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The Prevalence of Specific IgE and IgG to Reactive Dye-Human Serum Albumin Conjugate in Workers of a Dye Factory and Neighboring Factories

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Previous studies suggest that reactive dyes can induce IgE mediated bronchoconstrictions. To evaluate the significance of specific IgE and IgG antibodies in workers exposed to reactive dyes, we studied the prevalence of Black GR-specific IgG by enzyme linked immunosorbent assay, as well as Black GR-specific IgE by RAST, in 176 workers employed in 1 reactive dye factory and 4 neighboring factories. Six emplyees of reactive dye asthma who were working in factories near the reactive dye factories were noted. The prevalence of specific IgE antibodies in the neighboring factories was higher than in that of the reactive dye factory. The prevalence of specific IgG was highest in the reactive dye factory, and those of the neighboring factories were markedly lower. It was suggested that IgE mediated sensitization to reactive dye could have occurred in employees who were working in neighboring factories, and the prevalence of reactive dye-specific IgG antibody could be used as an in direct method of assessing the exposure of workers to reactive dye.

Key Words : *Reactive dye, Specific IgE antibody, Specific IgG antibody*

INTRODUCTION

Reactive dyes which bind covalently to textiles have been increasingly used as coloring agents in the textile industry, and some cases of occupational asthma due to these dyes have been reported(Luczynska and Topping, 1986 ; Docker et al.,1987 ; Park et al.,1988 ; Park et al.,1991). In 1989, we reported 9 cases of occupational asthma associated with exposure to 3 different reactive dyes in a dye factory(Park et al.,1988). Park et al.(1991) presented 13 cases of occupational asthma who showed significant bronchoconstriction on reactive dye bronchoprovocation test in a dye factory.

Black GR is known to be the most frequent sensitizer among various reactive dyes in this country. Positive skin tests and the presence of serum specific IgE antibodies to reactive dyes suggested that respiratory symptoms provoked by reactive dyes could be an IgE-mediated reaction(Park et al 1991). Park et al.(1991) suggested that the existence of specific IgG to reactive dye might represent a response to Black GR exposure and correlate better with the results of a reactive dye-bronchoprovocation test result.

In order to evaluate the significance of specific IgE and IgG antibodies in workers exposed to reactive dyes, we studied the prevalence of Black GR-

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specific IgG by enzyme-linked immunosorbent assay (ELISA), along with Black GR-specific IgE by RAST, in 176 workers employed in 1 reactive dye factory and 4 neighboring factories.

MATERIALS AND METHOD

Subjects

The subjects were consisted of all the employees—171 males and 5 females between age of 24 and 53 years—working in 1 dye industry(K factory) and 4 factories located around the reactive dye factory(E and K factory). The neighboring factories produce a direct dye(J factory), an intermediate form of dye and raw materials for paint(H factory), Fe_2SO_4(I factory), and vat dye(S factory). THe locations of all the factories were shown in Fig. 1.

Questionnaires

Doctors administered questionnaires (which are a National Heart and Lung Institute modification of the British Medical Research Council questionnaire, 1986) with additional questions at each visit. For the evaluation and comparison of data, the following definitions were used. 〈1〉 Lower respiratory symptoms : cough, sputum, chest tightness and shortness of breath 〈2〉 Symptomatic employees : workers who had suffered from lower respiratory symptoms during and after the dye exposure.

Preparation of Dye Solution for Skin Prick Tests

Black GR and the other chemicals produced in each factory were used for tests(Table 1). Ten mg of Black GR or each kind of chemicals were dissolved in 1 ml of modified Coca solution(NaCl 9gm, phenol crystal 4gm, NaHCO3 2.9gm in 1000ml of distilled water) containing 50% sterile glycerine for skin prick tests.

