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Immunoelectron-microscopic Localization of IgE Binding Site of Mugwort Pollen

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To elucidate the IgE binding site of mugwort (Artemisia vulgaris r.) pollen, pollen grains were frozen and fixed using a cryocut. They were incubated with antibodies according to the following sequence: Sera pool of individuals who showed mugwort-RAST class 3 or 4, biotin-labeled goat anti-human IgE antibody, streptavidin-peroxidase and diaminobenzidine. Then, they were observed under electron microscopy. The control section was incubated with the sera pool from individuals who showed a negative result on a skin prick test to mugwort pollen.

Antigenic activity (electrondense line) was noted on the surface of the exine. There was no activity in cytoplasm or the intine layer. The control section was completely free of activity.

It was suggested that the IgE binding site of mugwort pollen was present on the surface of the exine.

Key Words: *Mugwort pollen, IgE binding site.*

INTRODUCTION

Pollen allergens are present in the pollen grains of various plant species. On contact with wet human mucosa, they are rapidly released from the grains and, in susceptible human individuals, lead to the formation of specific reaginic antibody, IgE.

In attempts to localize the morphological sites of the allergens within the pollen grain, the approach was tried by Knox and Helslop-Harrison (1971) who found IgE binding sites on ragweed pollens using the indirect fluorescence, immunolabelling technique. Recently, other investigators (Grote et al., 1987) have tried to localize the IgE and/or IgG binding sites on birch pollens using the immuno-gold technique.

Mugwort (Artemisia vulgaris L.) pollen is one of the common cause of allergic reaction during late summer in northern Europe (Charpin et al, 1974). In Korea, the pollen of Artemisia, one of the most important allergens in this country (Park et al., 1989; Park et al., 1989) is reported to be abundant in the air of Seoul from the end of August through September (Hong et al., 1986). Many respiratory allergy patients surffer from aggravation of their symptoms during this season.

In this study, to observe the IgE binding sites within the mugwort pollens, we used the immunoelectron-microscopic method.

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MATERIALS AND METHODS

Human Serum

Sera were collected and pooled from 10 untreated patients allergic to mugwort pollens who showed class 3 or 4 response on the W_6 (mugwort) radioallergosorbent test (RAST, Pharmacia, Sweden). They were used as a positive sera pool. Sera from individuals who showed negative responses to 50 common inhalant allergens on skin prick tests were also obtained and pooled. They were used as a negative control.

Immunochemical Labelling

The mugwort pollens were collected from flowering plants during the autumn season. The dried pollens were defatted with diethylether. They were frozen at minus twenty degrees centigrade, sliced into 6 um thickness using a cryocut (2800 Frigocut, Reichert-Jung, Germany) and fixed on glass slides in a frozen state. One percent hydrogen peroxide was applied to exclude endogenous peroxide reaction within the pollens. Non-specific binding was blocked by incubating the pollens with 10% newborn calf serum (Sigma Chemical Co. USA) for 1 hour. Additionally, positive sera pool and negative control sera pool were incubated on the slides for 2 hours at room temperature. They were washed three times with phosphate buffered saline (PBS). Biotin conjugated goat anti-human IgE antibody (Sigma Chemical Co., USA) diluted to 1:1000 (w/v) with 10% newborn calf serum was incubated at room temperature four 2 hours and washed again. Streptavidin-peroxidase (Sigma Chemical Co. USA) diluted to 1:1000 w/v with PBS was incubated for 30 minutes and washed again. 0.06% diaminobenzidine-0.01% H_2O_2 (Kirkegaard & Peery Laboratories Inc. USA) was applied as a substrate solution for 10 minutes and washed again. Mugwort pollens without incubation with sera pool or labelling antibody were prepared, fixed according to the above method and observed. All slides, in which immunolabelling had been finished, were fixed for electronmicroscopic observation. At first, 1% O_sO_4 in 0.1M phosphate buffer was applied for 2 hours. They were dehydrated and embedded in epon resin. Ultrathin sections were cut using an ultramicrotome (Sorvall MT 6000, USA) into 60 to 80 nm thickness. They were stained with uranyl acetate and lead citrate. The sections were examined under a Hitachi H-600 transmission electron microscope (Japan) operated at 75 KV. Micrographs were taken using Agfa-Gevaert 23D56 film.

