

Increasing allergy potency of *Zinnia* pollen grains in polluted areas

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Received 25 November 2002; received in revised form 6 October 2003; accepted 8 December 2003

Abstract

There is much evidence that allergic symptoms represent a major health problem in polluted cities. The aim of this research is to elucidate some microscopic effects of air pollutants on pollen structure, proteins, and allergenicity. A scanning electron microscopy study of pollen grains indicated that in polluted areas, airborne particles accumulate on the surface of pollen grains and change the shape and tectum of pollen. Also, many vesicles are released from polluted pollen grains and the pollen material agglomerates on the surface of pollen grains. SDS–PAGE revealed that different proteins exist in mature and immature pollen grains. There were no significant differences between protein bands of polluted and nonpolluted pollen grains, but in polluted pollen, protein content decreases in response to air pollution, causing the release of pollen proteins. The results indicate that mature pollen have more allergenicity than immature pollen. According to the experiments polluted pollen grains are more effective than nonpolluted pollen grains in inducing allergic symptoms. Air pollutants can cause allergic symptoms, but when associated with allergen pollen grains, their allergenicity power is increased.

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Keywords: Air pollution; Allergy; Pollen; Allergotoxicology; Electron microscopy; Proteins

1. Introduction

Over the centuries, air pollution has increased from a local nuisance to a global problem. There is some evidence that confirms that air pollution may be responsible for the increase in pollen-induced allergies and asthma in highly polluted areas (Ishizaki et al., 1987; Ruznak et al., 1994; Behrendt et al., 1992, 1997). Grimfeld (1988) and Brun-Fahrland et al. (1992) reported increased asthma in children due to SO₂, cigarette smoke, and air pollution. Riedel et al. (1988) suggested that air contaminants such as SO₂ and other chemical pollutants are responsible for asthma developing in industrial regions. Additionally, irritant gases and/or diesel exhaust particles have been shown to express both adjuvant activity for sensitization against

common allergens and enhancing effects on allergic symptoms in sensitized individuals (Holt et al., 1986; Matsumara, 1970; Riedel et al., 1988; Helander et al., 1997; Behrendt et al., 1992, 1997). Pollen grains accumulate heavy metals, i.e., lead and cadmium, but also nitrate, sulfur, and other compounds (Behrendt et al., 1992, 1997; Sawidis and Reiss, 1995; Sawidis, 1997). Laboratory experiments have shown that NO₂, SO₂, and CO treatments can cause changes in the soluble protein composition of pollen grains. In this report, complex interaction between allergenic pollen grains and air pollutants is investigated.

2. Materials and methods

2.1. Sampling

Because it is a highly polluted city Tehran was chosen, as an ideal sampling site for these studies. We studied *Zinnia elegans* Faq.var.red (2n = 24) from the Astraceae

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family, which are grown in the central areas of Tehran, a polluted region, and also outside Tehran, a nonpolluted area (control). Fresh pollen grains were purified by passage through mesh with 30- μm -diameter pores. Some of the pollen grains were applied directly and others were exposed to Tehran's polluted air for 10–20 days. We collected immature pollen grains from immature anthers prior to release of pollen grains and mature pollen grains from plants that were growing in polluted areas.

2.2. Scanning electron microscopy

Polluted and nonpolluted pollen grains were studied by scanning electron microscopy. Purified pollen grains were fixed with glutaraldehyde, postfixed with osmium, dehydrated, and dried prior to gold coating. After being coated with gold, samples were analyzed using a scanning electron microscope (Model SEM-EDS, EX 300, Link, England).

2.3. Protein studies

Extraction of pollen protein from nonpolluted and polluted pollen grains was carried out separately at 4°C in Tris–HCl buffer, pH 7.6. Finally, 12% SDS–polyacrylamide gel electrophoresis was performed for the total soluble protein according to the method of Laemmli (1970). Soluble proteins were extracted in sample buffer (0.125 M Tris–HCl, pH 6.8, 4% SDS, 20% glycerol, 10% β -mercaptoethanol, 0.1% bromophenol blue dye) with heating for 3–4 min at 100°C before loading. The amount of protein was 10 μg per channel and the total current was 14 mA. The gel was run in Tris–glycine buffer (pH 8.3) with 0.1% SDS and calibrated with a marker protein obtained from Sigma. Protein concentration of the pollen extract was determined by using the method of Lowry et al. (1951).

