MACROPHAGE SPREADING PHENOMENON IN POLLINOSES

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Spreading of macrophages in experiments on guinea pigs with a model of pollinosis is delayed by specific allergen. Inhibition of spreading can also be obtained by treating macrophages of intact guinea pigs with allergens in combination with corresponding sera from patients with pollinosis. This phenomenon can be used in the writer's modification of a clinical diagnostic test for allergy.

KEY WORDS: pollinosis; spreading of macrophages; allergen

The property of macrophages to spread on a flat surface is one of the phenomena of phagocytosis [1, 10, 12]. In recent years this process has come to be used as an allergologic test. Spreading of macrophages in guinea pigs sensitized with tuberculin or certain bacteria can be delayed by the corresponding allergens [4, 7, 8].

In the investigation described below spreading of macrophages was studied in animals with a model of pollinosis by the method of Fauve and Dekaris [7], and also under clinical conditions using the same method in the writer's modification.

EXPERIMENTAL METHOD

A model of pollinosis was obtained in guinea pigs either by inhalation of an extract of ragweed pollen in a special chamber (3 courses) or by immunization by a single injection of a 5% suspension of ragweed pollen in Freund's incomplete adjuvant, into the limb [2, 3]. The animals of group 1 were tested after 1 and 3 courses of inhalations, and those of group 2, 1 week and 1.5 months after immunization in the limb. Macrophages were taken from the peritoneal cavity of guinea pigs killed by exsanguination, by washing them out with 5-6 ml of nutrient medium 199 containing heparin (5 units/ml). The washings contained $4 \cdot 10^5$–$6 \cdot 10^5$ cells/ml, mainly macrophages with a few (under 10%) lymphocytes. During the manipulations the cells remained viable. The macrophages were incubated with the corresponding ragweed allergen, either whole or in a dilution of 1 : 10. The allergen was prepared in the writer's laboratory by F. F. Lukmanova as a 6% extract of ragweed pollen in Coca's fluid.

In the control tests of series I the following materials were used: 1) macrophages without allergen; 2) macrophages with whole birch allergen, prepared by the same method as the experimental allergen; 3) macrophages with birch allergen in a dilution of 1 : 10. The macrophages and allergen were poured in a volume of 0.1 ml of each into centrifuge tubes, and the volume of liquid was made up to 1 ml with medium 199. The final dilution of the allergen was thus 1 : 10 in one case and 1 : 100 in the other. Incubation was carried out for 1 h at 37°C, after which one drop of the mixture was transferred to a Goryaev's chamber and incubation continued at room temperature in a moist Petri dish for 30 min. The number of spread macrophages was then counted and expressed as a percentage of 200 cells examined in the phase-contrast or light microscope. These numbers were compared for the different groups of experiments (statistical analysis) with the corresponding values obtained from the three control investigations. Spreading of the macrophages also was expressed as a spreading index (SI):

$$SI = \frac{\text{mean number of spreading macrophages during incubation with specific allergen (in \%)}}{\text{mean number of spreading macrophates in control (in \%)*}} \times 100.$$

In the experiments of series II tests were carried out on sera of pollinosis patients with a marked clinical picture and with positive skin tests to allergens from grass pollen. Mixtures of macrophages and specific

*Mean results for all three control tests in the denominator.

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TABLE 1. Spreading of Macrophages in Guinea Pigs with Model of Pollinosis

Procedure	n	Spreading of macrophages %	P	SI of macrophages, %
Immunization with 5% suspension of ragweed pollen in incomplete adjuvant				
1 week later				
Control	24	50,2±2,17	—	100
Whole specific allergen	8	48,5±4,18	>0,1	96,6
Specific allergen in dilution of 1:10	8	40,0±2,48	<0,01	76,7
1 month later				
Control	12	48,2±1,28	—	100
Whole specific allergen	4	43,5±3,6	>0,1	90,2
Specific allergen in dilution of 1:10	4	39,0±1,42	<0,01	72,6
1.5 months later				
Control	69	50,7±1,17	—	100
Whole specific allergen	23	41,0±2,12	<0,001	80,9
Specific allergen in dilution of 1:10	23	39,7±2,18	<0,001	78,3
Immunization by inhalations of ragweed allergen				
After 1 course				
Control	35	52,0±2,68	—	100
Whole specific allergen	13	40,8±2,66	<0,01	78,3
Specific allergen in dilution of 1:10	13	43,3±3,46	<0,05	83,0
After 3 courses				
Control	24	43,4±2,1	—	100
Whole specific allergen	10	28,2±2,96	<0,001	67,3
Specific allergen in dilution of 1:10	10	24,4±3,04	<0,001	56,2

Legend. Here and in Table 2 mean values of all investigations in control and corresponding investigations in tests with specific allergens are given; n) number of investigations.

