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The significance of specific IgG and IgG4 antibodies to a reactive dye in exposed workers

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Summary

To evaluate the significance of specific IgG and specific IgG4 in the development of work-related respiratory symptoms, specific IgG and specific IgG4 to Black GR–human serum albumin (HSA) conjugate were measured by ELISA in 309 dye-exposed workers and 63 unexposed patients as negative controls. A survey revealed that 78 (25·2%) had work-related lower respiratory symptoms with or without nasal, skin or eye symptoms. Specific IgG and specific IgG4 were detected in 23% and 14% of the exposed workers, respectively. The prevalence of specific IgG and specific IgG4 was significantly higher in smokers and workers with specific IgE or those with lower respiratory symptoms ($P < 0{\cdot}05$), but was not associated with work station, duration of dye exposure or atopy. These results suggested that the existence of specific IgG to Black GR–HSA might represent a response to Black GR exposure and be closely related with work-related respiratory symptoms.

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Introduction

Reactive dyes have molecular weights of 0·5–1 kD. They contain a chromogen linked to a reactive functional group designed to form irreversible covalent bonds with hydroxyl groups on cellulose fibres. Black GR has a β-sulphatoethyl sulphone group, which reacts by nucleophilic transfer onto unsaturated carbon bond [1]. Black GR, which is known to be the most frequent sensitizer among various reactive dyes, has been widely used as a colouring agent in the textile industry in Korea. Several investigators [2–4] reported the presence of specific IgE antibodies to the reactive dye–human serum albumin (HSA) conjugates. Our previous study [5] reported that the asthmatic symptoms of 13 (4·2%) workers among 309 employees were associated with reactive dye exposure and that the specific IgE antibodies to Black GR–HSA were detected in 17% of the dye-exposed workers with or without respiratory symptoms. In another report [6] only 12 (60%) workers out of 20 occupational asthma patients who showed significant bronchoconstrictions after exposure to Black GR, had Black GR-specific IgE antibodies. Therefore, we speculated that more than one mechanism might be involved in reactive dye-induced asthma.

In this study, in order to evaluate the significance of specific IgG (sIgG) and specific IgG4 (sIgG4) antibodies, we studied the prevalence of Black GR-specific IgG and IgG4 by ELISA in 309 Black GR-exposed workers and their relationship to respiratory dysfunction. The relationship between the existence of specific IgE and IgG antibodies was also investigated.

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Subjects and methods

Subjects

All 309 workers (275 men and 34 women, aged 19–63 years; mean 39·8 years), working in a single factory were included. Of these, 271 were dye process workers, seven were office workers, and 31 were laboratory workers. Symptomatic workers included workers who had lower respiratory symptoms such as cough, sputum, and shortness of breath during and after the dye exposure.

Sera

Sera from all workers were collected and stored at −20°C. Sera from 31 individuals who showed negative

response on skin-prick test with 50 common inhalant allergens, along with 32 atopic patients who had never been exposed to reactive dyes, were used as negative controls. Atopy was defined as a positive reactor to more than one common inhalant allergen on skin-prick test according to our previously described study [5]. A positive skin test was defined as the mean diameter of weal reaction larger than 2 mm and that of an erythema reaction larger than 21 mm on skin-prick test.

Preparation of dye–human serum albumin conjugate

According to our previously described method [2,5], 0·1 mg of Black GR was added to 2 mg/ml of HSA (Sigma Chemical Co., St Louis, MO) solution dissolved in 100 mM Na_2CO_3 (pH 11·0). It was incubated with stirring at 4°C for 18 hr and dialysed against 100 mM Na_2CO_3, with four changes, at 4°C for 48 hr. A similar technique was used to prepare a solution of HSA alone without reactive dye. The Black GR–HSA conjugate solutions were used as an antigen to detect specific IgG and IgG4 by ELISA.