2.4. Animals

Male 4–6 week-old guinea pigs weighing 350–500 g were sensitized and treated with *Z. elegans* pollen extracts. The animals were housed in an air-conditioned room at $25 \pm 2^\circ\text{C}$; they were fed a standard laboratory diet and given water with added vitamins. The experimental animal research section at Razi Institute provided these animals. Animals were sensitized and treated by injection of 100 μL pollen extract (containing 50 μg protein in phosphate-buffered saline) from immature, nonpolluted, and polluted pollen grains separately, with 50 μg $\text{Al}(\text{OH})_3$ added as an adjuvant. $\text{Al}(\text{OH})_3$ was suspended in saline buffer and added to pollen extract. Treatments were repeated five times, for 5 weeks, once each week.

2.5. Skin test

The antigenic components of *Zinnia* pollen grains are readily extractable and stable to proteolysis. Pollen extracts were prepared from all samples (including immature, mature, and polluted pollen) by the method of Sheldon et al. (1967), and skin prick tests were performed on guinea pigs. Each animal was tested with 100 μL of the total extract diluted with 0.05 M phosphate-buffered saline, pH 7.4, containing 50 μg protein without adjuvant. The negative control was buffered saline and the positive control was histamine acid phosphate. Skin reactions were read 1, 4, 8, and 24 h from the beginning of the test and quantified on the basis of wheal diameter.

2.6. Clinical and serological tests

Prior to each experiment, the guinea pigs were bled once. Blood samples were drawn directly from the heart (Aberg, 1989). After treatment with pollen extracts, the guinea pigs were anesthetized with chloroform, then blood was obtained directly from the heart. Sera from all samples were stored at -20°C until analyzed. The sera from 20 *Zinnia*-allergic sensitized animals were individually tested for IgE reaction to *Zinnia* nonpolluted pollen grains. In the next step, the sera of 20 sensitized animals were tested for polluted pollen grains. Also, 20 guinea pigs were tested for immature pollen. Sera from 20 animals that were not sensitized to *Zinnia* pollen were also tested as control sera. Total serum IgE was evaluated using the ELISA for blood. The standard Pharmingen IgE protocol for sandwich ELISA was used to quantify the total amount of IgE. The serum IgE level was expressed in nanograms per milliliter. Blood smears of healthy controls were compared with those of experimental animals. Total numbers of different kind of blood cells, especially eosinophils and neutrophils, were determined in all groups and then were statistically analyzed.

3. Results

3.1. SEM analysis

Pollen grains of *Z. elegans* are spherical and apolar and 20–25 μm in diameter; they possess a thick skin and three visible pores. SEM analysis showed that the tectum on pollen grains has long needles (Fig. 1). On the surface of nonpolluted pollen grains neither atmospheric fine dust nor agglomeration was visible. After contamination with polluted air the color of the pollen grains changed dramatically and darkened considerably. Moreover, when pollen grains were exposed to polluted air they became folded, vermicular particles accumu-

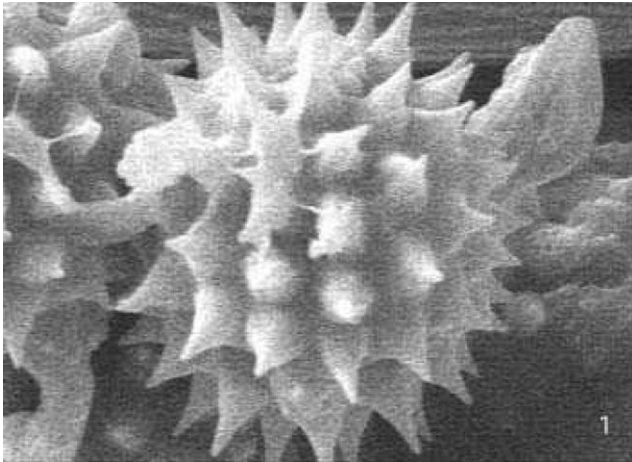


Fig. 1. Scanning electron micrograph of *Zinnia* pollen grains collected from nonpolluted regions. Pollen grains are spherical having long needles as tecta ($\times 1700$).

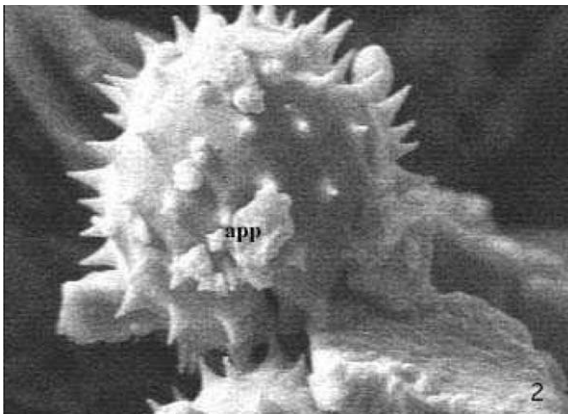


Fig. 2. Scanning electron micrograph of *Zinnia* pollen grains collected from polluted regions. Pollen grains are folded, tecta are disrupted, and airborne particles have accumulated on the surface of pollen grains. app, air pollutant particles ($\times 1700$).

lated on the surface of pollen grains (Fig. 2). Pollen grains collected from the polluted area were also covered with large amounts of pollutants, and the tectum of polluted pollen grains was disrupted. Interaction between pollen grains and particulate pollutants caused release of pollen constituents and agglomeration of organic particles on the pollen surface (Fig. 3).