RESULTS

The section of the native pollen grains (×17,000), without incubation with any sera or labelling antibody is shown in Fig 1. The outer layer is exine and under it, is the intine layer. In the middle of the pollen, there is cytoplasm. Fig 2. shows the section of mugwort pollen (×20,000), in which a positive sera pool was applied. The electron-dense line (arrow indicates) was noted on the surface of the exine layer (indicated by arrow). No discernible finding was noted in the intine layer or cytoplasm. Fig 3. shows the close-up section

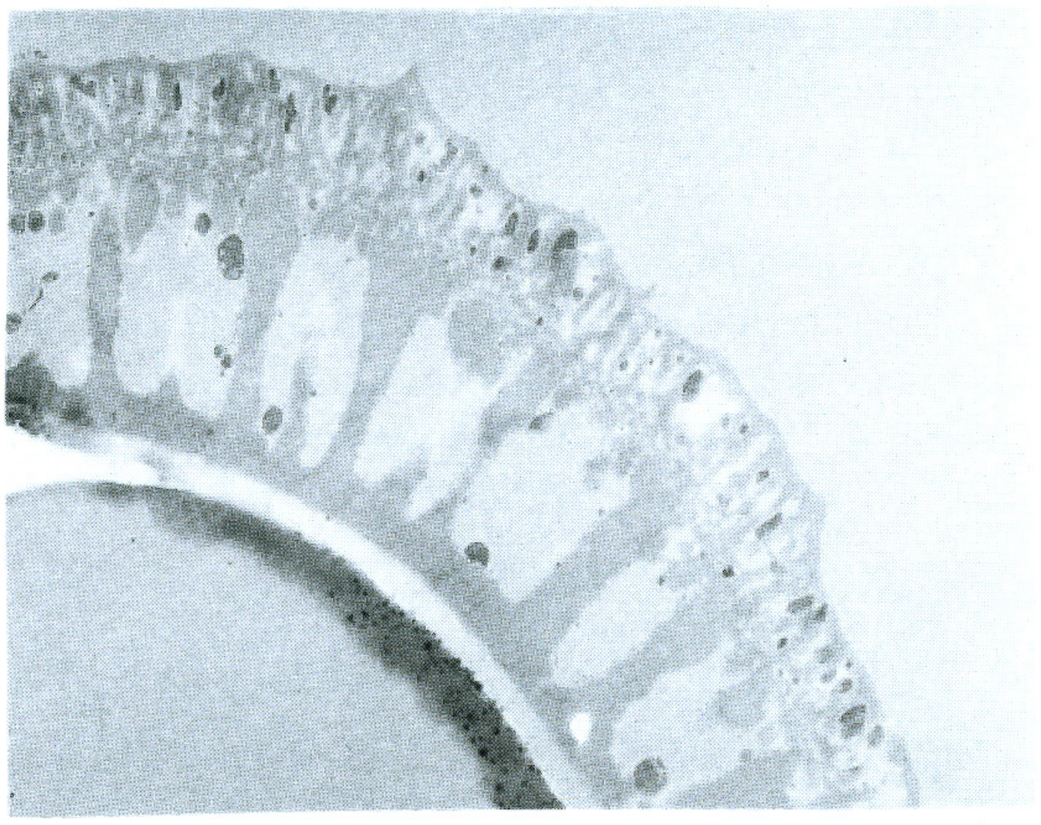

Fig. 1. Ultrathin sections of the native mugwort pollen without incubation with any sera or labelling antibody.

