EFFECT OF PHYTOHEMAGGLUTININ THERAPY ON PERIPHERAL BLOOD LEUKOCYTE RESPONSE TO WOUNDING IN RABBITS WITH DIFFERENCES IN DELAYED TYPE HYPERSENSITIVITY

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The positive therapeutic action of phytohemagglutinin (PHA) on the healing of experimental suppurating wounds [2] and encouraging clinical observations [4, 5] indicate that this substance has considerable promise. However, the dependence of the effect on delayed-type hypersensitivity (DTH) to PHA [2] requires further experiments in order to clarify the details of the mechanism of action of PHA and to discover any possible undesirable consequences of its use. The object of the present investigation was to determine how the dynamics of the numbers of all forms of pheripheral blood leukocytes and of the myeloperoxidase and phagocytic activity of the neutrophils depend on the strength of the DTH reaction to PHA during treatment of suppurating experimental wounds in rabbits with PHA.

EXPERIMENTAL METHOD

Experiments were carried out on 165 rabbits of both sexes and of various breeds, weighing 3.2 ± 0.06 kg. Each animal was tested by intradermal injection of PHA in a dose of 100 μg in 0.1 ml doubly distilled water. Standard circular wounds affecting skin and muscle, with an area of 4 cm^2, were inflicted on rabbits of three groups, subdivided according to the degree of their DTH reaction to PHA into those giving weak, moderate, and strong reactions. An ointment-emulsion with PHA (3.3 μg per gram base; from Difco, USA) was applied once a day throughout the period of healing. Blood was taken from the auricular vein immediately after trauma and on the 3rd, 5th, 7th, 10th, 14th, 18th, and 20th days. The numbers of neutrophils, lymphocytes, and monocytes in 1 μl blood were determined in 86 rabbits (24 giving strong, 41 giving average, and 21 weak reactions). Myeloperoxidase (MPO) activity and five phagocytic parameters of the neutrophils were studied [6] in 79 rabbits (22 with strong, 38 with average, and 19 with weak responses). MPO activity of the neutrophils was determined by the method of Graham, Knoll, and Lillie [3]. The results for enzyme activity were expressed by Kaplow's index [8]. To study the phagocytic parameters, a suspension containing $2 \cdot 10^9$ living *Staphylococcus epidermidis* cells in 1 ml culture was prepared and added to a mixture of blood with 5% sodium citrate solution in the ratio of 1:2:1. The mixture was incubated at 37°C for 1 h. The phagocytic parameters were determined in 100 neutrophils in fixed films. The results were subjected to statistical anslysis by Peters' method, using Mollenhauer's factor. According to most parameters (except those of phagocytosis) the groups of strongly and moderately strongly reacting animals were combined because no significant differences were found between them. This combined group will subsequently be described as strongly reacting.

EXPERIMENTAL RESULTS

Values of the leukocytic parameters determined in the intact animals were identical, i.e., they did not depend on the strength of the DTH reaction to PHA (Figs. 1 and 2; Table 1). Only monocytes, whose number was considerably smaller in animals with weak DTH, were the ex-

