EFFECT OF LEUKOCYTIC FACTORS ON EMIGRATION OF LEUKOCYTES IN INTACT AND IRRADIATED ANIMALS

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Leukocytic factors (lysosomes, granulocytic substance), isolated from polymorphonuclear leukocytes, as well as destroyed leukocytes of the peritoneal exudate of rabbits and rats, if injected intradermally, induce active emigration of leukocytes. Preparations from leukocytes obtained from irradiated animals possessed lower leukotaxic activity than preparations of leukocytes from intact animals.

In studies of the phlogogenic activity of leukocytes attention has been concentrated on their ability to induce the development of disturbances of permeability [7, 8]. Too little attention has been paid to the study of the property of leukocytes to induce emigration of cells. Experiments have shown that 40 min after intradermal injection of extracts of homologous polymorphs into rabbits, emigration of leukocytes develops. Extracts of other blood or tissue cells were ineffective [6]. In later investigations, emigration of leukocytes in the mesentery of rabbits and rats [7] was induced by lysosomal factors of polymorphs [3] and also by granulocytic substance [8].

The object of the present investigation was to continue the study of the leukotaxic activity of polymorphs of intact animals, and also of leukocytes from irradiated rabbits and rats, modified as a result of this procedure [1].

EXPERIMENTAL METHOD

The ability of homologous leukocytic factors (destroyed leukocytes, granulocytic substance, and lysosomes) from unirradiated and radiated rabbits and rats to induce emigration was estimated. Leukocytic factors were obtained from leukocytes of the peritoneal exudate [4]. Leukocytes were obtained from irradiated animals on the fourth day after x-ray irradiation in a dose of 800 R for rabbits and 600 R for rats (180 kV, 15 mA, filters: Al 3.5 mm; Cu 0.5 mm). The factors were injected intradermally in 0.1 ml physiological saline in doses (calculated per mg protein) leading to the development of disturbances of permeability of the skin vessels [2]. The animals were sacrificed 1 and 9 h after the injections. A piece of skin at the place where the preparations were injected was excised and fixed in 10% neutral formalin solution for 5-7 days, and then mounted in gelatin. Sections were cut from the blocks in a thickness of 20 μ on a freezing microtome. The sections were stained with hematoxylin-eosin. Each leukocytic preparation was tested on 5-8 animals. Leukocytes in the sections were counted under a magnification of 400 ×. To count the number of leukocytes per mm^2 tissue, 44 squares, the area of which was determined by an ocular micrometer, were examined. The number of leukocytes per mm^3 tissue was determined by Hjelman's formula [5] for counting mast cells: the leukocyte count was given by

$$n \frac{1000}{\alpha + d - 2h},$$

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TABLE 1. Emigration of Leukocytes after Intradermal Injections of Homologous Leukocytic Factors into Rabbits and Rats (number of leukocytes/mm^3 tissue)

Factor	Animals donating leukocytes	Rabbits		Rats (after 9 h)
		after 1 h	after 9 h	
Destroyed leukocytes	Unirradiated	560	3 600[1]	5 900[1]
	Irradiated		1 300[1,2]	1 700[1,2]
Granulocytic substance	Unirradiated	1 200[1]	4 700[1]	5 900[1]
	Irradiated		800[1,2]	2 600[1,2]
Lysosomes	Unirradiated	1 400[1]	3 000[1]	5 700[1]
	Irradiated		1 300[1,2]	2 000[1,2]
Physiological saline		—	600	500
No injection given		—	—	800

[1]$P < 0.05$ compared with control (injections of physiological saline).
[2]$P < 0.05$ compared with leukocytic preparations from leukocytes of unirradiated animals.

where n is the number of cells per mm^2, α the thickness of the section in μ (in these cases 20μ), d the mean diameter of the cells (in this case 10 μ), and h the resolving power, which in the case of the optical system used was equal to 1. Student's t test was used as the criterion of significance in the statistical analysis.

EXPERIMENTAL RESULTS

Emigration of leukocytes was observed 1 h after intradermal injections of all leukocytic preparations tested. The number of emigrating leukocytes was sharply increased 9 h after the injections (Table 1). The most intensive emigration occurred after intradermal injections of lysosomes from polymorphs. Emigration took place mainly into loose connective tissue of the stratum papillare of the skin, where most of the blood vessels are located. Emigration was less marked in the dense connective tissue of the stratum spinosum. The investigated area of tissue was edematous, loose in texture, and its blood vessels were hyperemic. Numerous polymorphs were seen outside the vessels, especially 9 h after injection of the leukocytic preparations. Some vessels were ruptured. The similar picture was observed in preparations of rat's skin.

In both rabbits and rats the ability of leukocytic factors isolated from the leukocytes of irradiated animals to induce emigration was considerably weakened.

These experiments thus revealed high leukotaxic activity of all the studied leukocytic factors from intact rabbits and rats. The development of emigration 1 h after injection of the preparations, and comparison of the results with data in the literature concerning the activity of other tissues [6] indicate the specific role of polymorphs. The cells which emigrated in most cases, even 9 h after injection, were polymorphs. These observations suggest that the leukotaxic effect of polymorphs is due to their lysosomal fractions. This is confirmed by the results of preliminary experiments in which acid phosphatase, the enzyme of lysosomes, was injected.

The ability of leukocytic factors of irradiated animals to induce emigration was considerably reduced. This decrease in the leukotaxic activity of preparations obtained from the leukocytes of irradiated rats and rabbits is evidently further proof of the development of qualitative changes in the leukocytes of irradiated animals.

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