# Natural Cytokine Complex in Therapy of Penetrating Corneal Wounds in Rabbits

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**Key Words:** *natural cytokine complex; cornea*

Reports have recently appeared on the possibility of enhancing regenerative processes in the injured cornea under the influence of cytokines [5]. Combinations of various cytokines - EGF and PDGF, basic and acid fibroblast growth factors in particular - have been shown to exert a greater influence on retinal cell proliferation than each of the cytokines alone [7]. The important role played by macrophage-like cells in regenerative and inflammatory processes permitted us to assume that the natural cytokine complex produced by the peripheral blood mononuclear cells may be effective in the treatment of eye injuries. Previously we developed a method for the experimental treatment of experimental cutaneofascial wounds based on the local application of a complex of endogenous immunopeptides secreted by the mononuclear cells, and suggested a method of autolymphokine therapy based on the use of an autologous complex of cytokines and its fractions obtained during peripheral blood cell stimulation [3].

The present research was aimed at an elucidation of the effect of a cytokine complex on regenerative processes in corneal tissue. The effect of the cytokine complex and its fractions on the course of rabbit corneal wound healing was studied.

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## MATERIALS AND METHODS

Blood was collected from the marginal vein of the rabbit ear. Mononuclear cells were isolated according to Boyum's method [4] and cultured in medium 199 with antibiotics (100 μg/ml streptomycin and 100 IU/ml benzylpenicillin, cellular concentration $5\times10^6$/ml). Phytohemagglutinin (20 μg/ml) (Difco, Usa) was added to the incubation medium. After 3 h incubation with phytohemagglutinin, the cells were twice washed in Hanks solution and incubated for 24 h. The cells were then sedimented by centrifugation and the resultant supernatant was collected. Supernatant fractions with a molecular weight of 80-60 and 30-10 kD were obtained by ultrafiltration through PM30 and PM10 membrane filters (Amicon, the Netherlands). The supernatant and its fractions were sterilized by filtration through Synpor filters (Czechoslovakia) with pore diameter 0.23 μ. Chinchilla rabbits were used in the experiments. Penetrating wounds of the cornea were inflicted under dicaine anesthesia. Before the injury the tested preparation was subconjunctivally injected in a dose of 0.3 ml. Further therapy consisted in instillations of the preparation, 1 drop 5 times a day for the first

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2 weeks and 3 times a day for the subsequent 6 weeks. The following variants of treatment were used: 1) cytokine complex therapy, group 1; 2) therapy with a complex fraction containing proteins with a molecular weight of 80-60 kD, group 2; 3) therapy with a supernatant fraction containing proteins with a molecular weight of 30-10 kD, group 3; 4) therapy with medium 199 with antibiotics used in cell culturing, group 4; 5) therapy with phosphate saline buffer (20 mM $KH_2PO_4$, 0.15 M NaCl, pH 7.35), group 5. There was one more group of animals treated with the cytokine complex one month after the wound was inflicted (after cicatrix formation), to which the preparation was instilled at the rate of one drop 3 times a day. The following parameters were assessed: wound size (width of edema and thickness of the forming cicatrix); mucous discharge and photophobia within the first two days after wound infliction; time of edema disappearance and recovery of the natural curvature and transparency of the cornea.

## RESULTS

Comparison of the results in controls and test group animals treated with the cytokine complex showed marked acceleration of eye recovery and the cessation of photophobia and mucous discharge within 2 days after wound infliction, a much finer cicatrix, and therefore better recovery of the natural curvature and transparency of the cornea. The scars had almost completely disappeared 8 month after the injury in the cytokine-treated animals, whereas in the controls scar width was virtually unchanged over the same period and corneal curvature and transparency remained abnormal (Figs. 1, 2; Table 1).

