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SENSITIZATION TO THE STORAGE MITE *TYROPHAGUS PUTRESCENTIAE* IN URBAN POPULATION OF UPPER SILESIA (POLAND)

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ABSTRACT. Exposure to indoor allergens, especially dust mites has been recognized as a risk factor for sensitization and allergy symptoms that in extreme conditions could develop into asthma. To determine specific antigens responsible for allergy in patients positive for mite allergy skin tests whole protein extracts from the cultured mite species *Tyrophagus putrescentiae* [TP], and from their excrements were obtained. The proteins were fractionated by SDS PAGE and identified by Western blot. The patient antibodies against particular antigens were identified in serum IgE fraction using anti-human anti-IgE monoclonal antibody. Western blot analysis revealed differences in reactivity of sera from patients positive for standard mite allergy skin tests with fractionated mite antigens. A total of 17 of 30 sera (56.7%) from patients positive to skin tests showed specific cross reactivity with antigens isolated from extracts of TP. The results revealed that 12 out of 30 tested sera (40.0%) reacted specifically with new antigens identified as protein fractions of extracts from excrements of TP. When assessing mite allergen reaction using mixture of mite proteins the results of the test are not satisfactory for determining the antigen causing patient allergy. The results obtained from studies reported here indicate significant discrepancy between the so called standard allergy skin test and what the patient is actually sensitive to. Also, a new class of immunizing protein of about 25 kDa has been identified in excrements from TP reacting with IgE from patients showing allergy to other mite extracts. A total of 4 of 30 sera (approx. 13.3%) from patients positive to skin tests showed specific cross reactivity with antigens isolated from mite excrements rather than from mite whole extracts.

Key words: allergenic mites, exposure, mite allergen, mite antigen, sensitization, storage mites, symptoms, *Tyrophagus putrescentiae*, urban population.

INTRODUCTION

Allergic disease is a common disorder affecting 40% of the world population. One of the most important events in the history of allergic disease was the discovery by Voorhorst et al. (1964) that dust mites are the major source of allergens in house dust. Dust mites are common in homes worldwide, and many species are the sources of potent allergens (Aki et al. 1995, Aalberse 1998, Arlian 2002), also in

Poland (Solarz 2001a, b). Sensitization to allergens derived from house dust mites is strongly associated with 3 diseases: asthma, perennial rhinitis, and atopic dermatitis. It is clear that mite bodies and mite faeces are the sources of many allergens. The allergens associated with mite fecal matter are enzymes that originate from the mite's digestive tract. Possible sources of other allergens include enzymes associated with the molting process that occurs as mites change from one life stage to the next. Some allergens may be components of mite saliva that is left in the environment on food substrates where mites feed. Mite allergens are divided into 15 specific groups (Arlian 2001, 2002) on the basis of their biochemical composition, sequence homology and molecular weight. Many species of storage mites occur in both residential and occupational environments and in processed foods and can cause allergic diseases. The most common mites found in houses in temperate climates are *Dermatophagoides farinae* and *D. pteronyssinus*. Large numbers of storage mite species such as *Tyrophagus putrescentiae* and *Lepidoglyphus destructor* may occasionally occur in dwellings. Sensitization to stored-product mites in urban dwellers has been reported in many countries. Generally the of storage mites in sensitizing and causing allergy in an urban setting is not known (Arlian 2002).

MATERIAL AND METHODS

Human sera. Whole sera were obtained from 30 atopic subjects (age range 10-40), known to be sensitive to house dust mites *Dermatophagoides* spp. (*D. pteronyssinus* and/or *D. farinae*), who live in Upper Silesia, in urban area where these mite species are prevalent (Solarz 2001a, b).

Mite body extract. Laboratory grown (25°C, 75% RH) adult male, female and larval stage house dust and storage mites were harvested from culture media (fish food) using a heat aggregation technique (in 45°C).

Faecal extract. Faecal enriched spent culture media was obtained from spent culture sieved through a 35 μm pore mesh. Microscopical examination of sieved material revealed only limited contamination by particulate matter from the mite body.

Sample preparation. Mite extracts were used fresh and stored for not longer than 16 h at 4°C before use. Mite bodies were heated (95°C, 5 min), homogenized in sample buffer (pH 6.8: 0.025 M Tris-HCl, 2% SDS, 10% glycerol) and further heated (95°C, 5 min). Faecal material was vortexed (4°C, 3 min) in sample buffer, passed through a 0.2 μm sterile filter and heated (95°C, 3 min). Identical samples were pooled and loaded 9-14 μg protein/track.

