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EXPERIMENTAL LYMPHOKINE THERAPY OF WOUNDS

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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.

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