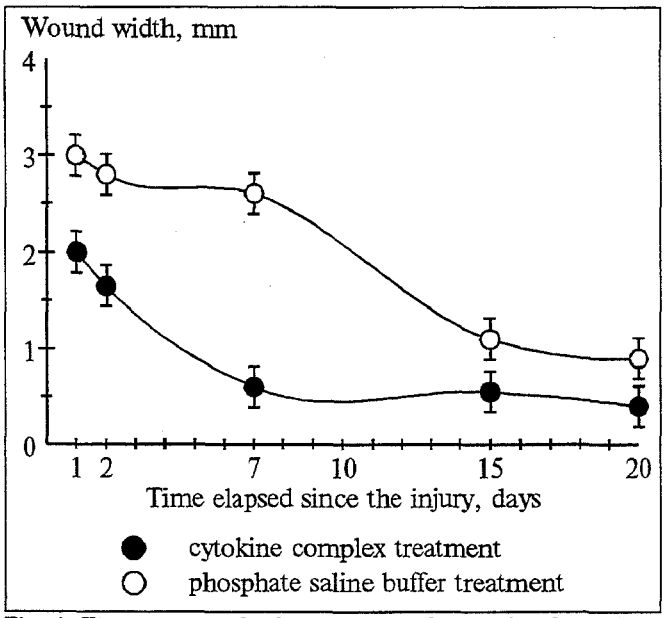


**Fig. 1.** Time course of healing of a wound treated with cytokine complex and phosphate saline buffer.

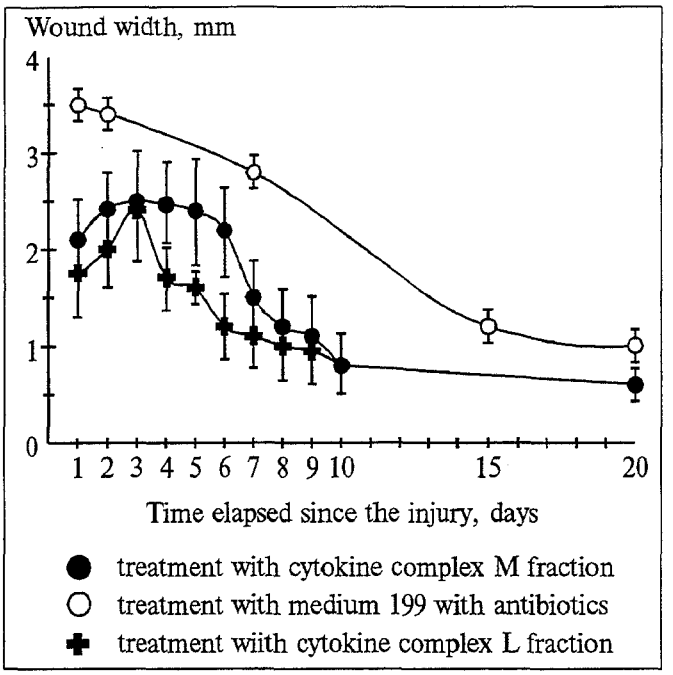


**Fig. 2.** Time course of healing of a wound treated with cytokine fractions and medium 199 with antibiotics.

The results were virtually the same in the two control groups of animals treated with medium 199 supplemented with antibiotics and with phosphate-saline buffer. Hence, the preparation was effective solely due to the cytokines, not to the negligible amounts of antibiotics in it.

Comparison of the results in the different test groups showed a much higher efficacy of the whole cytokine complex produced by the peripheral blood mononuclear cells than of different fractions of this complex. This is in line with current notions on the synergism of cytokine action. The diagrams presented in Figs. 1 and 2 demonstrate a much earlier wound healing and a finer scar in the animals administered the initial cytokine complex as compared to those treated with cytokine fractions. The effects of the different fractions are the same in the end, the difference being just in the time course of edema resolution and in the more frequent prevention by the M fraction, a protein fraction of molecular weight 30-10 kD, of mucous discharge within the first 24 h after the injury. Administration of fractions resulted first in edema enlargement, whereas the whole complex steadily reduced edema starting from the very first day. The M fraction, which inhibited macrophage migration *in vitro* [1], induced a marked edema reduction only 6 to 7 days after the injury, whereas treatment with the L fraction of molecular weight 80-60 kD, which inhibited leukocyte migration *in vitro* [2], reduced edema in 3-4 days. Note that the