Protein determination. Protein determination of mite body and faecal samples of each mite species were done using an adaptation or the Bradford assay.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was performed as described by Laemmli (1970) with same modifications using a mini-Protean II slab system (Biorad Ltd, U .K.). Each mite species was

represented on each gel along with a control track containing food culture media prepared as for faecal extracts. Electrophoresis was performed at 100 V for 60 min using 12% separating gels.

Electrotransfer. Proteins were electrotransferred by a modified method of Towbin et al. (1979) onto a polyvinylidenedifluoride (PVDF, Immobilon P, Millipore, U .K.) membrane using a mini-Transblot cell (Biorad Ltd, U.K.). Electrotransfer was carried out at 400 mA for 1 h with cooling.

Immunoprobing. Blotted membranes were blocked for 4 h at ambient temperature (22˚C) with 5% caseine milk in Tris-buffered saline (TBST, pH 7.4). Blocked membranes were incubated for 16 h at 4˚C with human sera diluted 1:100 in TBST, washed in TBST and incubated for 1 h at ambient temperature with anti-human IgE Clone GE-1 (Sigma-Aldrich), diluted 1:1000 in TBST.

Autoradiography. Air dried probed membranes were loaded in scanner.

RESULTS

Western blot analysis revealed differences in reactivity of sera from patients positive for standard mite allergy skin tests with fractionated mite antigens. A total of 17 of 30 sera (56.7%) from patients positive to skin tests showed specific cross reactivity with antigens isolated from extracts of *Tyrophagus putrescentiae* (Table 1). Comparing the two extracts examined, only 5/30 (16.7%) sera were recognizing proteins of the mite body extract. Eight and 23.3% of the sera had IgE binding to antigens of both extracts, whereas proteins of the faecal extract was bound by 4/30 (13.3%) of the examined sera (Table 1).

The results revealed that 8 out of 30 tested sera (26.7%) reacted specifically with new antigen identified as protein fractions of about 25 kDa in extracts from excrements of *T. putrescentiae*. Approx. 6.7 percent of the patients' sera (2/30) recognized allergens of about 72 kDa (Fig. 1).

DISCUSSION

The greatest exposure to storage mites usually occurs in an occupational and/or rural setting where allergies to these mites are of major importance (van Hage-Hamsten and Johansson 1998; Arlian 2001, 2002). Usually there is a little cross-reactivity between allergens of pyroglyphid house dust mites and and storage mites. CRIE analysis of *T. putrescentiae* extract utilizing sera from 24 occupationally exposed farmers in USA identified 14 allergens (Arlian et al. 1997, Arlian 2002). The number of individual allergens recognized by each patient ranged from 5 to 11; 5 allergens were recognized by all of the farmers' sera analysed. The number of allergens recognized and the sensitivity to individual allergens varied between patients. Immunoblotting using sera of farmers identified a 16-kDa protein as a major IgE-

binding component of *T. putrescentiae* extract (Johansson et al. 1991, Arlian 2002). Heterologous CIE and CRIE showed that few allergens of TP cross-react with those of *D. farinae* and *D. pteronyssinus* (Arlian et al. 1984, Arlian 2002). The antigen *Tyr p* 2 is a major allergen of *T. putrescentiae* (Arlian et al. 1984, 1997; Arlian 2002). This allergen shows more than 50% sequence identity with *Lep d* 2 of *L. destructor* and about 40% with allergens of *Dermatophagoides* spp. (Eriksson et al. 1998).

Table 1. The IgE response of the mite sensitive subjects examined against mite body and faecal extracts of *Tyrophagus putrescentiae*, using Western blotting

Subject	Mite body extract	Faecal extract
1	++	–
2	–	++
3	–	–
4	–	–
5	–	+
6	–	+++
7	–	–
8	+	++
9	+	++
10	–	–
11	+	++
12	–	–
13	–	–
14	+	–
15	++	–
16	+++	+
17	+	–
18	–	–
19	–	–
20	–	–
21	–	–
22	++	–
23	–	+
24	+++	–
25	+++	+
26	+++	+
27	++	+
28	–	+
29	–	–
30	–	–

The number of allergens actually identified in extracts of *T. putrescentiae* was comparable to the number that have been identified in the extracts of the house dust mites *Dermatophagoides* spp. (Aki et al. 1995, Aalberse 1998, Arlian 2002). These results revealed that 12 out of 30 tested sera (40.0%) reacted specifically with new antigens identified as protein fractions in extracts from excrements of TP. When assessing mite allergen reaction using mixture of mite proteins the results of the test