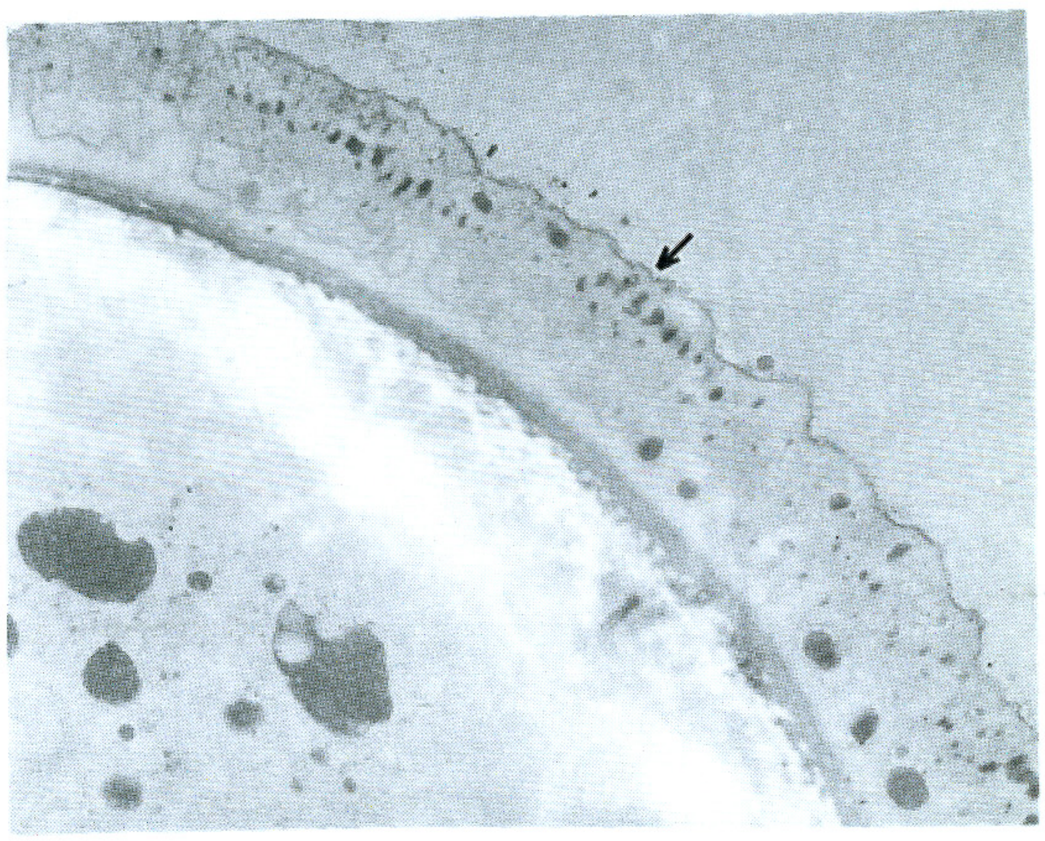

Fig. 2. Ultrathin section of mugwort pollen, in which a positive sera pool was incubated.

