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EFFECT OF THE STATE OF CELLULAR IMMUNITY ON PHARMACOLOGICAL IMMUNOCORRECTION OF WOUND HEALING BY PHYTOHEMAGGLUTININ IN RABBITS

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Great importance in the prevention of suppurative complications and in the treatment of wound has been attached in recent years to local and general mechanisms of immune defense of the body [3, 10, 11]. It has been shown [11] that a positive intradermal test to Thielmann's stain, tuberculin, and other bacterial antigens is associated with a low percentage of complications of wound healing (suppuration in 4% of cases), whereas a negative or weakly positive test is associated with a complicated course (suppuration in 21.5% of cases, development of infiltration in 59.2%). The change in the state of the wound from bacterial contamination to infection depends on the level of the body's immunologic defense [5].

In the last 10 years three principal trends in immunocorrection in vivo have been determined: hormones and mediators of the immune system, immune engineering, and pharmacological correction of immunity. The problem of wound healing and wound infection is also being tackled in accordance with these same three approaches [2, 6, 9]. A few investigations devoted to methods of immunologic stimulation of wound regeneration with the aid of the plant lectin phytohemagglutinin (PHA) have been published [1, 7]. The absence of any experimental justification for the use of this new immunostimulant for wound treatment prevents any objective evaluation of the method, still less any recommendation for PHA under clinical conditions.

Preliminary investigations to determine the PHA dose–effect relationship by the use of ointments and emulsions on 183 inbred C57BL/6 mice showed that a dose of 3 μg/g ointment base is optimal, when the times of wound healing are reduced by 33% compared with the control. The dose of PHA of under 3 μg/g had a suppressor effect; the times of wound healing during treatment with these doses were significantly longer than in the controls (25.5 ± 1.12 compared with 12.4 ± 0.55 days; $P < 0.001$). It has been shown [12, 13] that PHA (from Difco, USA, or Wellcome, England), in a dose of 3 μg/ml, causes maximal stimulation of DNA synthesis in a lymphocyte culture, raises the cyclic GMP level, and lowers the cyclic AMP concentration, effects which reflect maximal mitotic and functional activity of the lymphocytes.

The object of the present investigation was to study the stimulating effect of local application of PHA ointment and emulsion in a dose of 3 mg/g on wound healing in rabbits depending on the intensity of the reaction of cellular immunity revealed by the intradermal test to the same mitogens.

EXPERIMENTAL METHOD

The reaction of delayed-type hypersensitivity (DTH) objectively reflects the state of T cell immunity [8] and correlates with the reaction of blast transformation of lymphocytes in culture [4].

The intradermal test with PHA [1] was carried out on 255 rabbits of different breeds and of both sexes, weighing 3.2 ± 0.07 kg. All the rabbits were divided into three groups: those reacting strongly to PHA (32.9%;

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TABLE 1. Times of Complete Wound Healing (in days) in Rabbits Depending on DTH Reaction to PHA (M ± m)

Group of animals	No treatment	n	Treatment with PHA	n	P
Reacting strongly	24,0±2,63	7	25,5±1,30	8	>0,05
Reacting moderately strongly	23,0±1,21	16	26,2±1,89	11	>0,05
Reacting weakly	32,2±3,83	6	20,1±0,55	14	<0,01

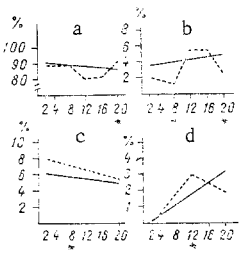

Fig. 1

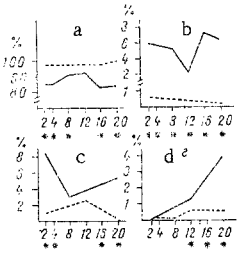

Fig. 2

Fig. 1. Cell counts in wound exudate during strong and moderately strong DTH reactions to PHA. Abscissa, time (in days), asterisks denote significance of differences between control and experimental values: broken line – control; continuous line – experiment. a) Granulocytes, b) lymphocytes, c) monocytes and macrophages, d) fibroblasts.

Fig. 2. Cell count in wound exudate during weak DTH reaction to PHA. Legend as to Fig. 1.