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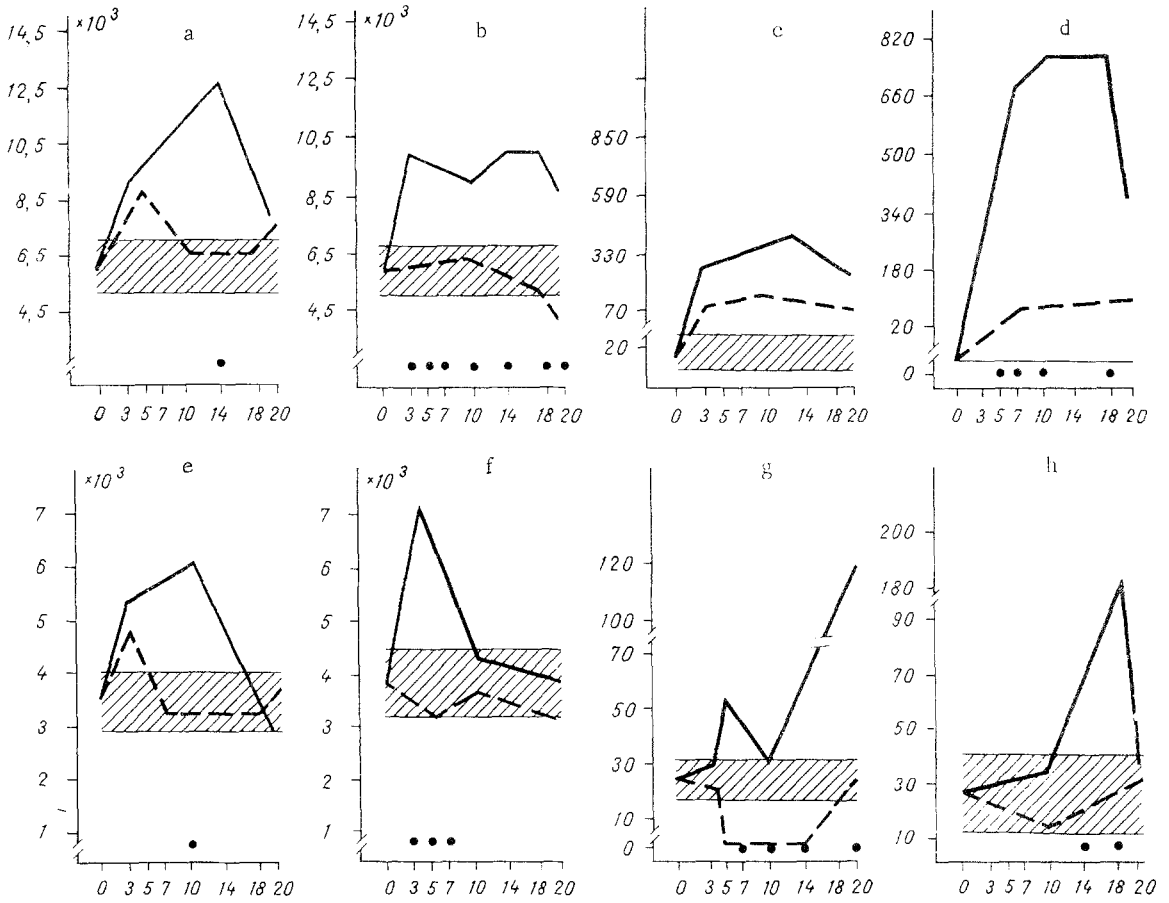

Fig. 1. Number of leukocytes (a, b), monocytes (c, d), lymphocytes (e, f), and transformed lymphocytes (g, h) in 1 μl blood. a, c, e, g) Strongly reacting animals; b, d, f, h) weakly reacting animals. Shaded area denotes confidence intervals of initial level. Broken line denotes control (wounding, without treatment), continuous line denotes experiment (treatment with PHA). Abscissa, number of cells in 1 μl blood; ordinate, time (in days). Dots indicate significance of differences between control and experiment.

ception (Fig. 1d). However, the response of the leukocytes to wounding in untreated animals is evidence of the unequal functional powers of these cells.

In the strongly reacting animals leukocytosis, which was observed for 7 days, was followed by restoration of the initial leukocyte count (Fig. 1a), whereas in weakly reacting rabbits there was no leukocytosis, and leukopenia developed by the 20th day (Fig. 1b). The monocytic reaction was equally high and stable in animals of both groups (Fig. 1c, d). However, the lower initial level of monocytes in the weakly reacting animals determines the slower rise of the response.

In the case of a strong DTH, lymphocytosis was found on the 3rd day (Fig. 1e), and withdrawal of the transformed lymphocytes from the circulation on the 5th-14th day (Fig. 1g). In the case of weak DTH the two parameters remained within their initial limits (Fig. 1f, h). Whereas in the strongly reacting animals stable neutrophilia was observed, the number of neutrophils in the weakly reacting animals was unchanged (Fig. 2a, b).

