OBJECTIVE PHOTOMETRY AS A METHOD OF ASSESSING ALLERGIC CHANGES IN LEUKOCYTES

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A method of photography of leukocytes stained with acridine orange and photometry of negatives is described. During investigation of leukocytes of patients with pollinoses, after contact with the specific allergen changes in intensity of luminescence of the modified cells were discovered to occur in two phases with an increase in intensity of luminescence of undeformed leukocytes and a decrease in luminescence of deformed, disintegrated cells.

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Changes in the intensity of luminescence of cells provide a sensitive test for detecting the early stages of tissue injury in various pathological processes [2-5]. The blood leukocytes undergo allergic modification during specific sensitization and structural changes after contact with the allergen both in vivo and in vitro [6-9]. However, until now these structural changes in preparations stained vitally with acridine dyes have been assessed only visually [1].

In the present investigation we attempted to study the character of luminescence of the structurally modified leukocytes at various stages of injury by using a method of objective (photographic) photometry.

EXPERIMENTAL METHOD

We studied 92 patients with pollinoses, hypersensitive to pollen of certain grasses (timothy grass, cocksfoot, fescue) and giving a positive allergic skin reaction to injection of allergen from timothy grass pollen. Blood was taken from the patients' finger. A 1.5% solution of the disodium salt of EDTA in 0.7% physiological saline was used as anticoagulant. Blood was mixed with anticoagulant in the ratio of 2:1.

Allergens from pollen of timothy grass (Phleum pratense) and oak pollen (Quercus robur), prepared in Cock's extracting fluid at the Research Laboratory of Allergology, AMN SSSR, were used in the experiments.

Blood in a volume of 0.45 ml was collected in silicone-treated Widal tubes, after which 0.05 ml of timothy grass pollen extract, diluted 1:10 in Simms' solution, was added to one tube. The same volume of nonspecific allergen (extract of oak pollen) was added to another tube. The contents of the tube were carefully mixed and kept at room temperature for 1 h.

Staining was carried out with a solution of acridine orange in saline concentrations of 1:20,000 and 1:100,000. After staining, the contents of the tubes were carefully mixed and 0.05 ml of dye was added to each (exposure 5 min). A drop of working mixture was placed on a slide and vital preparation made for investigation with the ML-2 luminescence microscope.

Luminescent neutrophils were photographed on the same RF-3 film using a magnification of 450 times and an exposure of 3 sec. The films were developed with metolhydroquinone for 10 min. The properties of the photographic film in different parts were determined by plotting characteristics curves. For this purpose, under identical working conditions, leukocytes were photographed on the film in a series of increasing exposures. From changes in the intensity of blackening, a curve was plotted and the region of normal intensity for the particular film determined. Under our experimental conditions the intensities of blackening to be investigated lay within the region of normal values of the characteristic curve.

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TABLE 1. Change in Intensity of Blackening of Negative Image of Leukocytes in Relation to Degree of Damage

Dye	Dilution	Intensity of blackening (S; M ± m)			P
		Control	experiment (leukocytes + specific allergen)		
			undeformed cells	deformed cells	
Acridine orange	1:100,000	0.226 ± 0.003	0.371 ± 0.005	0.171 ± 0.007	< 0.01
	1:20,00	0.405 ± 0.002		0.406 ± 0.002	> 0.9

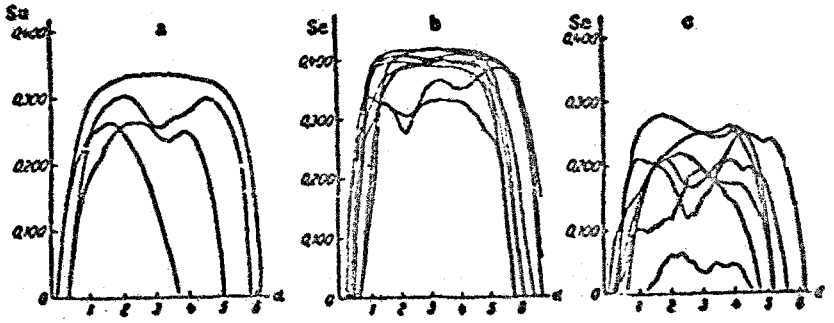

Fig. 1. Curves showing change in intensity of luminescence of modified leukocytes. Concentration of dye 1:100,000. a) Control: intensity of luminescence of group of leukocytes after contact with nonspecific allergen; b) experiment: intensity of luminescence of undeformed leukocytes after contact with specific allergen; c) experiment: intensity of luminescence of deformed leukocytes after contact with specific allergen; d) diameter of negative image of cell (in mm; recording 1:100). Remainder of explanation in text.

EXPERIMENTAL RESULTS

By means of a type MF-4 recording microphotometer two groups of films were investigated. One group corresponded to staining of the preparations with acridine orange in a concentration of 1:100,000 (75 films), and the other in a concentration of 1:200,000 (17 films). Leukocytes of control and experimental preparations were photographed on each film.

The leukocytes of the control preparations had a normal, unchanged shape with an emerald-green nucleus and bright red granules in the cytoplasm. In the experimental preparations the leukocytes were subdivided into two groups: undeformed, with no visible disturbances of cell structure, and deformed, with obvious disturbance of shape, rupture of the cell membrane, and liberation of granule.

The largest diameter of the cells in different directions was plotted on a logarithmic scale. The increase in density of blackening of the negative under identical conditions was regarded as the result of an increase in intensity of luminescence of the cell.

As Table 1 shows, with low concentration of dye (1:100,000) luminescence of cells with no external signs of injury in the experimental preparations was more intensive than that of the cells in the control. Luminescence of the deformed leukocytes was relatively weak. With an increase in concentration of fluorochrome in the working solution to 1:20 000 luminescence of the leukocytes in both the experimental and control preparations was identical, and they could be distinguished only by their structural changes (which were considerable in the experimental preparations).

The mean intensity of blackening (S_c in the control and S_e in the experimental) was calculated for each series of experiments. The difference between S_e and S_c for low concentrations of dye was statistically significant in every case, whereas with an increase in concentration of dye in the working solution the difference between S_e and S_c was not statistically significant.

As a result of automatic recording of the photometry a series of curves was obtained showing the total intensity of blackening of the cell and corresponding to different initial intensities of luminescence of the object. Analysis of these curves showed that the maximum of intensity of luminescence of the cells (peak of the curve) in both the experimental and control preparations occurred in the case of fluorochroming with the dye in a dilution of 1:20,000. With lower concentrations of dye in the working solution (1:100,000) the curves were different in character. For instance, a higher curve was obtained for undeformed leukocytes in the experimental preparations, whereas the maximum of intensity of luminescence of the deformed leukocytes was appreciably lower (Fig. 1).

Evidently, in the initial stage of structural modification of the leukocytes, before any marked morphological changes occur, the absorption properties of the cell cytoplasm are increased. The dye is fixed by the cell in large quantities and causes an increase in intensity of luminescence compared with the intact cell in the control. The deformed cell, having lost part of the substrate with which the dye is usually fixed, naturally gives weaker luminescence. With an increase in the total concentration of dye in the working mixture, this difference becomes negligeable.

The method of objective (photographic) photometry can thus be used to study allergic changes in leukocytes at the early stages of this process. The changes in intensity of luminescence of the modified leukocytes take place in two phases: an increase in intensity of luminescence of still undeformed cells and a decrease in intensity of luminescence of damaged, deformed leukocytes. If the concentration of dye is increased, the intensities of luminescence of the damaged and normal cells become equal.

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