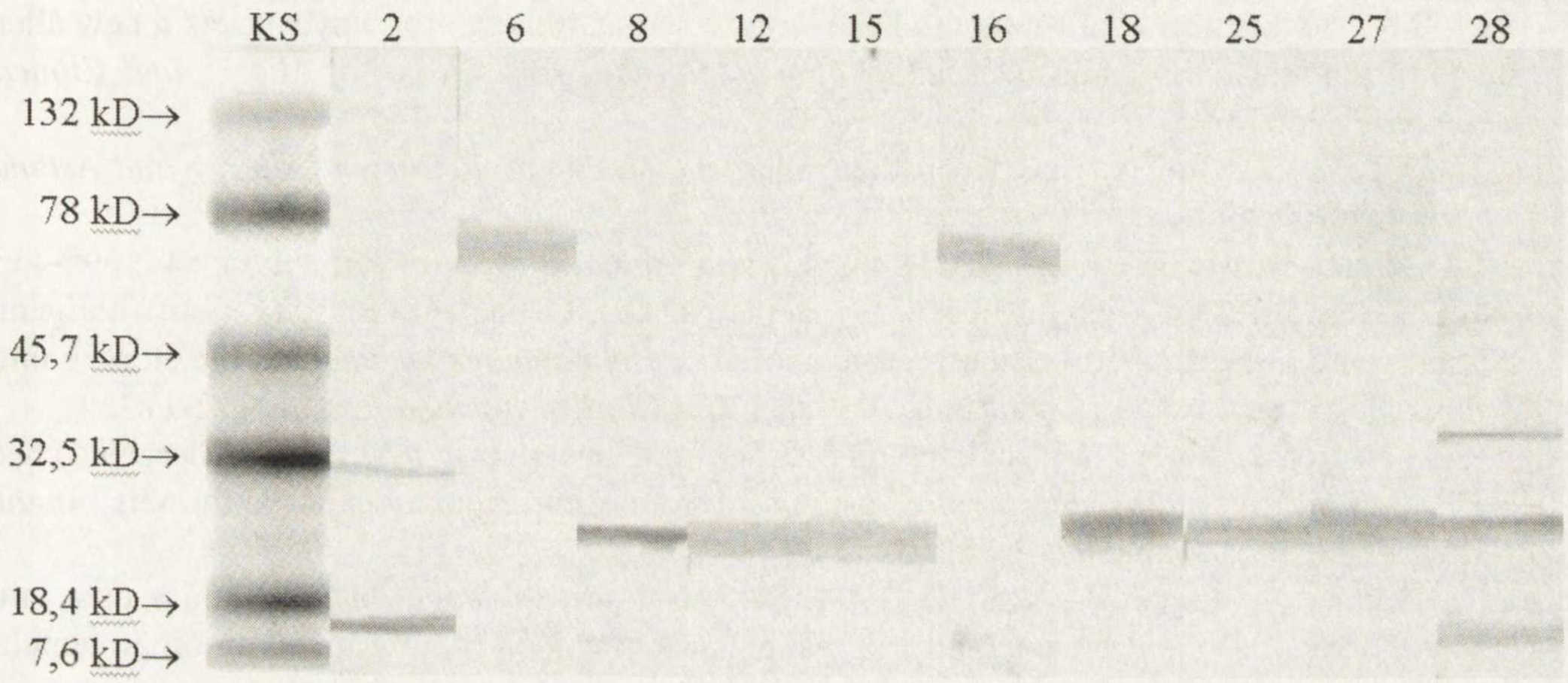

Fig. 1. The IgE responses of ten mite sensitive subjects against mite body and faecal proteins of *Tyrophagus putrescentiae* – representative immunoblots (few bands are unique to the mite species). KS – calibrated Molecular Weights of Kaleidoscope Standards; 2-28 – subjects shown in Table 1

are not satisfactory for determining the antigen causing patient allergy. The results obtained from studies reported here indicate significant discrepancy between the so called standard allergy skin test and what the patient is actually sensitive to. Also, a new class of immunizing protein of about 25 kDa has been identified in excrements from TP reacting with IgE from patients showing allergy to other mite extracts. A total of 5 of 30 sera (approx. 17%) from the examined patients showed specific cross reactivity with antigens isolated from mite excrements rather than from mite whole extracts.

Sensitization to stored-product mites in urban dwellers has been reported in Spain, Denmark, Germany, Croatia and USA (Korsgaard et al. 1985, Luczynska et al. 1990, Ebner et al. 1994, Garcia-Robaina et al. 1996, Macan et al. 1998, Gislason and Gislason 1999, Kanceljak-Macan et al. 2000, Arlian 2002).

CONCLUSION

Sensitization to the storage mite *T. putrescentiae* is present in the urban population of Upper Silesia in similar proportions as to pyroglyphid house-dust mites (*Dermatophagoides* spp.). Testing with storage mites should be considered routine allergological diagnostic procedure. In other words it is necessary to establish methods for identification and quantification of mites in the Upper Silesia.