Preparation of Dye-Human Serum Albumin (HSA) Conjugate Discs

To prepare the discs, 0.1mg of Black GR was

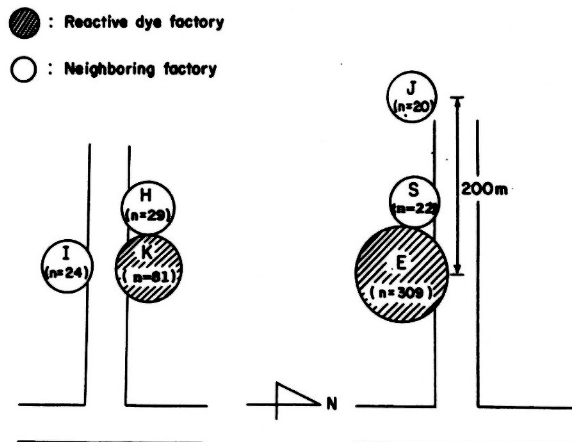

Fig. 1. Localization of all factories studied. The H and I factories were located near the K factory. The S factory was located near the E factory, and the J factory was 200m away from the E factory.

added to 2mg/ml of HSA(Sigma Chemical Co., St. Louis, Mo, USA) dissolved in 100mM Na2CO3 (pH 11.0) according to the previously described method(Park et al., 1989). It was incubated while stirring at 4℃ for 48 hours. These dye-HSA conjugate solutions were used in preparing RAST solid phases and used as an antigen to detect specific IgG by ELISA. Discs of nitrocelllose filter paper (NFP ; Millipore, Cat. No. HAHY 08250, 0.45um, Millipore Co., Bedford, MA, USA) were immersed into the dye-HSA conjugate solutions for 72 hours at 4℃ and dried at room temperature for use in the subsequent RAST assay.

RAST Assay

Sera from all the employees were tested with reactive dye(Black GR-HSA conjugate discs). The dried discs were blocked with 10% newborn calf serum for 1 hour and incubated with 50ul of sera for 6 hours at room temperature. The discs were washed 3 times with 2.5ml of 0.9% NaCl containing RAST washing solution additives(Pharmacia, Uppsala, Sweden). Then 50ul of 125-I-labeled anti-human IgE(Pharmacia) was added to each disc and left for 18 hours at room temperature. The discs were washed again, and the bound 125-I was measured using a gamma counter. All the assays were performed in duplicate. The results were expressed as percent binding, defined as a percentage of the added counts per minute(cpm) bound to the reactive dye-HSA conjugate discs. The cut-off value of positive IgE binding was determined as 2% according to the previous study(Park et al., 1991).

Measurement of Total IgE

The total IgE was measured using a Phadebas paper radioimmunosorbent test(PRIST) kit(Pharmacia) according to the manufacturer's directions. One anti-IgE disc was put into the bottom of each tube and incubated with 100ul of diluted serum at room temperature for 3 hours. The discs were washed 3 times with 2.5ml of 0.9% NaCl containing PRIST washing additibes. Then 100ul of anti-IgE-125-I PRIST tracer was added and the tube were incubated for 18 hours at room temperature and rinsed 3 times. The binding radioactivities were determined using a gamma counter, and absolute amounts of total IgE were determined on a standard curve.

Methacholine Bronchial Challenge Test

Symptomatic employees identified by our questionnaire were referred to the Allergy Clinic, Department of Internal Medicine, Yonsei University College of Medicine, while nonspecific bronchial hyperreactivity of them was determined by the previously described standard method(Chai et al., 1975). In addition, the reactive dye-bronchoprovocation test was performed in asthmatic employees whose methacholine PC_{20} was less than 5.0mg/ml according to the previously described method(Park et al., 1988 ; Park et al., 1989). We used Black GR solutions which were dissolved in 0.4% phenolized saline. The FEV1 and maximum mid-expiratory flow(MMEF) were measured by a spirometer before and 10 minutes, after, inhalation. The test solutions were delivered by a Vaponefrine nebulizer (Meiko Co., Japan) and connected to a compressed air souce. The patients were asked to breathe the nebulized aerosol 5 times up to their vital capacity. Serial increments in antigen concentration(0. 01, 0.1, 1.0, 2.5mg/ml) were given at 10-minute intervals until a 20% or greater decrease in FEV1 was obtained. The FEV1 and MMEF were measured frequently during the first hour and hourly thereafter for 9 or 10 hours.

