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EXPERIMENTAL LYMPHOKINE THERAPY OF WOUNDS

L. V. Koval'chuk, B. Bayart, and L. V. Gankovskaya UDC 617-001.4-085.362.112.94.017.1.063-036.8

Key words: lymphokines; mononuclear phagocytes; wound healing; cyclosporin A.

The problem of healing and treatment of wounds was and still remains one of the most urgent in contemporary surgery. Complication of wounds by the pathogenic microflora frequently leads to the development of a secondary immunodeficiency or, on the other hand, wounds which are slow to heal may be the result of a deficiency of the immune system.

Accordingly, the use of methods of immunocorrection during wound healing is of great importance.

The effect of lymphocytes on the regeneration of organs and tissues has been studied experimentally [1]. In recent years the role of lymphokines (interleukins, interferons) in regulation of the function of fibroblasts and epithelial cells has been extensively discussed. Disturbance of the secretion of these mediators is the main cause leading to the development of a severe inflammatory process and of indolent regeneration.

The study of the effect of lymphokines on regeneration in vivo is exceptionally interesting. This was the aim of the present investigation.

EXPERIMENTAL METHOD

A pure suspension of peripheral blood mononuclear cells from the rabbit ear was obtained by Böyum's method [7] and $5 \cdot 10^6$ of the isolated lymphocytes were stimulated with phytohenlagglutinin ("Difco") in a concentration of 10 μg/ml for 3 h. The cells were then washed to remove the mitogen and cultured for 20 h in medium with antibiotics: penicillin 100 μg/ml and streptomycin 100 μg/ml. After the end of culture the cells were removed by centrifugation and the supernatant was sterilized by filtration through membrane filters (pore diameter 0.22 μ, Whatman). Active fractions of lymphokines with mol. wt. of 20-30 kD (M fraction) and 60-70 kD (L fraction) were obtained from the supernatants of the peripheral blood lymphocyte cultures by gel-filtration on Sephadex G-100 [4]. The biological activity of the supernatants and of the isolated fractions was determined in a microversion of the macrophage migration inhibition test [5], and the phagocytic activity of the neutrophils was determined as in [6]. As an experimental model of wound healing, a skin-muscle wound with an area of 400 mm^2 was inflicted on noninbred rabbits in the scapular region (after anesthesia). Treatment of the wounds began on the 2nd day and continued for 7-8 days. The autologous supernatant of the lymphocyte cultures was used in a volume of 0.5 ml (protein concentration 50 μg/ml) and the M and L fractions of lymphokines in a dose of 100 μg/ml, by application to the wound. During the first 3 days treatment was carried out twice, in the morning and evening; subsequent treatments once a day for 4, 5, 6, 7, and 8 days. Lymphocytes (after culture for 20 h) were applied to the wound once at the rate of $7.0 \cdot 10^6$ cells/cm^2. The criteria of wound healing were: a) planimetric parameters: measurement of the area and determination of the rate of wound healing by Popova's method [5]; b) the time of complete epithelization; c) morphological investigation of the wound exudate by the squash preparations method [5]. The results were subjected to statistical analysis by Student's test.

Department of Immunology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 9, pp. 340-342, September, 1989. Original article submitted November 11, 1988.

0007-4888/89/0009-1313$12.50

TABLE 1. Effect of Autologous Supernatants of Cultures of Stimulated Intact Lymphocytes and of Lymphokine Fractions on Wound Healing in Rabbits

Parameter	Mean rate of healing, days	Mean time of healing, days	Number of wounds
Autologous supernatant of cultures of stimulated lymphocytes	13,4±4,5	17,6±4,6	8
Autologous supernatant of cultures of intact lymphocytes	6,7±4,1	26,6±2,6	6
Autologous stimulated lymphocyte	4,8±2,1	31,4±1,8	4
Autologous intact lymphocyte	5,9±3,7	27,6±0,6	4
Medium 199	6,0±2,5	32,5±2,5	10
Lorenden S	6,5±3,0	36,6±0,4	4
M fraction	12,5±3,6	18,5±3,6	12
L fraction	13,6±4,7	17,4±3,7	10

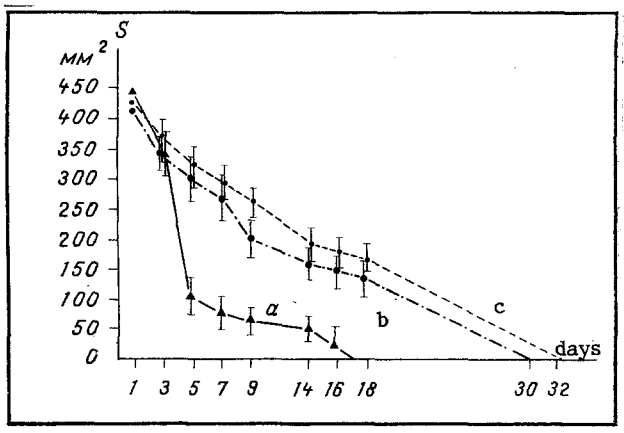

Fig. 1. Time course of contraction of wound defect in rabbits during treatment with autologous supernatants of cultures of stimulated and intact lymphocytes. Ordinate, area of wound (in mm^2); abscissa, times of wound healing (in days). a) Autologous supernatant of stimulated lymphocytes; b) autologous supernatant of cultures of intact lymphocytes; c) control.

EXPERIMENTAL RESULTS

Experiments to study the action of autologous supernatants of lymphocyte cultures and of the cells themselves on the wound were undertaken on various groups of animals.