ELISA

A 96-well EIA flat-bottomed plate (Costar, Cambridge, MA) was filled with 100 μl of a four-fold diluted solution of Black GR–HSA conjugate and 0·5 mg/ml HSA without dye in carbonate buffer (pH 9·6), which was preliminarily determined as the optimal concentration. After overnight incubation at 4°C, the plates were washed three times with 0·05 M Tween–phosphate-buffered saline (PBS–T). To each well were added 250 μl of 10% newborn calf serum (NCS), which was then incubated for 60 min at 37°C.

Anti-Black GR IgG ELISA

One-hundred microlitres 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 three assays were performed in duplicate. After incubation for 2 hr at 37°C, the wells were washed three times with PBS–T. One-hundred microlitres of horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Behring Diagnostics, San Diego, CA) diluted into 1/3000 (w/v) with 10% NCS, were added to each well. The plates were then incubated at 4°C for 2 hr. The wells were washed three times with PBS–T and then 100 μl of substrate solution were added, containing 0·01 M OPD–HCl in citrate phosphate buffer, pH 4·2, supplemented with 0·012% H_2O_2 before use. After incubation for 15 min at room temperature, 100 μl of 1 *N* H_2SO_4 were added to stop the reaction. The optical density of the solution at 490 nm was determined with a microtitre plate reader (MR 600, Dynamic Product, USA). The antibody titres were expressed as absorbances at 490 nm. 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). In cases of individual samples whose optical densities were higher than 0·062, the final absorbances were obtained by the subtraction of the absorbances of HSA-coated wells.

Anti-Black GR IgG4 ELISA

One-hundred microlitres of patient serum or negative control serum were added to each well coated with Black GR–HSA conjugate and incubated for 2 hr at room temperature. After the wells were washed three times with PBS–T, 100 μl of biotin-conjugated mouse monoclonal anti-human IgG4 (Sigma Chemical Co.) diluted to 1/1000 (w/v) with 10% NCS; were incubated for 2 hr at 37°C. The wells were washed three times with PBS–T. Then 100 μl of 1/100 diluted streptavidin–HRP (Sigma Chemical Co.) were added and incubated for 30 min. The wells were washed three times and 100 μl of substrate solution (55 mg of 2,2′-azido-di-3 ethylbenzthiazoline sulphonic acid; Sigma Chemical Co.) in 100 ml of 70 mM citrate phosphate buffer were added to each well. After incubation for 5 min, 100 μl of 2 mM sodium azide were added to stop the reaction. The optical density of the solution at 410 nm was measured with a microplate reader and the antibody titres were expressed as absorbance values. The positive cut-off value was determined as 0·05, because the mean and s.d. of 63 controls were very low (0·005 and 0·008, respectively).

Anti-Black GR IgE by RAST

To detect specific IgE antibodies to Black GR–HSA, we used RAST technique according to our previously described method [2,5,6]. The nitrocellulose filter paper (Millipore cat. no. HAHY, 08250, 0·45 μm; Millipore Co., Bedford, MA) discs were immersed in 0·1 mg Black GR–2 mg/ml HSA solution at 4°C for 3 days and air dried. They were blocked with 10% NCS for 1 hr, then incubated with 50 μl of serum for 6 hr and washed. Fifty microlitres of ^{125}I-labelled anti-IgE RAST tracer (Phamacia, Uppsala, Sweden) were added and left for 18 hr at room temperature. The discs were then washed again, and the bound ^{125}I was measured using a gamma counter. The results were expressed as percentage of bindings, defined as a percentage of the added c.p.m. bound to the Black GR–HSA conjugate disc.

Statistical analysis

The prevalence of serum antibodies between study groups was compared with the chi-square test.