TABLE 1. Comparison of the Effects of Variuos Cytokine Complex Factors and Fractions and Control Media on Cornea Wound Healing

| Comparison criteria | Phosphate saline buffer | Medium 199 with antibiotucs | Whole cytokine complex | M fraction | L fraction |
|---|---|---|---|---|---|
| Time of complete edema resolution, days | 14 | 14 | 7–8 | 7–8 | 6–7 |
| Mucous discharge, days | +(1–2) | +(1–2) | – | – | ± |
| Photophobia, days | +(1–2) | +(1–2) | – | – | – |
| Cicatrix width 3 weeks after wound infliction (Student test), mm | 1.5–0.1 | 1.5–0.1 | 0.3–0.1 | 0.5–0.1 | 0.5–0.1 |
| Recovery of normal corneal curvature and transparency 5 months after wound infliction | – | – | + | ± | ± |

effect of the cytokines is most pronounced during the first week after the injury, and scar size changes very slowly after 2 weeks.

Histological analysis of cicatrix structure in control animals and in those treated with the cytokine complex M and L fractions was carried out at the Helmholtz Institute of Ophthalmology. Scars were examined 6 weeks after the injury. In control animals mature scars with a clear-cut laminar structure were formed (Fig. 3, *a*). The epithelium in the cicatrix zone was slightly thickened, and the endothelium; was completely restored. Cicatrix structure was the same in both test groups of animals. Residual epithelial plugs were found, and the structure of the deeper layers of scar tissue was characterized by a more pronounced cellular pattern without clear-cut lamination (Fig. 3, *b*). Active large keratoblasts predominated among the cells.

Hence, cytokines promoted a certain delay of corneal cut wound cicatrization in experimental animals as against controls that may be due to the effects of cytokines on the early reparative processes in

