ALLERGIC CHANGES IN LEUKOCYTES IN PATIENTS WITH POLLINOSES

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Recently, in the study of allergy considerable attention has been paid to the blood leukocytes, which undergo profound allergic structural changes during sensitization of the organism to the allergen. These sensitized leukocytes, if they come into contact with the specific allergens in vivo or in vitro, become deformed and undergo lysis, liberating histamine [9, 10, 12].

Allergic sensitization of the leukocytes has been observed both in reactions of the immediate type [1, 3, 5, 7, 8] and in cases of slow hypersensivity [4].

It was reported in 1947 that the leukocytes of patients sensitive to ragweed pollen underwent intensive lysis after incubation with the specific allergen [13]. These findings were subsequently confirmed by other authors [5, 11].

In the present investigation the allergic sensitization of the leukocytes of patients with pollinoses and the specificity of the reaction were studied by means of the method of staining with acridine orange.

Compared with other methods of staining leukocytes, the fluorescence method is capable of demonstrating finer morphological changes in the cells and it can be used on whole blood. The leukocytes are stained differentially by acridine orange and fluoresce brightly against the black background of the microscope field. The erythrocytes do not fluoresce because the luminosity is extinguished by hemoglobin.

EXPERIMENTAL

The allergens investigated were obtained from the pollen of timothy grass (Phleum pratense), speckled alder (Alnus incana), ragweed (Ambrosia artemisiafolia), and oak (Quercus robur), and they were prepared by the usual method in extracting fluid at the Allergologic Research Laboratory, Academy of Medical Sciences of the USSR.

Blood was taken from the finger of the hay fever patients. The anticoagulant used was a 1.5% solution of the disodium salt of EDTA (Chelaton-3) in 0.7% physiological saline. The pH of the solution must not exceed 8.0 to avoid nonspecific lysis of the leukocytes. The blood and anticoagulant were mixed in the ratio 2:1.

Into each of three silicone-treated Widal tubes 0.45 ml of blood was poured, after which 0.05 ml of the freshly prepared allergen in a dilution of 1:10 was added to tube No. 1 (the allergen used was that which gave the strongest allergic skin reaction with the corresponding patient). An equivalent dose of nonspecific allergen was added to tube No. 2. The control test was carried out with Simms' solution, with which the allergen was diluted. The contents of the tubes were carefully mixed and allowed to stand at room temperature for 1 h.

Before the staining was carried out the tubes were again shaken and 0.05 ml of a 1:20,000 saline solution of acridine orange was added to each. The duration of exposure to the dye was 3-5 min. One drop of the mixture from each tube was placed on a slide and a vital preparation made. This was at once examined under the luminescence microscope. One hundred granulocytes were counted in a field of vision and the percentage of deformed cells was calculated.

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Allergic Changes in Leukocytes of Patients with Pollinoses

Patient	Course of treatment	Allergic skin reactions with dust allergens: Timothy grass	Orchard grass	Fescue	Alder	Birch	Hazel	Oak	Ragweed	Changes in leukocytes after reacting with allergens (in %): Timothy grass	Alder	Oak	Ragweed	Simms' solution
N	1	++++	++++	++++	+	+	+			60		21		8
S	He Untreated	++++	++++	++++	+	+	+			73		33		20
B	3	++++	++++	++++	+	+	+			31		6		5
P	He Untreated	++++	++++	++++	+	+	+			40		15		6
K	" "	++++	++++	++++	+	+	+			73		17		9
D	" "	+								16	52			14
I	" 1									11	55			10
R	"									12	45			4
K-v	He Untreated				+	+	+			8	20			3
Kh	1				+	+	+			10	37			10
K-n	1											10	30	3
M	2											7	52	3
Ya	1											16	32	12
P-v	He Untreated				+	+	+					47	73	30
M-v	1											15	63	7

EXPERIMENTAL RESULTS

In normal conditions the nuclei of leukocytes when stained with acridine orange are bright green, the cytoplasm is dull green, and the granules in the cytoplasm are brick red. This type of fluorescence was observed in the control preparations. It is explained by the property of acridine orange of reacting differentially with nucleic acids. The RNA of the cytoplasmic granules fixes the dye in large amounts and forms orange-red complexes, while the DNA fixes less of the dye and gives a bright green fluorescence.

When the changes in the cell are profound, just before death, an increase in the adsorption of dye is observed and its luminescence changes from the green to the orange-red part of the spectrum (the "concentration effect" of Strugger). In the experimental preparations, for instance, leukocytes were observed which had lost their normal round shape and which had ragged, fringed borders. The modified cells also included some with no visible nuclear structure and with total or partial disappearance of their cytoplasmic granules. The luminescence of the cell components had moved into the yellow-orange region of the spectrum. All these changes are evidence of profound intramolecular disturbances of the cell metabolism under the influence of the allergen.

The blood investigated was taken from 100 persons (47 females, 53 males) aged from 11 to 64 years, including 85 patients with hay fever and 15 healthy subjects.

The patients were divided into two groups: group 1 consisted of patients who had received 1-4 courses of specific desensitizing therapy (57 patients) and group 2 of untreated patients (28 patients).

Increased sensitivity to weed pollen (timothy grass, orchard grass, fescue) was discovered in 65 patients to pollen of weeds and trees (alder, hazel, birch) in 8 patients, to tree pollen only in 7 patients, and to ragweed dust in 5 patients.

Since the pollens of related grasses (timothy grass, orchard grass, fescue) and the pollens of trees (alder, hazel, birch) possess certain common antigenic properties detectable by the precipitation reaction in agar [2], only one specific allergen was used—that giving the strongest allergic skin test in the patient.

It became apparent during the investigation that the mean percentage of deformed leukocytes following administration of the specific allergen reached 52 in the patients of group 1 and 55 in group 2, compared with 15 and 9 respectively in the controls. The nonspecific allergens, which did not give positive skin tests in the patients, caused nonspecific injury to 13-18% of the leukocytes (close to the control value). Selected results obtained in the course of investigation of 15 patients are given in the table.

A similar series of tests was carried out with the blood of 15 healthy persons who were not sensitive to the

pollen of weeds and trees. Only a small percentage of deformation of the leukocytes (17 and 22%) was obtained in these tests in both the control and the experimental samples.

The results suggest that leukocytolysis develops not because of toxic substances present in the allergen, but as the result of a more complex immunochemical process [6].

The leukocytes of the sensitized patients, when they react with the specific allergen in vitro, thus undergo intensive structural changes, and when subsequently stained with acridine orange the luminescence of the cell components is modified. Allergic sensitization of the leukocytes takes place in both treated and untreated patients. The method of detection of allergic sensitization of the leukocytes in vitro by luminescence microscopy is a simple test for the diagnosis of the allergic state in such patients.

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