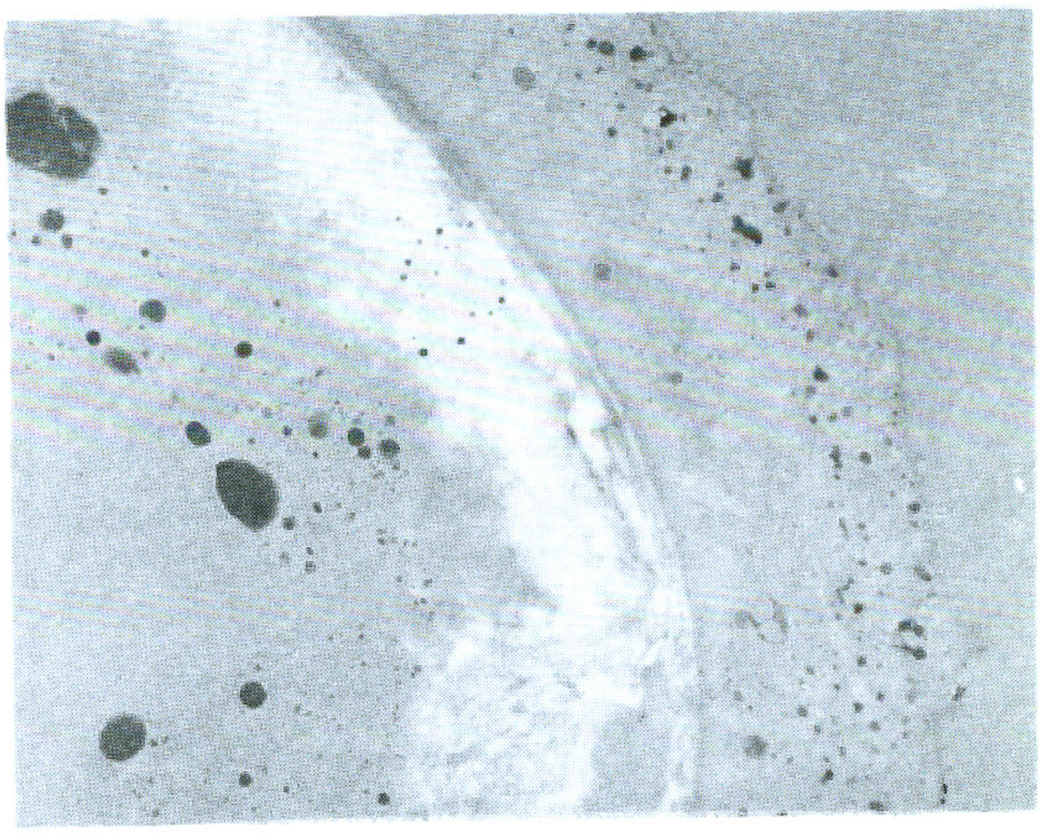

Fig. 3. The close-up finding of an ultrathin section of a positive control.

of the positive control (×17,000). An electrondense line (arrow indicates) was noted on the surface of the exine. Fig 4. shows the section (×17,000), in which a negative sera pool was applied. There was no electron dense line in the exine layer or in any other site of the pollen. The electron-dense bodies in the exine layer were observed in both positive and negative samples as well as in the native pollen.