84 animals), those reacting moderately strongly (39.6%; 101) and those with a weak response (27.5%; 70 rabbits). By means of a specially constructed dissector, standard circular wounds of skin and muscle, 4 cm^2 in area, were inflicted under sterile conditions on the rabbits in the scapular region. The cytological composition of the wound exudate was studied in 104 rabbits: in 23 responding strongly (11 control and 12 experimental), in 41 responding moderately strongly (16 and 25 respectively), and in 40 responding weakly (18 and 22). Analysis of squash preparations from the wound surfaces was carried out on the 2nd, 4th, 8th, 12th, 16th, and 20th days of healing. Films were stained with azure II and eosin. The cell formula in the squash preparations was based on counting 500 cells. Histological sections through the skin from 105 rabbits were studied (39 reacting strongly, 34 moderately strongly, and 32 weakly) on the 5th, 10th, 15th, and 20th days of healing. The material was fixed in Bouin's solution. Paraffin sections were stained in the same way as the squash preparations. An ointment-emulsion of PHA (from Difco) was applied daily to the wounds of the experimental animals in a dose of 3 μg/g ointment base. Ointment base alone was applied to the wounds of the control animals. The results were subjected to statistical analysis by means of the Wilcoxon–Mann–Whitney nonparametric U test.

EXPERIMENTAL RESULTS

Investigation of the times of wound healing and of the cytology of the wound exudate of the rabbits after local application of ointment-emulsion with PHA showed that the therapeutic effect depended on the type of the animals' reaction to the intradermal test with PHA.

As Table 1 shows, a clear stimulating effect on the times of wound healing was found in the group of animals reacting weakly to PHA in the intradermal test. Healing was speeded up on average by 38% (by 12 days). The rate of wound healing per DM during treatment for 20 days was almost twice the rate of healing in the control (4.5 ± 1.45 compared with 8.5 ± 1.30; P = 0.05). No significant acceleration of wound healing took place in the animals with strong and moderately strong DTH reactions to PHA in the course of treatment; the wounds healed at the same time as in the control.

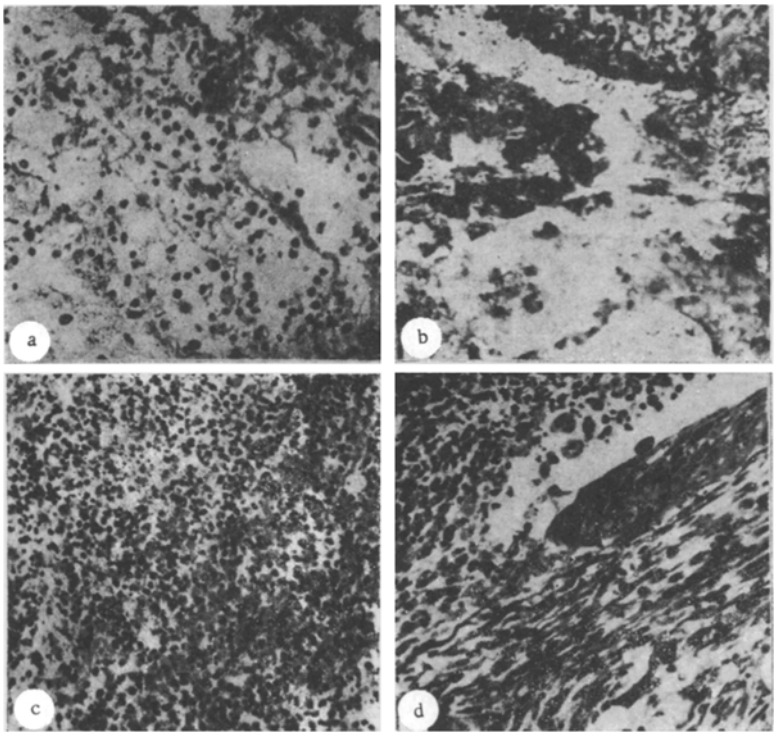

Fig. 3. Sections through skin in wound region in animals with weak DTH reaction to PHA. a, b) No treatment: infiltration with few cells (a), abundance of necrotic masses, absence of epithelization (b); c, d) after application of PHA emulsion ointment; abundant cellular infiltration (c), epithelial layer spreading above the layer of oriented fibroblasts (d). a, c, d) 5th day, b) 20th day of healing. Stained with azure II and eosin, 200×.