REFERENCES

Aalberse R.C. 1998. Allergens from mites: implications of cross-reactivity between invertebrate antigens. *Allergy* 53(Suppl. 48): 47-48.

Aki T., Kodama T., Fujikawa A., Miura K., Shigeta S., Wada T., Jyo T., Murooka Y., Oka S., Ono K.

1995. Immunochemical characterization of recombinant and native tropomyosins as a new allergen from the house dust mite, *Dermatophagoides farinae*. *The Journal of Allergy and Clinical Immunology* 96: 74-83.

Arlian L.G. 2001. Dust mites: Update on their allergens and control. *Current Allergy and Asthma Reports* 1: 581-586.

Arlian L.G. 2002. Arthropod allergens and human health. *Annual Review of Entomology* 47: 395-433.

Arlian L.G., Geis D.P., Vyszenski-Moher D.L., Bernstein I.L., Gallagher J.S. 1984. Cross antigenic and allergenic properties of the house dust mite *Dermatophagoides farinae* and the storage mite *Tyrophagus putrescentiae*. *The Journal of Allergy and Clinical Immunology* 74: 172-179.

Arlian L.G., Vyszenski-Moher D.L., Johansson S.G.O., van Hage-Hamsten M. 1997. Allergenic characterization of *Tyrophagus putrescentiae* using sera from occupationally exposed farmers. *Annals of Allergy, Asthma & Immunology* 79: 525-529.

Ebner C., Feldner H., Ebner H., Kraft D. 1994. Sensitization to storage mites in house dust mite (*Dermatophagoides pteronyssinus*) allergic patients. Comparison of a rural and an urban population. *Clinical and Experimental Allergy* 24: 347-352.

Eriksson T.L.J., Johansson E., Whitley P., Schmidt M., Elsayed S., van Hage-Hamsten M. 1998. Cloning and characterisation of a group II allergen from the dust mite *Tyrophagus putrescentiae*. *European Journal of Biochemistry* 251: 443-447.

Garcia-Robaina J.C., Torre-Morin F., Bonnet-Moreno C.G., Antonin-Arias J., Perez-Santos C., Sanchez-Covisa A. 1996. House dust mites and Der p 1 in Tenerife (Canary Islands, Spain): the relative importance of other non-*Dermatophagoides* spp. mites. *Allergologia et Immunopathologia* 24: 135-138.

Gislason D., Gislason T. 1999. IgE-mediated allergy to *Lepidoglyphus destructor* in an urban population – an epidemic study. Short communication. *Allergy* 54: 878-883.

Hage-Hamsten M. van, Johansson S.G.O. 1998. Clinical and immunologic aspects of storage mite allergy. *Allergy* 53(Suppl. 48): 49-53.

Johansson E., Borga A., Johansson S.G.O., van Hage-Hamsten M. 1991. Immunoblot multi-allergen inhalation studies of allergenic cross-reactivity of the dust mites *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clinical and Experimental Allergy* 21: 511-518.

Kanceljak-Macan B., Macan J., Buneta L., Milkovic-Kraus S. 2000. Sensitization to non-pyroglyphid mites in urban population of Croatia. *Croatian Medical Journal* 41: 54-57.

Korsgaard J., Dahl R., Iversen M., Hallas T. 1985. Storage mites as a cause of bronchial asthma in Denmark. *Allergologia et Immunopathologia* 13: 143-49.

Laemmli U.K. 1970. Cleavage of structural proteins during assembly of the head of the bacteriophage T 4. *Nature* 227: 680-685.

Luczynska C., Griffin R., Davis R.J., Topping M.D. 1990. Prevalence of specific IgE to storage mites (*A. siro*, *L. destructor* and *T. longior*) in an urban population and cross-reactivity with the house dust mite (*D. pteronyssinus*). *Clinical and Experimental Allergy* 2: 403-406.

Macan J., Kanceljak-Macan B., Zuskin E., Milkovic-Kraus S. 1998. Sensitization to storage mites in urban working environment. *Archives of Industrial Hygiene and Toxicology* 49: 27-32.

Solarz K. 2001a. Risk of exposure to house dust pyroglyphid mites in Poland. *Annals of Agricultural and Environmental Medicine* 8: 11-24.

Solarz K. 2001b. Pyroglyphidae (Acari: Astigmata) in Poland. Distribution, biology, population ecology and epidemiology. *Acta Zoologica Cracoviensia* 44: 435-528.

Towbin H., Staehelin T., Gordon J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences of the United State of America* 76: 4350-4354.

Voorhorst R., Spieksma-Boezeman M.I.A., Spieksma F.T.M. 1964. Is a mite (*Dermatophagoides* sp.) the producer of the house-dust allergen? *Allergy and Asthma* 10: 329-334.

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