TABLE 2. Spreading of Macrophages of Intact Guinea Pigs on Incubation with Sera of Patients with Pollinosis (grass pollen) and Specific Allergens and in Control Tests

Procedure	n	Spreading of macrophages %	P	SI of macrophages, %
Control	36	55,4±1,27	—	100
Whole allergen	20	39,8±2,06	<0,001	71,5
Allergen in dilution of 1:10	25	45,0±2,54	<0,001	81,5

allergens for the patients were prepared in the same way as for the previous experiments, and the whole test serum was added to them. Macrophages from intact guinea pigs were used. The scheme of these experiments was the same as for the previous series. All components were taken in a volume of 0.1 ml, after which the volume of liquid was made up to 1 ml with medium 199. To compare the percentages of spreading of the macrophages and the values of SI, the mean result of 4 or 5 investigations was used as the control: 1) macrophages with specific allergens, whole and in a dilution of 1 : 10 (final dilutions 1 : 10 and 1 : 100); 2) macrophages with

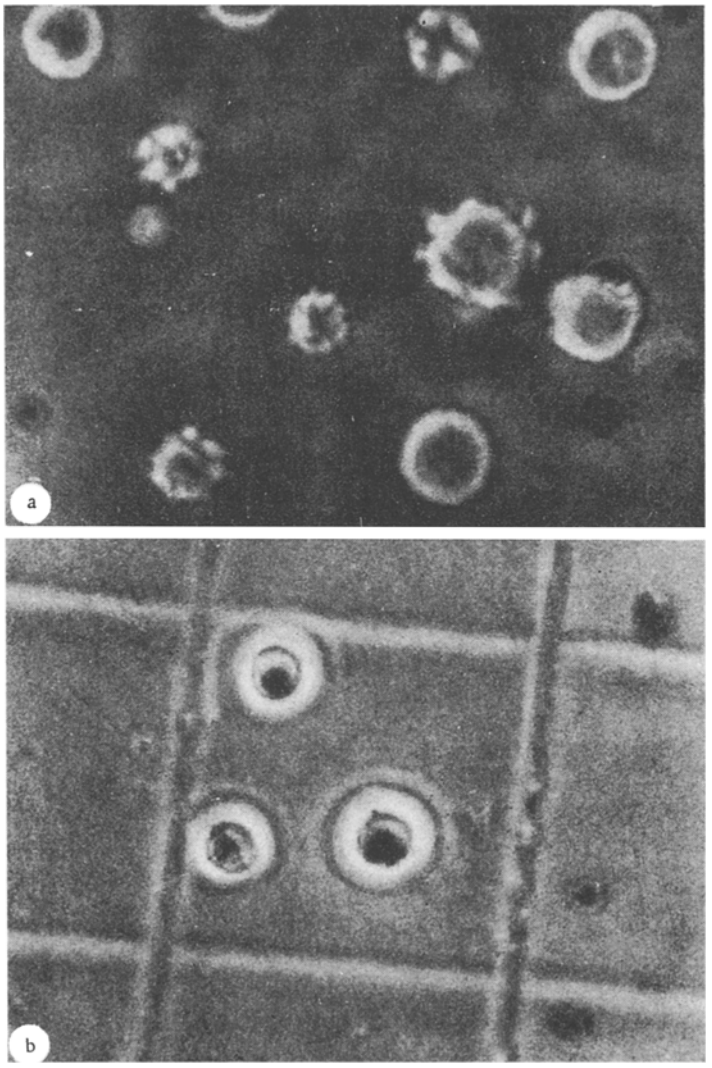

Fig. 1. Spreading of macrophages in pollinosis: a) spreading macrophages after incubation with patient's serum (skin sensitivity to allergen from timothy grass pollen) and control allergen from alder pollen; b) inhibition of spreading of macrophages after incubation with serum of same patients and specific allergen from timothy grass pollen. Azure–eosin, 200 ×.

patient's serum; 3) macrophages with patient's serum and control allergens in the dilutions adopted; 4) macrophages in medium 199; 5) in some experiments, macrophages with healthy human serum and with specific allergens in the dilutions adopted.

EXPERIMENTAL RESULTS

In the experiments of series I on guinea pigs with the model of pollinosis (Table 1) the number of spreading cells in the control was relatively stable between 48.2 and 50.7%. Incubation of the macrophages with the specific allergen (ragweed) caused inhibition of spreading. After immunization in the limb the allergen gave the greatest inhibition in a dilution of 1 : 10 (35 guinea pigs), when the number of spreading cells fell to 39-40% (SI = 79.7-72.6%). Inhalation of the allergen led to even greater inhibition of spreading, especially after the third course, when there were 28% of spreading cells with whole allergen (SI = 67.3%) and 44.4% with diluted allergen (10 guinea pigs; SI = 56.2%).

In the experiments of series II with sera of patients with pollinosis (Table 2), when macrophages of intact guinea pigs were used the mean number of spreading cells in the control was 55.4%. Incubation of the macro-

phages in medium 199 alone gave a lower percentage of spreading, and addition of the allergens increased it. Incubation of the macrophages with the patients' sera together with the corresponding allergens led to inhibition of spreading (Fig. 1). The mean number of spreading cells from 20 sera with whole allergen was 39.8% (SI= 71.5%) and from sera with diluted allergen it was 45% (SI=81.5%).

The ability of macrophages to carry out phagocytosis (to spread) in sensitized guinea pigs is thus regularly inhibited by specific allergens. The same inhibition of spreading of macrophages, but this time in intact guinea pigs, takes place in response to a combination of allergens and the corresponding patients' sera. The addition of nonspecific allergens or of allergens from noncorresponding sera increases the degree of spreading a little, for they evidently act as protein irritants. Inhibition of spreading of macrophages is caused by allergens specific for macrophages or sera which participate in the immunological process. It can tentatively be suggested that loss of the phagocytic function by a high proportion of macrophages is connected with their switch to the other side of their activity: cooperative interaction with T and B lymphocytes [9, 13]. The cessation of phagocytosis may perhaps reflect the readiness of the macrophages to receive information from lymphocytes. The suggestion has recently been made that inhibition of spreading of macrophages is caused by lymphokines [5, 6, 11, 12]. In that case, there is perhaps a change in the function of macrophages connected with the accumulation of antigens on their receptors.

The sera of allergic patients can be used as a diagnostic test in the study of spreading of macrophages.

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