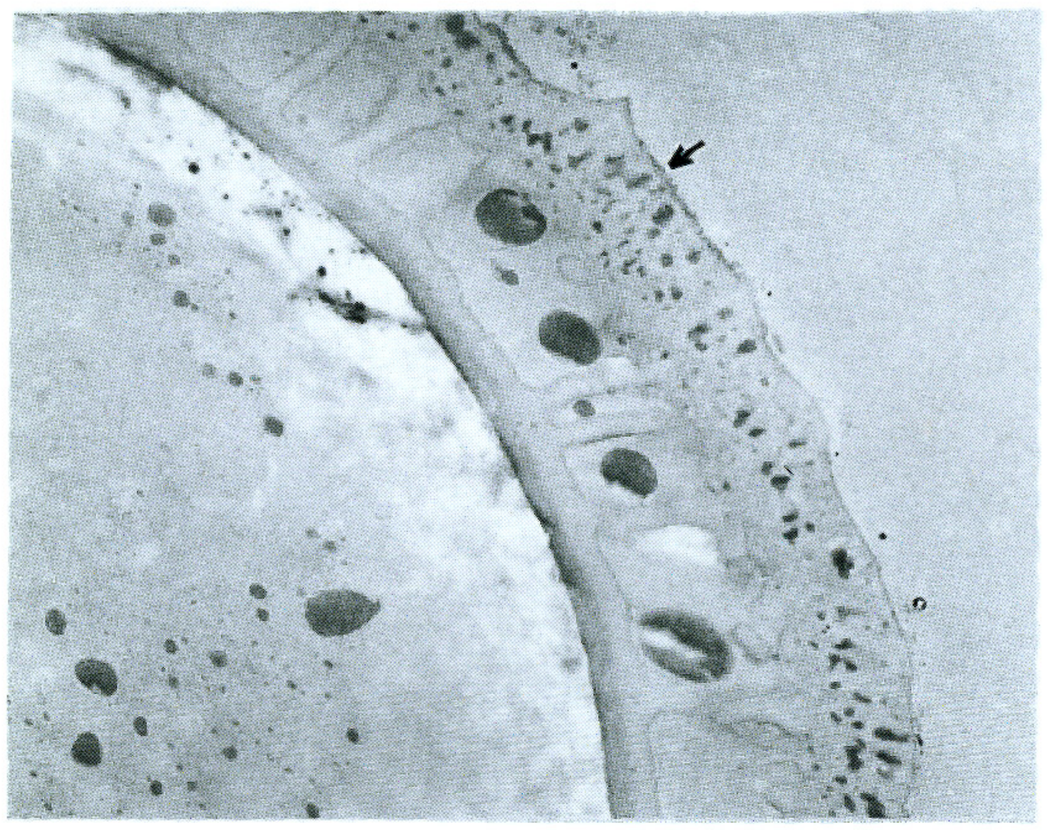

Fig. 4. Ultrathin section of mugwort pollen, in which a negative sera pool was incubated.

DISCUSSION

The present study has the first time localized on the ultrastructural level the IgE binding site on mugwort pollen using sera from human atopic individuals with the immunolabelling technique. The assumption is that the positive labelling of the section (electrondense line) might be due to specific binding of IgE antibodies to endogenous pollen substances.

Concerning the distribution pattern of IgE binding sites within pollens, there is some disagreement with the results published by other investigators (Knox and Heslop-Harrison, 1971; Grote and Fromme, 1984; Grote and Fromme, 1986; Grote et al., 1987). Knox and Heslop-Harrison (1971) reported that antigen E, the most active human allergen of Ambrosia (ragweed) pollen, was present in the intine layer by use of an immunofluorescence method. Howlett et al. (1973) detected diffusible immunoreactive proteins within the exine and intine layers of Ambrosia (localization of Antigen E). Some investigators (Knox and Heslo-Harrison, 1970; Vithanage et al., 1980; Howlett et al. 1981; Vithanage et al., 1982) localized 'Antigen A' and 'Group I allergen' not only in Lolium perenne pollen in the exine and intine layers, but also within some cytoplasmic sites of pollen grain. Grote and Fromme (1986) reported that the IgE binding sites of birch pollens (Betula pendula) were noted in the bacular cavities, and in the cytoplasm. There was no labelling of the intine. They used cetylphyridinium chloride (CPC) and paraformaldehyde as fixatives to preserve the diffusable proteins in birch pollens. In this study, the IgE binding sites of mugwort pollens were noted on the surface of the exine layer. There was no labelling of the intine layer or cytoplasm. This is a different finding from other pollens and there would be at least three possible explanations. First, we used the pre-embedding technique, in which frozen pollens were incubated with labelling antibody, and then were fixed and embedded in epon resin. This method can result in the disadvantage of antibody molecules having to diffuse into cut or whole cells, and they therefore may be unable to penetrate into uncut cells through the plasma membrane, giving false negative results in the intracelluar compartment (Knox et al., 1980). Freeze-dried specimens are brought into contact with aqueous incubation or washing media for shorter or longer periods of time; this might result in the danger of losing antigens. Secondly, in contrast to other pollen species, there might be no IgE binding sites within the intine and cytoplasm of mugwort pollens. It might be suggested that the IgE binding sites of mugwort pollens might be slightly different according to maturation of pollens. Thirdly, previously reported results-perhaps due to a different preparation from that used in this study-might present a 'snap shot' of the dynamic process of antigen migration. Previous descriptions of antigens within the intine and within both the cytoplasm and intine could show already mobilized antigens on their way from the cytoplasm through the intine and exine to the surface of the grain. Further investigations are required to compare the results using different preparation methods such as post-embedding technique and different fixation methods as Grote and Fromme (1984) suggested.

In conclusion, it was suggested that the IgE binding sites of mugwort pollen might be present in the exine layer. Further investigations are needed to study results based on different preparation methods.

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