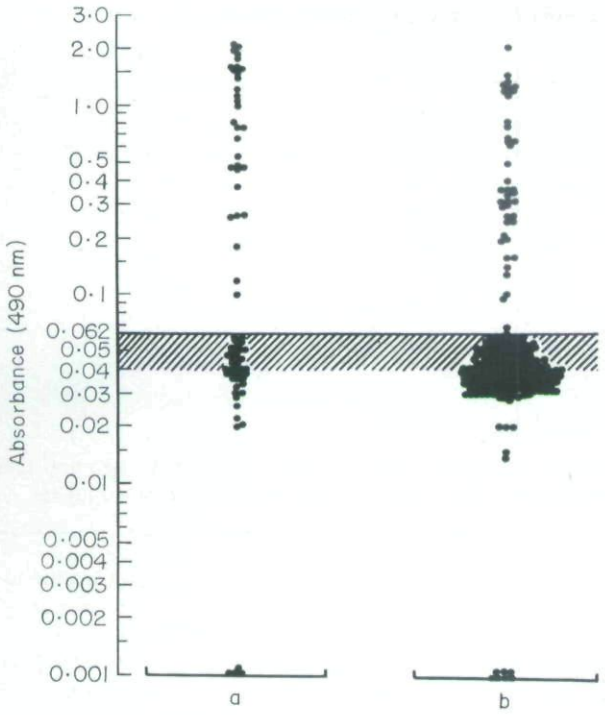

Fig. 1. The specific IgG antibodies to Black GR–human serum albumin conjugate in exposed workers with (a; $n=78$) or without (b; $n=231$) lower respiratory symptoms.

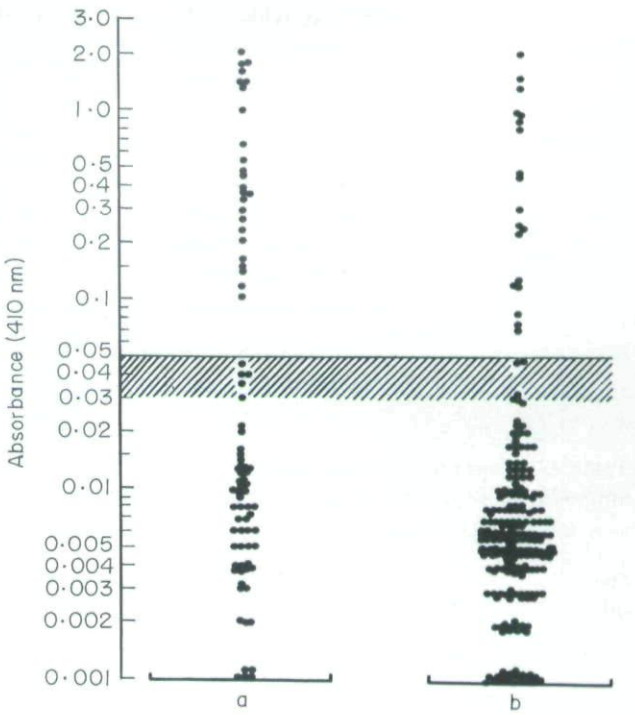

Fig. 2. The specific IgG_4 antibodies to Black GR–human serum albumin conjugate in exposed workers with (a; $n=78$) or without (b; $n=231$) lower respiratory symptoms.

Results

Immunologic studies

Figures 1 and 2 illustrate the specific IgG and IgG4 antibodies to Black GR–HSA conjugate in all employees. Table 1 summarizes the prevalence of specific IgE, sIgG, and sIgG4 antibodies to Black GR–HSA. Of the 78 symptomatic workers, 23 (30%) had high sIgE bindings to Black GR–HSA, whereas 29 (13%) out of 231 asymptomatic workers were positive ($P<0{\cdot}05$). There was also a close relationship between respiratory symptoms and the prevalence of sIgG and sIgG4 antibodies in that 32 (41%) out of 78 symptomatic workers had sIgG, and 25 (32%) out of 78 symptomatic workers had sIgG4 antibodies to Black GR–HSA ($P<0{\cdot}05$). Two individuals (one was an atopic patient) out of 63 control patients who had never been exposed to Black GR had sIgG antibody. Some asymptomatic workers had specific IgE (13%), sIgG (18%) or sIgG4 (8%) antibodies. The prevalence of sIgG and sIgG4 was significantly higher in workers who had specific IgE ($P<0{\cdot}05$), as shown in Table 2. The sensitivity and specificity for sIgG were 52% and 94% and those for sIgG4 were 64% and 95%, respectively, in workers with sIgE antibodies. The sensitivity and specificity for sIgG4 were 80% and 86% in workers with sIgG antibodies, respectively. The correlation was poor between specific IgE and sIgG levels ($r=0{\cdot}23$).