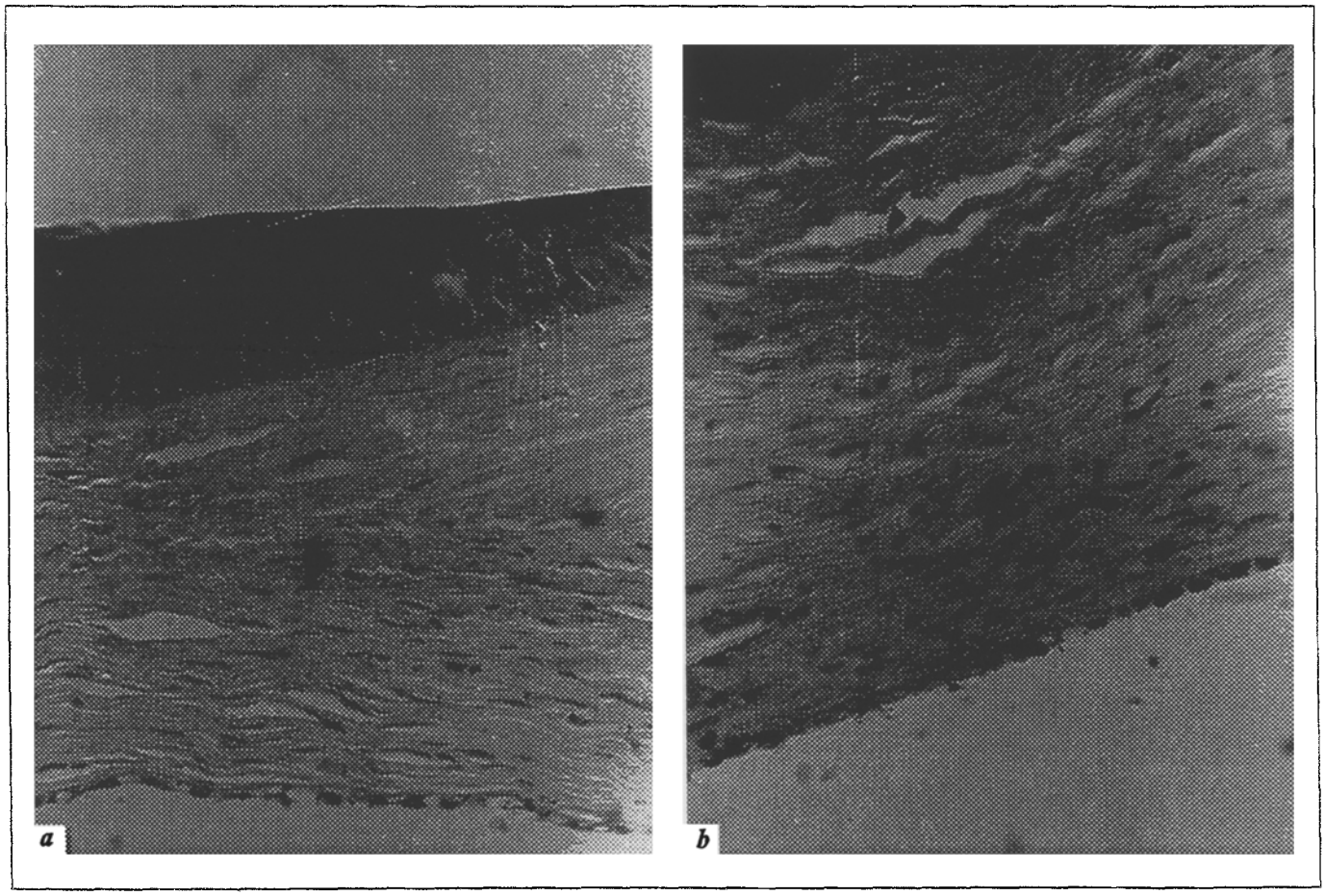


Fig. 3. Histological control. *a*) laminar structure of cicatrix consisting of mature keratinocytes. Control. Magnification 160. Hematoxylin–eosin staining. *b*) cellular pattern of cicatrix. Young keratinocytes in deeper layers. Cytokine therapy. Magnification 160. Hematoxylin–eosin staining.

the cornea. It is possible that the cytokine-effected delay of fibrin resolution by macrophages at the earlier stages of healing results in a latter filling of the wound canal with newly formed scar tissue characterized by a higher keratoblast content.

The fact that cytokines affect the earliest stages of inflammatory and regenerative processes was confirmed by further experiments with cytokine complex administration one month after the injury. The preparation was used for a month without any effect on scar size being noted. Previously we demonstrated that the cytokine fraction of molecular weight 30-10 kD noticeably changed the metabolic profile of phagocytes and regulated their mobility, activized phagocytosis, potent biooxydant generation, production of immunopeptides (interleukin-1, tumor necrosis factor, etc.), and regulated fibroblast functions [6]. It may be assumed that the effect of the cytokine complex was manifested in a depression of fibroblast excessive proliferation and inflammatory reaction inhibition due to inhibition of macrophage and leukocyte migration toward the focus of injury from the limbic area. It is also possible that exogenously introduced cytokines induce immunopeptide production by the cells and normalize the healing processes.

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# EXPERIMENTAL GENETICS

# Characteristics of T-Suppressors Responsible for Cellular and Humoral Immune Response Competition in the Spleen

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The possibility of competence between delayed-type hypersensitivity (DTH) and antibody production (ABP) T-effectors at the stage of mature antibody-producing cells in the spleen has been previously demonstrated [1] with antigen-specific DTH T-suppressors acting as contrasuppressors with respect to the ABP productive phase in the spleen [1,3].

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