EXPERIMENTAL SENSITIZATION WITH EXTRACT OF *Dermatophagoides pteronyssinus*

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The immunogenic properties of the mite *Dermatophagoides pteronyssinus* on subcutaneous injection of the antigen were studied in experiments on guinea pigs and rabbits. The experiments showed a marked change in immunological reactivity of the recipient animals, accompanied by the production of agglutinating, mast cell-sensitizing, and precipitating (in rabbits) antibodies and by the development of hypersensitivity of immediate and delayed type.

KEY WORDS: *Mites; antibodies; hypersensitivity of immediate and delayed type.*

The view that mites present in house dust (from bedclothes) play an important role in the etiology and pathogenesis of bronchial asthma and other allergic diseases came into prominence after a series of investigations by Voorhorst and Spieksma [9, 10]. They found that the skin test with an extract of mites of the genus *Dermatophagoides* is positive in a high proportion of patients with bronchial asthma with increased sensitivity to house dust. An investigation has shown [5] that in almost 100% of cases mites of the genus *Dermatophagoides*, mainly *D. pteronyssinus* are present in dust from the bedclothes of children with neurodermatitis and urticaria.

Some workers [4, 7, 10] have stated that mites of the genus *Dermatophagoides* are universally distributed throughout the world except in districts subjected to severe drought for long periods. Data on mites of the genus *Dermatophagoides* are given in a number of papers [2, 6], but the immunogenic properties of the mite *Dermatophagoides pteronyssinus* have received little study.

The effect of sensitization of animals of various species with an extract of the mite *D. pteronyssinus* was studied.

EXPERIMENTAL METHOD

Two series of experiments were carried out on 50 guinea pigs, 30 rabbits, and 15 rats. In series I (control) 12 guinea pigs (group 1) and 12 rabbits (group 2) were sensitized with a saline extract of human hair (beard) cuttings from an electric razor and of the yeast *Saccharomyces cerevisiae*. The reason for sensitizing control animals with an extract of this mixture was that it is a culture medium for the mite *D. pteronyssinus*. In series II 20 guinea pigs (group 3) and 18 rabbits (group 4) were sensitized with the experimental antigen (mites + medium). All animals were sensitized by five subcutaneous injections, with an interval of 4-5 days between consecutive injections. The scheme of sensitization for the guinea pigs of all series was 0.5, 1, 2.5, 3, and 3.5 ml, and for the rabbits 1, 2, 3.5, 4.5, and 5 ml. A 5% saline extract of the antigen was used for sensitization. The control antigen contained 0.03-0.05 mg/ml protein nitrogen and the experimental antigen 0.2-0.3 mg/ml. Coca's fluid in phenol, pH 7.2-7.4, was used as the extracting fluid. Extraction was carried out at 4°C for 3 days with periodic shaking by hand. After centrifugation at 6000 rpm for 30 min the antigen was filtered through a fine-pore filter. Each batch of antigen was kept for not more than 15 days in a refrigerator. Antigen also was prepared from bedding dust (without mites) for the cross reaction. Circulating antibodies in the blood were determined

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by Boyden's passive hemagglutination test (PHT) in the modification of Ado and Pol'ner [1] on the 12th-14th day after the beginning of sensitization and on the 14th day after the fifth injection. Cross reactions were carried out with extract of bedding dust and horse serum in twofold dilution. Shelley's indirect test [8], the indirect mast cell degranulation test (IMCDT) [3], and Ouchterlony's gel-diffusion test were also carried out. At the height of sensitization passive transfer of reactions of immediate and delayed type was carried out. For passive transfer of the reaction of delayed type a suspension of lymph node lymphocytes containing $2.5 \cdot 10^7$ cells was transferred to the recipient. All numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

On the second to third day after the third sensitization the PHT in all the experimental guinea pigs became positive. The antibody titer varied from 1:64 to 1:256, with a mean value of 1:138±18.75 ($P<0.001$). In one guinea pig the antibody titer was as high as 1:512. The mean antibody titer in the control guinea pigs did not exceed 1:8. At the same time the antibody titer in the experimental rabbits varied from 1:32 to 1:256 (1:102±19.27), and in the control rabbits its mean value was 1:5±1.21. The PHT with bedding dust was positive undiluted and in a dilution of 1:2 in 25% of the experimental rabbits and guinea pigs; this suggests that there is negligible similarity between the antigenic determinants of the separate components of mites and of this particular bedding dust. The PHT with the sera of the animals of group 1 and dust antigen was negative. The PHT with the sera of all series of animals and horse serum (cross reaction) also was negative.

In Shelley's indirect test (IMCDT) spontaneous changes in the cells took place as a rule in the control preparations. In the present case this index was 12±0.66%. On the second to third day after the third sensitization the percentage of degranulated cells in the experimental guinea pigs reached 38±2.03 ($P<0.001$) and in the rabbits 36±2% ($P<0.001$).

In the IMCDT with the sera of the experimental guinea pigs the percentage of modified mast cells was 29±1.31 ($P<0.001$) compared with 12±1.07 in the control. In the experimental rabbits this index did not exceed 21.5±0.81% ($P<0.001$), compared with 11±0.51% in the control rabbits; the rate of spontaneous destruction of mast cells was 5.0±0.82%.

The gel-diffusion test was negative in all cases. A rapid increase in the titer of circulating blood antibodies occurred at the height of sensitization. In the guinea pigs of series II (experiment) the mean antibody titer in the PHT reached 1:916±48.8 ($P<0.001$), with fluctuations from 1:512 to 1:1024. The antibody titer against extract of bedding dust was 1:4 in four guinea pigs, but in the rest the reaction was positive with whole serum only. The antibody titer of the experimental rabbits in the PHT varied from 1:256 to 1:1024, with a mean value of 1:731±76.6 ($P<0.001$). In all animals of series I the PHT with dust extract was negative.

On the 14th day after the fifth sensitization the percentage of degranulated basophils with the guinea pigs' sera was 63±1.66 ($P<0.001$) and with the rabbits' sera 59±1.75 ($P<0.001$). The percentage of degranulated mast cells with the sera of the experimental guinea pigs was 56±1.24 ($P<0.001$) compared with 16±0.98% in the control. This index in the experimental rabbits was 44.4±1.27% ($P<0.001$) and 16.5±0.63% in the control rabbits. Consequently, by the simultaneous use of the indirect tests of degranulation of mast cells and basophils, the indices of the two tests consistently followed a parallel course.

Transferring immune lymphocytes from the experimental guinea pigs to intact recipients showed that a skin reaction began after 10-12 h and reached a maximum after 24-48 h. The reactions were positive in all recipients with antigen in dilutions of 1:4-1:8. After passive transfer of immune serum into recipient guinea pigs the skin test was positive with antigen in dilutions of 1:8 to 1:32. The direct intradermal test was positive in all the experimental animals.

The gel diffusion test at the height of sensitization gave only one thin precipitation line in 40% of the experimental rabbits. Cross reactions were negative. The percentage of positive reactions with the experimental guinea pigs did not exceed 10, and only one hardly detectable precipitation line appeared.

Mites of the species *Dermatophagoides pteronyssinus* contained (in this case cultivated) in bedding dust thus possess considerable sensitizing properties and are potential allergens.

After artificial subcutaneous sensitization of animals the latter formed in their bodies agglutinating antibodies, antibodies sensitizing cells of the fine connective tissue (mast cells and basophils), and precipitating antibodies (10-40%). However, further investigations are required to shed light on the mechanisms of immunogenesis and the functional characteristics of the antibodies produced by them.

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