Relationship between intensity and length of exposure and serum antibodies to Black GR

This study included 209 workers who directly participated in the dye process (group II) and 100 workers (group I) whose work stations were distant from the dye process, including office workers, laboratory workers or electrical technicians. The prevalences of sIgG and sIgG4 were not significantly different between group I and group II, as shown in Table 3. The length of exposure varied from 1 month to 74 months. There was no correlation between duration of dye exposure and sIgG and sIgG4 level ($r=0{\cdot}008$ with sIgG, $r=0{\cdot}079$ with sIgG4, data not presented).

Association between smoking and atopy and specific IgG and IgG4 antibodies

Table 4 shows that there was no association between atopy and the presence of sIgG and sIgG4 antibodies. The prevalence of sIgG was 26% in atopic workers who were identified by skin-prick test with common inhalant aller-

Table 1. Specific antibodies to Black GR among workers with or without respiratory symptoms on dye exposure

	IgE antibody prevalence (%)	IgG antibody prevalence (%)	IgG_4 antibody prevalence (%)
Symptomatic workers	23/78 (30)*	32/78 (41)†	25/78 (32)‡
Asymptomatic exposed workers	29/231 (13)*	41/231 (18)†	19/231 (8)‡
Unexposed patients	0/13 (0)	2/63 (3)	0/63 (0)

*,†,‡ Prevalences were significantly different from symptomatic group and asymptomatic exposed group ($P < 0{\cdot}05$).

Table 2. Association between specific IgE and IgG or IgG_4 antibodies to Black GR–human serum albumin conjugate

Specific IgE antibody	IgG antibody prevalence (%)	IgG_4 antibody prevalence (%)
Negative reactor	34/255 (13)*	16/255 (6)†
Positive reactor	39/54 (72)*	28/54 (52)†

*,† Prevalences were significantly different from negative reactor and positive reactor on Black GR RAST.

Table 3. Exposure and specific antibodies to Black GR–human serum albumin conjugate

Group	IgE antibody prevalence (%)	IgG antibody prevalence (%)	IgG_4 antibody prevalence (%)
Group I	17/100 (17)*	18/100 (18)†	9/100 (9)‡
Group II	37/209 (18)*	55/209 (26)†	35/209 (17)‡

Group I, 100 workers whose work stations were far from the dye process; Group II, 209 workers who directly participated in the dye process.
*,†,‡ No significant difference between the two groups.

Table 4. Association between atopy and specific IgG and IgG_4 antibodies to Black GR–human serum albumin conjugate

Atopy* by skin test	IgG antibody prevalence (%)	IgG_4 antibody prevalence (%)
Presence	31/121 (26)†	16/121 (13)†
Absence	40/182 (22)†	26/182 (14)†

* Individuals who had a positive skin test to one or more common inhalant allergens.
†,‡ No significant difference between the two groups.

Table 5. Association between smoking and specific IgG and IgG_4 antibodies to Black GR–human serum albumin conjugate

Smoker	Specific IgG antibody* prevalence (%)	Specific IgG_4 antibody† prevalence (%)
Non-smoker	13/94 (14)	5/94 (5)
Former smoker	6/46 (13)	9/46 (20)
Current smoker	54/167 (32)	30/167 (18)

*,† Significant differences were found ($P < 0{\cdot}05$).

gens and 22% in non-atopic workers ($P < 0{\cdot}05$). Comparable findings were found in the prevalence of sIgG4 between atopic and non-atopic workers. However, Table 5 shows that the prevalence of sIgG and sIgG4 was significantly greater in smokers ($P < 0{\cdot}05$).

Discussion

Previous studies [2–5] demonstrated the presence of Black-GR-specific IgE antibodies by RAST employing the Black GR–HSA conjugate as the solid-phase antigen in dye-exposed workers. In this study, we succeeded in detecting sIgG and sIgG4 antibodies to Black GR–HSA by ELISA. The RAST system was thought to be a very effective method for IgE antibody detection, while the ELISA technique proved suitable for IgG antibody detection because there were non-specific bindings of IgG in the Black GR IgG-RAST system of our preliminary experiments as Layton *et al.* [7] had previously described.

