulmonary characteristics of the study subjects with bronchial hyperreactivity with other workers<sup>§</sup>**

	Bronchial hyperreactive subjects <sup>§</sup>	Facility employees with (+) SPT <sup>a</sup>	Facility employees with (-) SPT	Office workers with (+) SPT	Office workers with (-) SPT
Eosinophils	4.3 $\pm$ 1.9	4.7 $\pm$ 4.9	2.4 $\pm$ 1.5*	3.9 $\pm$ 3.1	1.7 $\pm$ 1.0*
Basophils	3.4 $\pm$ 3.6	1.3 $\pm$ 1.4*	0.9 $\pm$ 0.5*	0.6 $\pm$ 0.3*	0.6 $\pm$ 0.4*
IgE	1,501 $\pm$ 1,682	1,948 $\pm$ 2,531	183 $\pm$ 321*	1,336 $\pm$ 2,238	173 $\pm$ 326*
IgG4	1.23 $\pm$ 1.18	1.28 $\pm$ 1.27	0.71 $\pm$ 0.46	2.07 $\pm$ 2.30	2.87 $\pm$ 3.33
IL-4/IFN $\gamma$ ratio	3.5 $\pm$ 3.7	20.1 $\pm$ 40.5	14.4 $\pm$ 26.1	2.3 $\pm$ 1.6	4.3 $\pm$ 9.6
FEV1	2.73 $\pm$ 0.48	3.09 $\pm$ 0.57	3.07 $\pm$ 0.42	3.36 $\pm$ 0.55	3.09 $\pm$ 0.81
FEV1/FVC	77 $\pm$ 15	87 $\pm$ 6*	86 $\pm$ 6*	88 $\pm$ 5*	89 $\pm$ 5*

<sup>§</sup>The results are expressed as means  $\pm$  SD. <sup>§</sup>The subjects showing a 20% or more reduction in FEV1 as described in Table 5, and the parameter units are as those described in Table 5. <sup>a</sup>The bronchial hyperreactive subjects were excluded from the facility employees with skin prick test positive. \*Significantly different from the study subjects with bronchial hyperreactivity.

elderly nursing home. The immunological and pulmonary functional profiles of these four bronchial challenge-positive subjects were then compared with those of the bronchial challenge-negative employees or office workers (Tables 5 and 6). They had significantly higher eosinophil and basophil frequencies in their peripheral blood than the SPT-negative employees or office workers. In addition, basophil frequency in the bronchial challenge-positive subjects was also significantly higher than the SPT-positive employees or office workers with negative response to the bronchial challenge test. However, it should be noted that the average increase in basophil frequencies of the four bronchial challenge-positive subjects was due to one subject having a very high basophil frequency (8.7%). The four bronchial challenge-positive subjects also had significantly elevated plasma IgE levels compared to the SPT-negative employees or office workers. Moreover, they had significantly lower mean FEV1/FVC value than the bronchial challenge-negative employees or the office workers irrespective of SPT positivity.

Thus, overall, when compared with the bronchial challenge-negative workers irrespective of work place, the

bronchial hyperreactive public facility employees had immuno-pulmonary characteristics (namely, elevated eosinophil and basophil frequencies and plasma IgE levels and lowered FEV1/FVC values) that suggest the presence of a respiratory allergic disease.

## Discussion

Here we examined for the first time in Korea whether immuno-pulmonary parameters that reflect the occurrence of respiratory allergic diseases are elevated in employees that work at public facilities. Despite growing concern in Korea about indoor air quality in public facilities, the effects on worker health of indoor air pollutants, especially biological contaminants, in public facilities has not been investigated systematically. House dust mite was the allergen that evoked responses most frequently in our study subjects, followed by fungus and animal furs. Such animal or microbiological allergens are major indoor biological allergens that are known to induce asthma or allergic rhinitis in humans worldwide, including in Korea<sup>1, 19–24)</sup>. In contrast, plant allergens brought from



the outdoor into the indoor environment were found to be minor allergens in terms of the allergic sensitization of both the facility employees and the office workers.

That the public facility employees and office workers did not differ in their aeroallergen-positive rates and distribution of sensitizing aeroallergens suggested that public facility indoor environments may not increase the risk of developing biological allergen-linked respiratory allergic diseases compared to the indoor environments in offices. However, we found that compared to the allergen-negative employees or the office workers, the aeroallergen-positive public facility workers had substantially higher levels of various immunological parameters that are known to reflect respiratory allergic hyperreactivity, namely, peripheral eosinophil frequencies, plasma IgE levels, and IL-4/IFN $\gamma$  production ratios (Table 4). Respiratory allergic disease is characterized by the predominance of type-2 helper T cell (Th2)-mediated immune responses, which produce IL-4 and thereby downregulate the production of IFN $\gamma$  by type-1 helper T cells<sup>25</sup>). Thus, public facility workers who are aeroallergen-sensitized could have a potential towards the manifestation of allergic respiratory disease than the aeroallergen-negative public facility employees or the office workers. It is possible that various chemical indoor pollutants, such as formaldehyde, environmental tobacco smoke, volatile organic compounds, carbon monoxide, or nitrogen dioxide, could exacerbate or influence the development of asthma in the aeroallergen-sensitized public facility workers<sup>26–28</sup>). Exposure to endotoxin from gram negative bacteria may also be a factor promoting the manifestation of asthma in the aeroallergen-sensitized public facility workers<sup>6</sup>). Future studies should examine whether public facilities are more polluted with such indoor contaminants than office workplaces, and whether this is responsible for the enhancement in public facility workers of immune parameters that reflect respiratory allergy progression. Moreover, concomitant analyses determining genetic predisposition to respiratory allergic diseases will help to more accurately determine the true health risks to which public facility workers are subjected.

It has been shown that elevated plasma IgE levels and eosinophil frequencies in the peripheral blood are immunological biomarkers that correlate well with the occurrence of respiratory allergies<sup>16, 25</sup>). Indeed, we found that of all the biomarkers we used to determine respiratory allergic hyperreactivity, the plasma IgE levels and eosinophil frequencies in the peripheral blood correlated best with the results of the aeroallergen sensitization and methacholine bronchial challenge tests (Tables 4 and 5). Thus, the propensity of particular human populations towards developing respiratory allergic hyperreactivity may best be measured by performing the following tests: a SPT to

aeroallergens, quantitative measurement of IgE and eosinophils, and the methacholine bronchial challenge test.

We noted that of the four subjects with positive methacholine challenge test results, three were children daycare center employees. The average FEV1 value of the children daycare center employees was lower than those of the other employees or the office workers. In addition, compared to the daycare center employees with negative SPT results, the SPT-positive daycare center employees had elevated plasma IgE and IgG4 levels and a greater frequency of eosinophils and basophils in their peripheral blood. It has been reported that IgG4 production can be enhanced by IL-4 or IL-13, which are two classical cytokines involved in the induction of immediate hypersensitivity<sup>29</sup>). Increase in serum level of allergen-specific IgG4 has been reported from subjects with respiratory allergic disease<sup>30, 31</sup>). Thus, of all the public facilities examined in our study, children daycare centers seem to be the most risky workplace in terms of developing and/or exacerbating respiratory allergic diseases. This notion is supported by several studies that have suggested there is a causal relationship between biological allergen exposure at children daycare centers and the development of respiratory allergic diseases in child attendants<sup>8, 32</sup>).

In conclusion, while our present study was performed with relatively few study subjects and should be interpreted cautiously, our central observation was that aeroallergen-sensitized employees at public facilities did have a more risk of developing respiratory allergic diseases than non-sensitized employees or office workers.

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