REACTIVE CHANGES IN LEUKOCYTES IN ANIMALS SENSITIZED WITH HOMOLOGOUS TISSUE ANTIGENS

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After intraperitoneal injection of heart, kidney, and spleen antigens into guinea pigs and grafting of skin from donor guinea pigs, the recipient animals develop reactive changes in the blood neutrophils, manifested by a specific increase in their sensitivity to antigens of these organs (the leukocytolysis reaction), accompanied by the appearance of accessory nuclear lobules in the nuclei of the neutrophils (nonspecific reactive changes).

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Medawar and other investigators [9, 14, 15] have explained the similarily between the reactions of transplantation immunity and the "delayed" allergy of tuberculin type. It has now been shown that the most accurate test for diagnosis of bacterial allergy is the leukocytolysis reaction (deformation of leukocytes by the action of specific bacterial allergens in vitro) [1, 2, 4, 7, 8, 17]. The high sensitivity of this reaction is explained by direct contact of the allergens with the sensitized leukocytes, which at the same time can transmit sensitization to intact animals [1, 10, 12, 14]. It has also been found that accessory nuclear lobules of the "drum-stick" type in neutrophils identified by several workers [3] as sex chromatin, characteristic of women and female animals, in various human allergoses and in animals sensitized with bacterial allergens, as Sakharov and Kudrina [5, 6] have shown, are reactive changes in the nuclei of neutrophils. The number of neutrophils with accesssory nuclear lobules of class A (to use Kozenov's [13] classification) is increased (depending on the degree of sensitization) in men and women, and also in male and female experimental animals.

In the present investigation reactive changes in leukocytes were studied in animals following homografting of skin and other organs in order to detect a state of sensitization in the recipients to homologous tissue antigens.

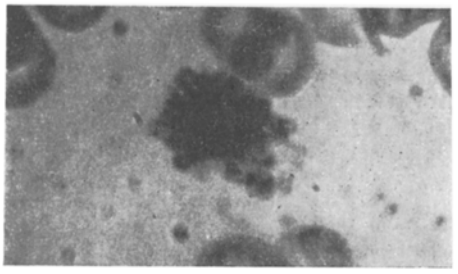

Fig. 1. Deformation of neutrophilic granulocyte from blood of a recipient guinea pig sensitized with donor guinea pig heat antigen, resulting from the action of specific antigen.

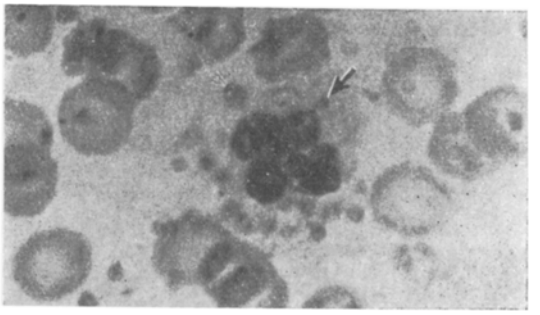

Fig. 2. Accessory nuclear lobules (indicated by arrow) in neutrophilic granulocyte from blood of a recipient male guinea pig sensitized with antigen from donor guinea pig heart.

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TABLE 1. Number of Neutrophils with Accessory Nuclear Lobules and Number Undergoing Deformation Under the Influence of Specific Tissue Antigens in Blood of Guinea Pigs Before and After Sensitization (in %)

Days of observation	Sensitivity of the antigen							
	kidneys		spleen		heart		skin (homografting)	
	accessory nuclear lobules	Leukocytolysis	accessory nuclear lobules	Leukocytolysis	accessory nuclear lobules	Leukocytolysis	accessory nuclear lobules	Leukocytolysis
Before expt.	2,3± 0,027	1,6± 0,095	2,5± 0,062	1,9± 0,034	3,0± 0,098	2,9± 0,096	3,0± 0,099	4,0± 0,102
After sensitization								
1—5	24,6	27,3	25,9	26,7	—	22,5	7,2	12,4
6—10	19,4	19,6	19,0	17,1	21,0	18,6	7,4	16,0
11—15	14,5	19,0	15,6	13,5	18,9	15,8	10,3	17,0
16—20	—	—	—	—	—	—	13,2	17,3
21—30	9,7	9,5	7,5	8,4	9,6	10,1	4,1	7,0
Mean . . .	17,0± 0,781	17,2± 0,933	17,0± 0,838	16,4± 0,800	16,5± 0,555	16,7± 0,608	8,4± 0,344	14,1± 0,372

Note. Number of animals investigated indicated in section "Experimental Method."