Table 1. Study Subject

Factory	Dyes Produced	Number of Employees		Prevalence of Occupational
		Male	Female	Asthma to Black GR(%)
K	Reactive	80	1	11 (14%)
I	FeSO4	24	0	3 (13%)
S	Vat	20	2	3 (14%)
H	Paint	29	0	0 (0%)
J	Direct	18	2	0 (0%)

* Four kinds of reactive dyes were produced in the K factory, and among them Black GR was the most frequently handled dye.

Table 2. Prevalence of Specific IgE and IgG Antibodies to Black GR-Human Serum Albumin(HSA) Conjugate in Study Subjects

Factory	Specific Antibodies to Black GR-HSA		Number of Workers
	Specific IgE(%)	Specific IgG(%)	with Increased tIgE*
K	19/81 (23)	40/81 (49)	47/81 (58)
I	12/24 (50)	4/24 (17)	20/24 (83)
S	10/22 (45)	4/22 (18)	13/22 (59)
H	12/29 (41)	7/29 (24)	17/29 (59)
J	5/20 (25)	0/20 (0)	9/20 (45)

* tIgE : total IgE level
* Increased total IgE : more than 160 IU/ml of serum total IgE.

Anti-Black GR IgG ELISA

A 96-well EIA flat bottom plate(Costar, Cambridge, MA) was filled with 100ul of a four fold diluted solution of Black GR-HSA preliminarily determined as the optimal concentration(Park et al., 1989). After overnight incubation at 4 C, the plates were washed 3 times with 0.05 M Tween phosphate buffered saline(PBS-T). Added to each well were 250 microliters of 10% newbrorn calf serum (NCS), which was then incubated for 60 minutes at 37℃ and not washed. One hundred microliters of diluted patients' serum or negative control serum (1 : 100 in diluent buffer : PBS-T containing 2% NCS) were added to each well coated with Black GR-HSA conjugate. All of these assays were performed in duplicate. After incubation for 2 hours at 37℃, the wells were washed 3 times with PBS-T. One hundred microliters of horseradish peroxidase (HRPO) conjugated goat anti-human IgG(Behring Diagnositcs, San Diego, CA) diluted into 1 : 3000 w/v with 10% NCS, were added to each well. Then the plates were incubated at 4℃ for 2 hours. The wells were washed 3 times with PBS-T and then 100ul of substrate solution was added, containing 0.01 M O-phenylenediamine-HCl in citrate phosphate buffer, pH 4.2, supplemented with 0.012% H202 was added to stop the reaction. The optical density of the solution at 490nm was determined with a microtiter plate reader(MR 600, Dynamic Product, USA). The antibody titers were expressed as absorbances at 490nm. The positive cut-off value was determined from the mean and two-fold standard deviation of 63 negative controls (Mean+2 S.D.=0.062).

RESULTS

Prevalence of Asthmatic Workers

As shown in Fig 1, the H and I factories are located nearby from K factory, in which reactive dyes including Black GR were produced. The S factory was located near E factory, the largest reactive dye factory in Incheon, Korea and the J factory is 200 meters away from E factory.

Table 1 summarizes various kinds of dyes produced and the prevalence of asthmatic workers in each factory. The prevalences of asthmatic workers who showed positive responses on Black GR-bronchoprovocation test were 14% in the K factory, 13.6% in the S factory, 12.5% in the I factory. Some workers whose methacholine PC_{20} level was ranged from 5 to 25.0mg/ml and had Black GR-specific IgE antibodies were found.