Comparison of the cell counts in the wound exudate from animals with strong and moderately strong DTH reactions in the control and experimental series showed that the course of the inflammatory reaction was identical (Fig. 1). The lymphocytic and monocytic-macrophagal reactions were at a high level at all periods of observation and the number of fibroblasts increased steadily to a considerable height. Significant differences between the control and experimental groups were observed only at certain times. This active type of cell dynamics ensured rapid clearance of purulent and necrotic masses from the wounds and created the necessary conditions for granulation tissue formation and early epithelization.

Analysis of cell counts in the wound exudate of animals with a weak DTH reaction to PHA showed a significant change in cell characteristics in the experimental rabbits compared with the control (Fig. 2). Whereas in the control animals at all stages of viewing (from the 2nd through the 20th day), the predominant cells in the wounds were neutrophils with signs of weak functional activity (Fig. 2a), and the intensity of the lymphocytic-macrophagal reaction was low (Fig. 2b, c; Fig. 3a), local applications of PHA emulsion ointment reduced the number of granulocytes but sharply increased the number of lymphocytes (including their active forms), monocytes, and macrophages. A significant increase in the number of these cells could be seen almost throughout the period of regeneration (Fig. 2b, c; Fig. 3c). The active function of the neutrophils and macrophages ensured rapid clearance of purulent and necrotic masses from the wound surface and filling of the wound defect by young granulation tissue. Whereas in the control, weakly responding animals the conditions for the formation of a layer of fibroblasts above which the epithelium has to proliferate did not arise until the 10th day (Fig. 2d; Fib. 3b), by the 5th day an increase in the number of fibroblasts and their distinct orientation in the granulation tissue could be observed in the wounds of the experimental rabbits, and layers of proliferating epithelium were spreading over the granulations (Fig. 3b).

The principal and fundamental results of this investigation was thus that PHA, in a dose of 3 μg/g ointment base, if applied locally to infected wounds in rabbits, creates a selective positive effect which depends on the strength of the DTH reaction, as measured by the intradermal test to PHA. The mitogen causes acceleration of wound healing by 33% in inbred C57BL/6 mice and by 38% in rabbits with a weak DTH reaction to PHA. Very small doses of PHA have a suppressor effect. The results of cytological and histological investigations of wound healing are evidence of the high compensatory powers of animals with strong and moderately

strong DTH reactions in the control and of the absence of any significant changes in the cellular and tissue dynamics during wound treatment with PHA. Positive changes in the cellular and tissue dynamics took place in animals with a weak DTH after application of PHA emulsion ointment. Judging from data in the literature, some workers have attempted to extend the practice of wound treatment with PHA under clinical conditions [7]. In this connection it must be emphasized that the intradermal test for PHA must be carried out for diagnostic (the state of cellular immunity) and also, possibly, for prognostic (the course of wound healing in the post-operative periods) purposes. Treatment of wounds with the PHA preparation is indicated if the intradermal test is negative or weakly positive.

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A MOUSE CELL LINE WITH INHERITED STABLE COLCHICINE RESISTANCE

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Resistance of mammalian somatic cells to antitubulins and, in particular, to colchicine and colcemid arises as a rule as a result of changes in permeability of the cell plasma membrane, leading to a decrease in accumulation of the mitostatics [3, 6, 9]. An interesting feature of these membrane changes is their unstable inheritance in a series of cell generations when the cells are cultured under nonselective conditions [1, 2, 9]. It has accordingly been suggested that such changes in membrane permeability may arise not as a result of gene mutations, but because of other genetic changes [1, 2, 9].

The present investigation was devoted to the description of a new subline of mouse cells resistant to colchicine.

EXPERIMENTAL METHOD

Mouse cells of lines L [5] and B-82, a subline of L cells deficient in thymidine kinase [8], were used. The cells were cultured in medium containing 45% Eagle's medium, 45% lactalbumin hydrolysate, 10% bovine serum, and 100 i.u./ml monomycin.

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