3.2. Protein analysis

SDS-PAGE protein profiles of different pollen samples (immature, polluted, and nonpolluted) are shown in Fig. 4. SDS-PAGE revealed that several protein bands exist in the immature pollen grains, and are different from bands observed in mature pollen grains. Pollen grains collected from the polluted area have the same bands as nonpolluted pollen grains, and also bands of pollen grains treated with polluted air were identical to normal ones. Furthermore, in all

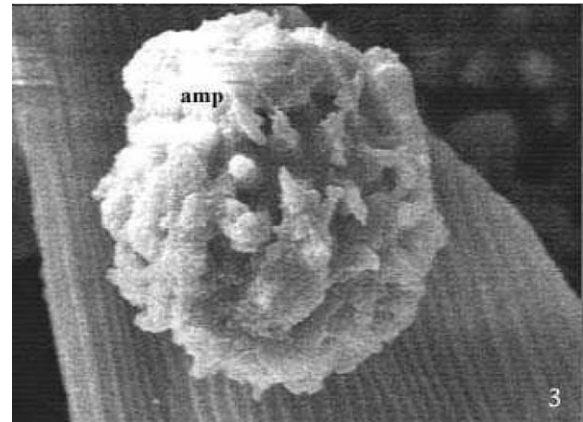


Fig. 3. Scanning electron micrograph of *Zinnia* pollen grains that were exposed to Tehran polluted air for 20 days. Pollen grains are folded, airborne particles have accumulated on the surface of pollen and pollen, material has been released by pollen and has agglomerated on the surface of pollen grains. amp, agglomerated material on the surface of pollen ($\times 1700$).

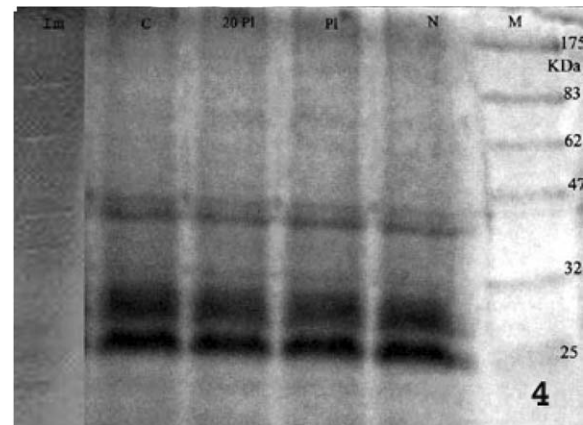


Fig. 4. Protein profile of different pollen grains. Results show that polluted and nonpolluted pollen grains have the same bands, but immature pollen grains have several different bands. M, protein markers; Im, bands of immature pollen grains; N, bands of normal mature pollen grains; PI, bands of pollen grains collected from polluted area; 20PI, bands of pollen grains exposed for 20 days to pollution; C, bands of pollen grains exposed for 20 days to clean air.

polluted pollen grains, protein content decreased in response to air pollution. Protein content of extracts from pollen grains collected on the same day from plants growing in the nonpolluted area and plants growing in the polluted area and pollen grains aerated with polluted air, all collected on the same day, is summarized in Table 1. Significant changes ($P < 0.01$) were noted in total concentration of soluble protein per gram of pollen, between polluted and nonpolluted pollen grains.

3.3. Skin test

Results of the skin test in guinea pigs for different samples are summarized in Table 2. Pollen extract had

Table 1

Total protein content of polluted and nonpolluted pollen grains of *Zinnia elegans*

Specimen	Protein content (mg/g)
Normal pollen grains	4.6 ± 1.8
Pollen grains exposed to pollution for 10 days	3.7 ± 1.5
Pollen grains exposed to pollution for 20 days	2.8 ± 0.9

Table 2

Results of skin test for different pollen extracts

Sample	Wheal diameter (cm)
C ^a	1.1 ± 0.5
Im	1.95 ± 0.9
N	2.5 ± 0.7
Pl	4.3 ± 0.9

^aC, control group treated with saline; Im, group treated with immature pollen extract; N, group treated with nonpolluted pollen extract; Pl, group treated with polluted pollen extract.