EXPERIMENTAL METHOD

The leukocytolysis reaction was carried out as follows: blood was taken from a vein or the heart of the experimental animals (guinea pigs) into a test tube containing 5% sodium citrate solution (in the proportion of 1 : 4). Next, 0.3 ml of the citrated blood was poured into each of a series of tubes and 0.1 ml antigen added, while physiological saline was added to the control tube. The antigen was prepared from different organs (heart, kidney, or spleen) which were ground in a mortar with the addition of physiological saline in the proportion of 1 : 10. The suspension obtained after centrifugation (2500 rpm) was used as antigen. Tubes containing citrated blood and antigen were incubated at 37° for 1 h. Thin films on slides were then made from the mixture of blood with antigen and stained by the Pappenheim – Kryukov method.[1] The films were examined under immersion (1200 ×). Deformed neutrophils with injury to their cytoplasm and nucleus were counted (Fig. 1).

Accessory nuclear lobules in the neutrophils were determined in blood films incubated with the addition of tissue antigens and also without antigen. These films were also stained by the Pappenheim – Kryukov method and with orcein. Only those accessory nuclear lobules were counted which had the typical "drum-stick" appearance and which corresponded to Kozenov's class (Fig. 2). Nuclear formations belonging to classes B, C, and D were not counted.

The total number of male guinea pigs investigated by these methods was 200, 30 of which received skin homografts while the rest were sensitized with antigens of the homologous organs: kidney (70 guinea pigs), spleen (60 animals), heart (40 animals).

EXPERIMENTAL RESULTS

The results of a study of the leukocytolysis reaction and of blood neutrophils possessing accessory nuclear lobules in guinea pigs after skin homografting and after intraperitoneal injection of kidney, spleen, and heart antigens taken from donor guinea pigs are summarized in Table 1. In all the experiments, male guinea pigs were used. The mean numerical results of the leukocytolysis reaction in the animals before the experiment did not exceed 1.6-4%, i.e., they were within the limits of the normal control. The mean number of accessory nuclear lobules of "drum-stick" type in the guinea pigs before the experiments was between 2.3 and 3%. In a group of 10 control guinea pigs investigated separately, the mean number of neutrophils with accessory nuclear lobules was 2.65 ± 0.094%. Their number in healthy female guinea pigs, on the other hand, was 8.79 ± 0.073%.

After immunization of the animals with kidney, spleen, or heart antigens, a parallel increase was observed in the percentage of deformed neutrophils, estimated by the leukocytolysis reaction, and in the number of neutrophils possessing accessory nuclear lobules. These indices rose to a maximum in the experiments in which animals were sensitized

TABLE 2. Percentages of Leukocytolysis Reaction and Accessory Nuclear Lobules in Neutrophils of Male Guinea Pigs in Crossed Tests with Tissue Antigens