The role of sIgG and sIgG4 in reactive dye asthma should be elucidated. Our previous study [6] revealed that sIgG to Black GR–HSA was detected in 85% of 20 occupational asthmatic patients due to Black GR. Indeed, 60% of them had Black-GR-specific IgE. Cartier *et al.* [8] noted that the level of sIgG to isocyanate (hexamethylene diisocyanate and methylene diisocyanate) bore a satisfactory association with the results of specific inhalation challenges, but the levels of specific IgE did not. Forster *et al.* [9] reported that the sIgG to tetrachlorophthalic anhydride (TCPA) was detected in all asthmatic workers and some of the exposed asymptomatic workers. Moreover, the sIgG4 antibody increased in five out of seven occupational asthmatic workers due to TCPA, but was not detected in any of the asymptomatic individuals. In this study, the prevalence of sIgG and sIgG4 to Black GR–HSA in 309 exposed workers was significantly higher in workers with respiratory symptoms than in asymptomatic workers. The prevalence of sIgG (41%) in symptomatic workers was higher than that of specific IgE (30%), but there was no statistical significance between them. The prevalence of sIgG and sIgG4 antibodies to Black GR in 14 asthmatic workers, who showed significant bronchoconstriction after inhalation of reactive dyes, were 86% and 57%, respectively. Of individuals who did not complain of symptoms on exposure to reactive dyes, 18% and 8% had sIgG and sIgG4, respectively, in their serum. There were a few IgG reactors (3%) of the control individuals who had never been exposed to reactive dyes. These results suggested that the existence of sIgG to Black GR might represent a response to Black GR exposure and that the presence of sIgG could be used as a method for assessing the exposure group. Although we observed that the prevalence of sIgG was higher in group II workers (participants in the dye process) than in those of group I, no statistical significance was found, suggesting that the intensity of the exposure within the factory did not influence the development of sIgG and IgG4 antibodies to Black GR–HSA. The correlation between length of exposure and sIgG level was poor. On rather limited data, it appears that the duration of exposure is not important.

This study revealed the association between smoking and sIgG and sIgG4 to Black GR–HSA conjugate. Comparable findings were found between smoking and specific IgE antibody to Black GR in our previous study [5]. The reason for this is unknown, but a possible explanation is that smoking increases antigen access to immunocompetent cells [10]. Smokers' lungs are more permeable to inhaled antigens, probably because of the inflammation in the airways produced by cigarette smoke [11]. However, no association was found between atopy and sIgG and sIgG4 antibodies in this study, which was a comparable finding to that of specific IgE in our previous study [5], suggesting that pre-existing atopy did not affect the development of sIgG and sIgG4 antibodies to Black GR–HSA.

Most of the IgE antibody responders were also sIgG and sIgG4 antibody responders, which suggested that specific IgE and IgG antibodies to Black GR–HSA conjugate might be a parallel immune response to inhaled antigens as other investigators have reported in grass and mite allergens [12–14]. However, 34 (11%) out of 309 workers had sIgG without specific IgE. The reason why reactive dye-exposed workers produce sIgG and sIgG4 antibody to Black GR-HSA, although they do not have specific IgE, is unknown. It is unusual with pollens or mite allergens to find non-allergic or skin test negative individuals who have any detectable IgG antibody, because the genetic factors controlling inhalant allergies might influence production of both IgG and IgE antibodies [15,16]. Recently, Platts-Mills *et al.* [17] described in a study of specific IgE and sIgG antibodies to rat urinary allergen that the exposure to rat urinary allergen in laboratory workers was quantitatively greater than exposure to pollen or dust mite allergens, and that the production of sIgG in asymptomatic individuals might represent a response to a relatively high dose exposure in individuals who would probably fail to respond to a lower dose.

The existence of specific IgG antibody to Black GR–HSA conjugate might represent the sensitization to Black GR exposure and be closely related with respiratory symptoms.

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