Table 3

Results of serological studies: determination of numbers of eosinophils and neutrophils and evaluation of blood IgE in different experimental and control animals

	C ^a	Im	N	Pl
Eosinophils ($\times 10^4$ cells/mL blood)	5.5 ± 1.3	9.8 ± 2.2	21.6 ± 3.9	58.7 ± 5.6
Neutrophils ($\times 10^4$ cells/mL blood)	35 ± 1.9	6.8 ± 3.4	9.4 ± 2.8	9.3 ± 3.7
IgE (ng/mL)	9.5 ± 2.6	15.2 ± 4.7	104 ± 12.2	168.2 ± 18.2

^aSee Table 2 for abbreviations.

an allergenic effect. During the skin prick test, the maximum allergenic sensitivity was observed for pollen grains collected from the polluted area, with an average wheal diameter of about 5 cm. Allergic reactions to normal pollen grains collected from the nonpolluted area, were relatively large, with an average wheal diameter of 3 cm. Statistical analysis showed that the effect of polluted pollen grains is about two times that of normal pollen extracts ($P < 0.05$).

3.4. Clinical and serological tests

Results of blood smears from different groups are summarized in Table 3. The numbers of eosinophils and neutrophils were increased in the animals treated with pollen extract. The total number of eosinophils in guinea pigs treated with polluted pollen grains was about two times that of guinea pigs treated with nonpolluted pollen grains and several times greater than that of guinea pigs treated with saline, as the control group (Fig. 5).

Data for determination of IgE in different groups are given in Table 3 and Fig. 6. All pollen extracts caused an increase in IgE levels in the blood of treated animals.

Eosinophilia in the peripheral blood of different experimental groups that treated by saline or pollen extracts

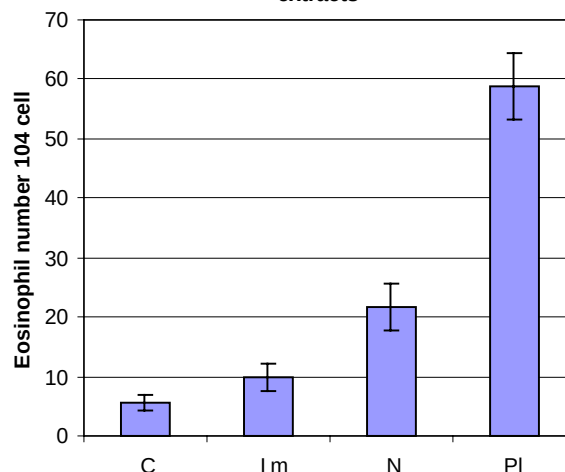


Fig. 5. Eosinophilia in the peripheral blood of guinea pigs treated with different pollen extracts or saline. Polluted and nonpolluted pollen extracts caused an increase in eosinophils characteristic of allergic, but polluted pollen grains were more effective. C, control group treated with saline; Im, group treated with immature pollen extract; N, group treated with nonpolluted pollen grains; Pl, group treated with pollen grains collected from polluted area.

Increasing of total blood IgE in animals that treated by pollen extracts

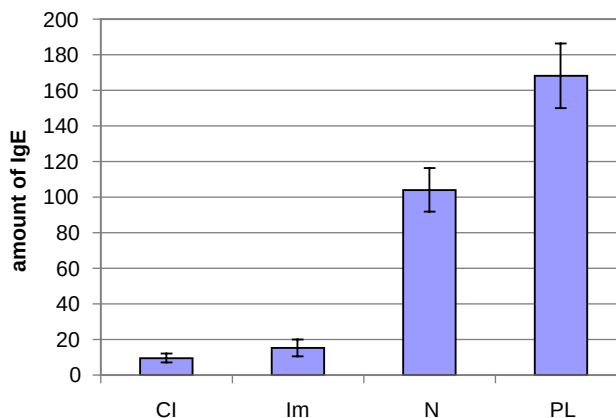


Fig. 6. Amount of IgE (ng/mL blood) in blood of animals treated with different pollen extracts or saline. Result show that both nonpolluted and polluted pollen extracts induce an increase in blood IgE as allergy inducers but polluted pollen grains with more effective. C, control group treated with saline; Im, group treated with immature pollen grains; N, group treated with nonpolluted pollen grains; Pl, group treated by pollen grains collected from polluted area.