Prevalence of Specific IgE and IgG to Black GR-HSA Conjugate

Table 2 reveals the prevalence of specific antibodies to Black GR-HSA conjugate and total IgE antibodies. The prevalence of specific IgE antibodies in the H and I factories were 41% and 50% respectively, and those of the S and J factory were 45% and 25%, respectively. The prevalences of specific IgG antibody in the H and I factories were 24.% and 17%, respectively. The prevalence of S factory and J factory were 18% and 0%, respectively. Many of workers had increased total IgE level (> 160 IU/ml), ranging from 45% to 83%, and 48 workers(27% of those studied) had increased total IgE without dye-specific IgE antibodies as shown in Table 2.

DISCUSSION

Previous studies (Park et al., 1989 ; Park et.al. 1991) showing that inhalation challenge tests with reactive dyes induced asthmatic responses and specific IgE antibodies to Black GR-HSA conjugate were detected by RAST employing Black GR-HSA conjugate disc as a solid phase in reactive dye-exposed workers suggested that their respiratory symptoms could be mediated by IgE-mediated reaction. In this study, several employees who were working in factories near reactive dye factories were noted. As the bronchoprovocation test with Black GR showed asthmatic responses and specific IgE antibody to Black GR-HSA was detected by RAST, their asthmatic symptoms might be caused by reactive dye.

Park et al. (1991) revealed that 17% of employees had serum specific IgE antibodies to Black GR-HSA conjugate in 309 employees of E factory. In this study, the prevalences of specifie IgE antibody to Black GR-HSA conjugate in the S and J factories were higher than that of the E factory. The prevalences of specific IgE antibodies to Black GR-HSA in the H and I factories were higher than that of the K factory. It might be caused by that all emplyees, including office workers and laboratory technicians, were included in study of E factory and only dye process workers, most of whom participated in the study of the H, I, S, and J factory were smokers. Our previous study(Park et al., 1991) suggested that the development of reactive-dye specific serum IgE antibody was associated with smoking status. It was suggested that the sensitization to Black GR could occur in employees working far from dye making process.

The role of specific IgG and IgG4 in reactive dye asthma should be elucidated. THe prevalence of specific IgG and IgG4 to Black GR-HSA conjugate in exposed workers was significantly higher in workers with respiratory symptoms than in those with no symptoms(Park et al., 1991). Our other studies(Park et al., 1989 ; Park et al., 1989 ; Park et al., 1991) suggested that another immunological mechanism, an IgG mediated reaction might be involved in the induction of asthmatic symptoms of dye exposed workers. These studies also showed that specific IgG antibodies to Black GR-HSA bear a closer association with the results of specific inhalation challenge, as other investigators suggested in patients with TDI-induced occupational asthma(Cartier et al., 1989). Park et al.(1991) showed that there was no statistical significance in the prevalence of specific IgG antibody to Black GR-HSA between those who participated in dye making process and those who worked far from dye process within a factory. The intensity of exposure within the same factory did not influence the development of specific IgG and IgG4 antibody to Black GR-HSA conjugate. The correlation between length of exposure and specific IgG was poor. The results in this study revealed that the prevalence of specific IgG was highest in the K factory which produce reactive dyes and intensity of the H and I factory were markedly lowered compared to that of the K factory. The prevalence of IgG in S factory was lower than that of the E factory(23% in our previous study). The prevalence of the J factory, which was located 200m away from E factory was 0%. It was suggested that there was an increase in the prevalence of specific IgG antibody in relation to the increased intensity of exposure to the reactive dye. Serum specific IgG to Black GR-HSA conjugate appeared to be a marker of reactive dye exposure.

In conclusion, IgE mediated sensitizaton to reactive dyes have occurred in employees who were working in neghboring factories, and the prevalence of reactive dye-specific IgG antibody could be used as an indirect method of assessing the exposure of workers to reactive dyes.

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