Group 1 consisted of animals treated with autologous supernatant of cultures of stimulated lymphocytes; group 2 of animals treated with autologous supernatant of intact cells; intact and stimulated lymphocytes were introduced into the wound of the rabbits of groups 3 and 4. Animals treated with medium 199 and with lorenden S. The experimental results are given in Table 1.

The healing time of wounds treated with autologous supernatant of cultures of stimulated lymphocytes was shortened from 32.2 ± 2.5 to 17.6 ± 4.8 days. During treatment with autologous supernatant the size of the wound defect was reduced by 80-90% on the 9th day, compared with only 30-40% in the control (Fig. 1). Autologous supernatant of unstimulated lymphocytes had no such effect.

Autologous supernatant of cultures of stimulated lymphocytes is known to contain a wide spectrum of lymphokines [11].

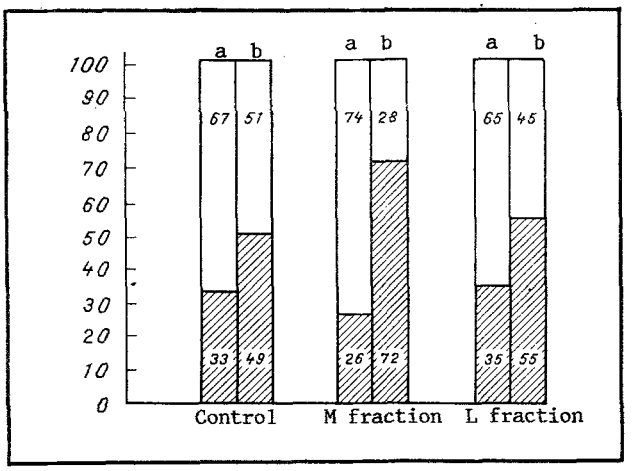

Fig. 2. Cellular composition of wound exudate during treatment with lymphokine fractions. Ordinate, number of cells (in %); abscissa, preparations used for treatment; a) before treatment, b) on 6th day of treatment. Unshaded columns — granulocytes; shaded — mononuclears.

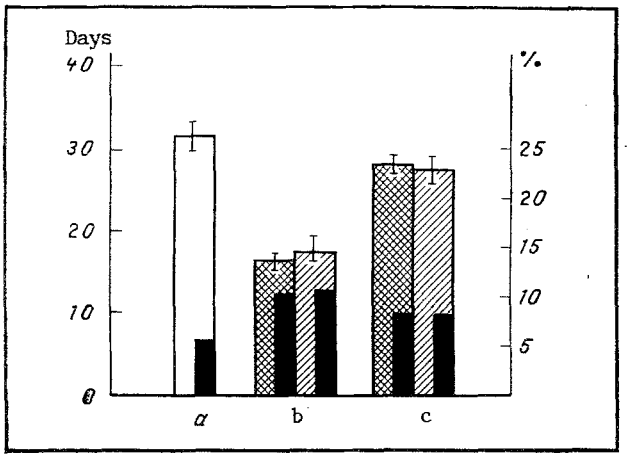

Fig. 3. Effect of lymphokine fraction obtained from supernatants of cultures of stimulated B lymphocytes (a) in the presence (c) and absence (b) of cyclosporin A on wound healing in rabbits. Ordinate, times of complete epithelization (in days) and rate of healing of wounds (black columns); abscissa, preparations used for wound treatment: unshaded columns — control; obliquely shaded — M fraction; cross-hatching — L fraction.

Determination of the biological activity of the supernatants revealed activity of macrophage migration inhibiting factor (MMI 38.9 ± 4.2) and of a factor activating leukocyte function (increased by 20-30%).

To confirm fully the role of lymphokines in stimulation of wound healing experiments were carried out in which active fractions of lymphokines isolated from the supernatant were used: the M fraction with molecular weight of 20-30 kD, stimulating the functional activity of cells of the macrophagal-monocytic series, and the L fraction with molecular weight of 60-70 kD, activating leukocyte function [4]. The times of complete epithelization in the case of treatment with M and L fractions of lymphokines were 18.5 ± 3.6 and 17.4 ± 3.7 days respectively.

Morphological analysis of the wound exudate showed that on the 6th day of treatment with autologous supernatant and with lymphokine fractions the number of mononuclear cells was 2-3 times greater than the number of granulocytes. This early increase in the number of mononuclears leads to rapid cleansing of the wound, a switch of the regeneration process into the proliferative phase of healing, and shortening of its times [5].

Further proof that lymphokines may help to accelerate regeneration is given by experiments involving culture of stimulated lymphocytes with cyclosporin A. Cyclosporin A, a known immunodepressant, acts on effector cells of immunity and, in particular, it inhibits secretion of the some lymphokines (interleukin 2, interferon [8-10]). Lymphocytes stimulated by phytohemagglutinin were cultured with cyclosporin A for 15 h in a dose of 1 μg/ml in medium 199 ($5 \cdot 10^6$ cells/ml). The lymphokine fractions thus obtained, after gel-filtration on Sephadex G-100, had no stimulating action on wound healing in rabbits (Fig. 3). The complete epithelization time was 28.4 ± 4.5 and 29.0 ± 6.6 days. This is further confirmation that lymphokines play a role in regeneration.

Accelerated wound healing in rabbits may be due to the direct effect of lymphokines on fibroblasts and on cells of the phagocytic system, playing an active role in regeneration.

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