Serum of animals treated with nonpolluted pollen extract contained more IgE than serum of control animals. The amount of IgE in the serum of animals treated with immature pollen extract was more than that in the control group, but less than in the other experimental groups. The results indicate that the amount of IgE increased significantly in the animals

treated with polluted pollen extract. Statistical analysis indicates that differences between polluted and non-polluted groups are significant ($P < 0.05$).

4. Discussion

Ultrastructural studies (Figs. 1–3) revealed that air pollution affects pollen grains. On the surface of nonpolluted pollen grains neither atmospheric fine dust nor agglomeration was visible. When pollen grains were exposed to polluted air they became folded, and vermicular particles accumulated on the surface of the pollen grains (Fig. 2). Pollen grains collected from the polluted area were covered with large amounts of pollutants and the tectum of polluted pollen grains was also disrupted (Fig. 3). Agglomeration of airborne particles was observed on the surface of pollen collected from the polluted area (Figs. 2 and 3), which is in agreement with observations reported by Behrendt et al. (1988), Ghanati and Majd (1995), Majd and Kiabi (1997), and Knox and co-workers (Knox and Suphioglu, 1996; Knox et al., 1997). The results of 12% SDS–PAGE analysis for soluble proteins (Fig. 4) demonstrated the same bands in pollen grains that collected from both polluted and nonpolluted areas. It seems that air pollution could not affect the developmental process of pollen grains and protein synthesis, which is different from findings of Ruffin et al. (1983), Kainka-Stanicke et al. (1989), Ruznak et al. (1994), and Chakraborty et al. (1997) but is in agreement with the findings of Behrendt et al. (1997). According to the present results, total protein of pollen grains significantly decreased in all polluted groups (Table 1). This is in accordance with the findings of Behrendt et al. (1997), but not those of Ruffin et al. (1983). Release of pollen material is the major cause of the decrease in pollen protein content in polluted areas.

The rapid development of major cities together with heavy vehicle exhausts leads to many difficulties for people. There is evidence that air pollution not only enhances the risk of developing asthma but may also be responsible for the increasing prevalence rates of pollen allergies in highly polluted cities (Ishizaki et al., 1987; Behrendt et al., 1992). The allergenic potential of polluted and nonpolluted pollen grains was measured by wheal diameter, leukocyte number changes, and total blood IgE. Clinical and serological results indicated that number of eosinophils, average wheal diameter, and amount of IgE are greater in guinea pigs treated with extracts of fresh pollen than in the control group (Tables 2 and 3). Statistical analysis indicated that *Z. elegans* is an allergen plant and an attempt was made to detect its allergens. The results indicated that mature pollen grains are more allergenic than immature pollen. This means that a significant amount of allergens are produced in

the developmental process of pollen formation that later accumulate in mature pollen grains.

The different results, including skin tests, evaluation of IgE, and determination of blood cell number, indicated that allergenic potential of polluted pollen grains is greater than that of nonpolluted pollen grains, is accordance with some prior findings (Behrendt et al., 1992, 1997; Ruznak et al., 1994; Knox et al., 1997; Emberlin, 1998).

According to the current observations organic substances that exist in the air pollutants mediate agglomeration of particles on the pollen surface. Under appropriate conditions, i.e., humidity, water-soluble compounds of air pollutants may induce local allergen release and agglomeration of pollen material including pollen proteins on the surface of pollen grains. It seems that air pollutants increase the solubility of pollen proteins, similar to detergents. Furthermore, air pollutants damage the membrane and cell wall of pollen grains, which, in turn, maximizes the release of pollen materials. A consequence of the release of allergen molecules into aerosol is that the molecules are free to interact with other types of airborne particles, such as diesel exhaust particles, that are associated with air pollution. The findings of Ito et al. (1996), Majd and Kiabi (1997), Behrendt et al. (1992), Behrendt and Becker (2001), and Knox and co-workers (Knox and Suphioglu, 1996; Knox et al., 1997) support this idea. Therefore, one has to take into consideration that in regions with high pollution, air pollutant particles are not only a carrier of pollutants, but are also a carrier of allergens and probably altered allergens, and that pollens are not only carriers of allergens, but also carriers of pollutants (Behrendt et al., 1992).

5. Conclusion

The results of this study indicate that, in polluted areas, air pollutant particles are adsorbed and accumulate on the surface of pollen grains, therefore modifying pollen shape and structure. Air pollutants induce allergen release and agglomeration of pollen material, including pollen proteins, on the surface of pollens. Releasing pollen allergens on the surface of pollen grains is one of the important reasons allergy is frequent in polluted areas. On the other hand, pollen allergens adsorbed by airborne particles alter their properties, and modification of these properties increases allergy symptoms in polluted cities.

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