MPO activity in animals with strong DTH fell and subsequently recovered, to exceed the control values a little (Fig. 2c). In animals with weak DTH only a decrease in this parameter was observed (Fig. 2d). The ingestive power of each neutrophil (phagocytic number, Fig. 2e), the absolute phagocytic index (Fig. 2g) and, toward the end of the period of observation, the bacterial disintegration index (the percentage of neutrophils completing phagocytosis, Fig, 2h) were increased in the group of moderately strongly reacting animals. Strongly reacting animals (not the combined group) were characterized by very strong individual variability of the parameters with a tendency for most of them to increase. In the case of weak DTH

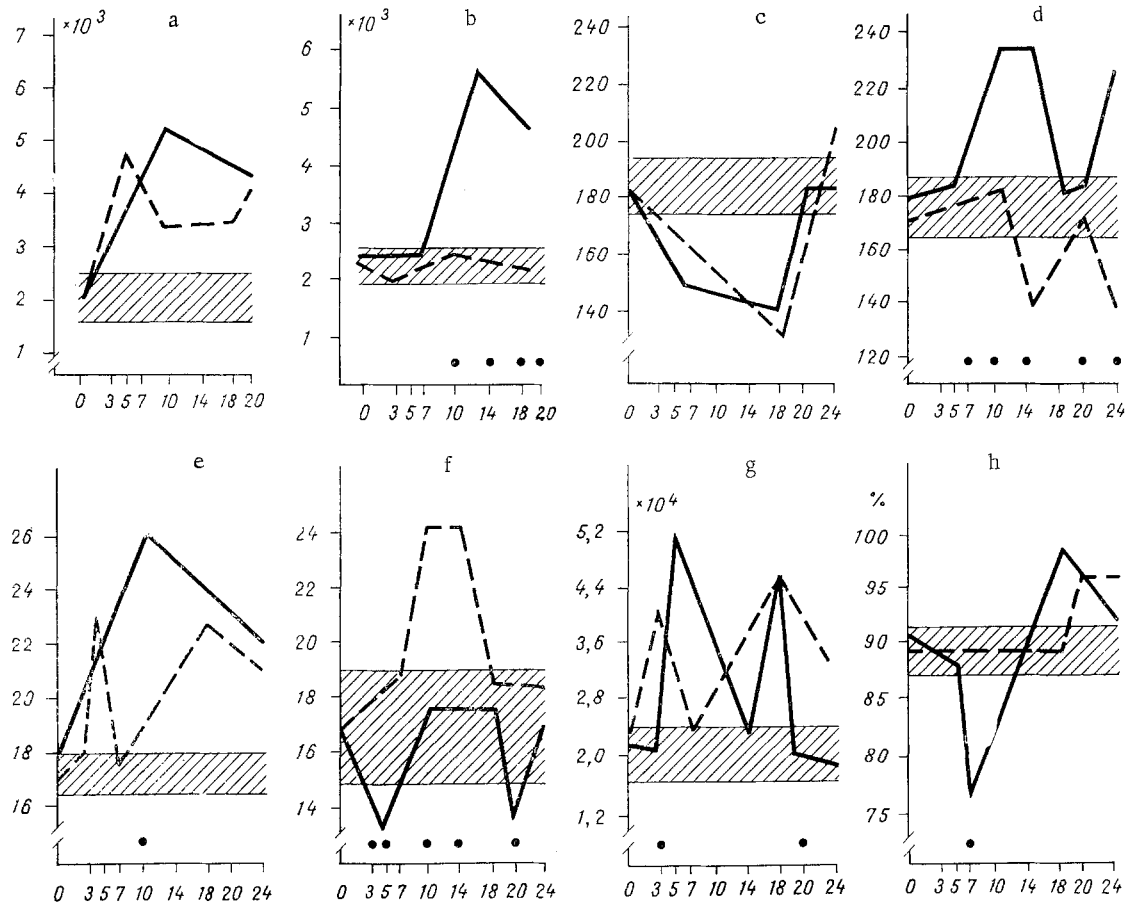

Fig. 2. Neutrophil counts (a, b), myeloperoxidase activity (c, d), and phagocytosis (e, f, g, h). a, c) Strongly reacting animals; b, d, f) weakly reacting animals; e, g, h) moderately strongly reacting animals. Abscissa: a, b) number of neutrophils in 1 μl blood; c, d) MPO activity (Kaplow's index); e, f) phagocytic number; g) absolute phagocytic index; h) % of neutrophils completing phagocytosis. Remainder of legend as to Fig. 1.