Organ used to prepare antigen	Index studied	Antigens for leukocytolysis reaction				
		heart	kidneys	spleen	skin	blood films without antigen (control
Heart	Deformation of leukocytes	22,5±0,436	16,0±0,280	13,4±0,235		3,2± ±0,018
	Accessory nuclear lobules	16,5±0,256	16,0±0,235	16,8±0,248		16,0± ±0,295
Kidneys	Deformation of leukocytes	10,2±0,193	17,3±0,315	11,5±0,198		2,7± ±0,015
	Accessory nuclear lobules	17,3±0,360	17,0±0,318	16,5±0,295	17,0± ±0,320	16,8± ±0,390
Spleen	Deformation of leukocytes	11,3±0,280	8,7±0,115	17,3±0,309		1,0± ±0,012
	Accessory nuclear lobules	15,0±0,218	16,2±0,203	17,0±0,318		16,3± ±0,380
Sensitization by skin grafting	Deformation of leukocytes	8,5±0,017	5,5±0,016	8,2±0,025	10,0± ±0,083	4,0± ±0,039
	Accessory nuclear lobules	9,0±0,093	8,4±0,033	8,2±0,040	8,4± ±0,053	8,1± ±0,062
Control	Deformation of leukocytes	2,3±0,010	2,8±0,042	4,0±0,053		0,8± ±0,004
	Accessory nuclear lobules	2,4±0,018	2,3±0,052	2,5±0,063	2,1± ±0,035	2,8± ±0,061

with kidney, spleen, and heart antigens during the first 5 days of the experiment. On the 6th day they began to fall. By the 21st-30th day the number of neutrophils with accessory nuclear lobules had fallen to 7.5-9.7%, and the mean percentage of the leukocytolysis reaction had fallen to 9.5-10.1%. Mean percentages for the whole period of these experiments were 16.5-17.0 for neutrophils possessing accessory nuclear lobules of the "drum-stick" type and 16.4-17.2 for the leukocytolysis reaction. Statistical analysis of the results showed that $P < 0.007$, so that the results obtained are significant.

These experiments thus showed conclusively that the increase in the percentage of accessory nuclear lobules in the neutrophils of male guinea pigs is due to a parallel rise and fall in the percentage of neutrophils undergoing deformation.

Skin homografting, as the results in Table 1 show, produced less marked reactive changes in the neutrophils. The number of accessory nuclear lobules in the neutrophils after skin grafting increased relatively slowly until the 20th day, reaching 13.2%. This was followed by a decrease in the number of "drum-sticks" in the neutrophils on the 21st-30th day to 4.1%. Their mean number during the months after skin grafting was 8.46%. The reaction of deformation of leukocytes with skin antigens also increased in intensity until the 20th day inclusive, to reach 17.3%. On the 21st-30th day the percentage of neutrophils undergoing leukocytolysis fell to 7.05. The mean percentage of leukocytolysis during the period of the experiment was 14.16.

Comparison of the results obtained after intraperitoneal injection of kidney, spleen, and heart antigens into guinea pigs and of skin homografting of these animals shows a significant difference in the leukocyte reaction, for after intraperitoneal injection of tissue antigens the maximal increases in the number of

neutrophils with accessory nuclear lobules and with deformation were found during the first 5 days of the experiment, whereas after skin grafting they occurred on the 16th-20th days. The indices of accessory nuclear lobules and deformation of leukocytes were much higher in experiments with intraperitoneal injection of antigens, moreover, than after skin grafting. This was evidently because the antigenic stimulation was stronger after intraperitoneal inoculation than after skin homografting.

It can thus be concluded that accessory nuclear lobules in neutrophils are reactive structures due to sensitization. This conclusion is also confirmed by results of studies of the direct action of allergizing antigens on the nuclear substance of leukocytes and, in particular, on their DNA [11, 12, 16, 18].

It must also be noted that tissue antigens have a well marked specificity of action on sensitized leukocytes of experimental guinea pigs, whereas accessory nuclear lobules in the neutrophils are nonspecific reactive structures, reflecting general sensitization of the organism irrespective of the sensitizing antigen. These facts are reflected in Table 2, in which several series of experiments on guinea pigs sensitized by homologous heart, kidney, and spleen antigens and by skin homografting are summarized.

The results obtained by sensitization of guinea pigs with tissue antigens agree in principle with the facts discovered in bacterial allergy [7, 8]. The only difference is that during bacterial sensitization, reactive changes in the leukocytes are much stronger than in the case of sensitization of the animals with homologous tissue antigens.

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