TABLE 1. Parameters of Phagocytic Activity of Neutrophils in Intact Rabbits (M ± m)

DTH response to PHA	Percent of phagocytic neutrophils	Phagocytic capacity	Phagocytic number	Absolute phagocytic index	Index of completion of phagocytosis
Strong	73,6±6,1	1451±233	18,4±1,4	$2,4\pm0,5\cdot10^4$	90,5±3,4
Average	73,2±1,9	1824±269	16,8±1,2	$2,1\pm0,4\cdot10^4$	89,1±2,6
Weak	76,7±5,4	1852±247	17,6±2,0	$3,3\pm0,7\cdot10^4$	93,1±2,4

all the phagocytic parameters were unchanged except the phagocytic number, which was increased (Fig. 2f).

An adequate response to wounding thus occurred only when a strong DTH to PHA was present. In animals with weak DTH a multiple defect of the leukocyte system was found. Judging from the high level of the monocytic response, attained despite very low initial values, it can be concluded that monocytes were the best preserved part of this system. However, in the absence of response of the other cells, they were unable to cleanse the wound. The mortality from wound infection among weakly reacting animals was 68.4% compared with 32.3% among strongly reacting animals. The periods of wound healing also were considerably longer than those in animals with strong DTH (32.2 ± 3.8 days compared with 23.5 ± 1.6 days).

Treatment of the wounds on strongly reacting animals with PHA did not affect the monocytic response (Fig. 1c) or neutrophilia (Fig. 2a) but led to the appearance of a second lymphocyte peak on the 10th day (Fig. 1e) and to an increase in the number of leukocytes on the 14th day (Fig. 1a). The number of circulating transformed lymphocytes also was considerably

increased (Fig. 1g). MPO activity (Fig. 2c) and the phagocytic parameters showed no significant change. The times of healing remained at the control level.

In weakly reacting animals the effect of PHA on most leukocytic parameters was strong. A stable and well-marked leukocytosis was observed (Fig. 1b) and also a simultaneous early and marked increase in the numbers of monocytes (Fig. 1d) and lymphocytes (Fig. 1c). On the 14th and 18th days a considerable number of transformed lymphocytes came out into the circulation (Fig. 1h). Neutrophilia was observed only on the 10th day (Fig. 2b). MPO activity rose sharply (Fig. 2d), but the phagocytic parameters showed no significant change. Only a decrease in the ingestive power of the neutrophils occurred (Fig. 2f). Thanks to the neutrophilia, however, the absolute phagocytic index remained at its initial level. On the whole, therefore, the phagocytic function of the neutrophils was not activated.

Cytological and histological investigations of the wound showed that in weakly reacting animals PHA makes the process of wound healing similar both in the character of its course and in the times of healing (shortening to 20.1 ± 0.6 days) to that observed in untreated animals with a strong DTH to PHA [2]. However, weakly reacting animals treated with PHA differed in some special features.

The delayed neutrophilia and incompleteness of the phagocytic function of the neutrophils are evidence of the serious defect of these cells and of the stimulating effect of PHA mediated through other cells (through monokines and lymphokines). Preservation of the monocytic response in untreated animals and also the early, intensive and stable monocytosis during treatment suggest direct stimulation of the monocyte system by PHA [11] and a leading role of monocytes in the correction of the wound healing process.

Considering the weak response of lymphocytes to PHA in the intradermal test it can be tentatively suggested that the high and prolonged lymphocyte peak is the result of a response mediated by monokines [10, 12, 13]. The ability of the monocytic–macrophagal system to cleanse the wound even when neutrophil function is defective is evidence of the stimulating action of lymphokines on this system [7]. Monocytosis and lymphocytosis also are responsible for the optimization of regenerative processes [1, 9, 10, 12].

Since in weakly reacting animals PHA did not affect the course of wound healing, the changes in certain leukocytic parameters (the additional peaks of lymphocytes and leukocytes, the release of transformed lymphocytes into the circulation) can evidently be interpreted as a side effect. An increase in the number of transformed lymphocytes in the circulation also was observed in animals with weak DTH. Our preliminary studies of the late action of PHA (1.5 and 3 months after treatment) on the structure of the organs of immunity indicates that these side effects are nonpathogenic.

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