Experimental Study of Cytokine Regulation of Postburn Regeneration of the Cornea

L. V. Gankovskaya, L. V. Koval'chuk, T. V. Radygina, N. A. Konstantinova, I. P. Khoroshilova-Maslova, L. V. Ilatovskaya, and M. E. Bakhtulova

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A complex of cytokines with interleukin-1, interleukin-6, tumor necrosis factor-α, and growth-transforming factor-β_1 activities promoted resorption of debris and proliferation of keratoblasts in eye burns.

Key Words: *endogenous cytokines; cornea; burn; regeneration*

The key role of cytokines in the regulation of pathological processes in the eye is extensively discussed in recent years [8]. A positive effect of cytokines in penetrating wounds of the eye has been demonstrated experimentally and clinically. Application of a cytokine complex (CC) decreases inflammation, prevents the formation of coarse cicatrices, and decreased autoimmune reactions to eye-specific antigens [1].

Formation of coarse intensively vascularized corneal leukomas in burn disease are often responsible for keratoplasty failure, because of transplant opacity or rejection [4], which necessitate the search for new approaches to regulation of postburn regeneration of the cornea.

We investigated the effect of CC on inflammation and regeneration processes on a model of post-burn regeneration of rabbit cornea.

MATERIALS AND METHODS

Citokine complex was were prepared by culturing porcine blood leukocytes stimulated with phytohemagglutinin [2]. Cytokine-containing fraction with interleukin-1, interleukin-6, tumor necrosis factor-α (TNF-α), and growth-transforming factor-β_1 (GTF-β_1) activities was used. Protein concentration (700 μg/ml) was measured according to Bradford [5]. The concentration of GTF-β_1 was determined using a Quantikine enzyme immunoassay kit. Activity of interleukin-1 was evaluated in biological test [8]. Activity of TNF-α was tested in L-929 tumor fibroblast culture [6]. Activity of macrophage migration inhibitory factor (MIF) was evaluated using a Migroscreen kit (Niarmedik, Moscow).

Alkali burn of the cornea was inflicted by 20-sec application of a 8-mm filter paper disc soaked with 10% NaOH after total droperidol analgesia and local dicaine analgesia. Experimental rabbits were treated by fractionated instillations of CC (one drop 5 times a day during the first 2 weeks). To controls, medium 199 was instilled according to the same protocol.

Histological and morphometrical analyses and clinical evaluation of the efficacy of CC were carried out on days 1, 3, 7, 14, and 30. Clinical criteria of healing were the time course of healing of corneal erosion, ulceration of the cornea, neovascularization, and inflammation. On day 14 after burn, fibroblasts and keratocytes in histological sections of the cornea were counted. The results were statistically processed using Wilcoxon's *U* test [3].

RESULTS

Cytokine activity in CC was estimated by biological testing and expressed in arb. U/mg protein. It was

Department of Immunology, Russian State Medical University; Helmholtz Institute of Eye Diseases, Moscow

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TABLE 1. Number of Fibroblasts and Keratocytes in Histological Sections of Rabbit Cornea on Day 14 after Burn (M±m)

Specimen	Number of fibroblasts and keratocytes		
	surface layers	middle layers	deep layers
Control	11±4.479	8±3.319	7±1.99459
Experiment	24±5.464	23±6.118	26±5.158

3200±530 for interleukin-1, 700±100 for interleukin-6, 320±80 for MIF, and 212±53.1 for TNF-α. The concentration of GTF-β_1 in CC was 470 pg/ml.

Cytokine complex instilled to experimental rabbits contained (in arb. units per 50 μl): 32±4 interleukin-1, 2.1±1 TNF-α, 35±5 interleukin-6, and 16±4 MIF. The concentration of GTF-β_1 in CC was 24 μg/ml.

Treatment with CC for 7 days decreased the size of erosions and the degree of vascularization of the cornea in comparison with the control. Histological study of corneal burn revealed penetration of solitary cells (including macrophages and keratoblasts) into the burn focus. Morphological analysis demonstrated the difference between the status of the burn focus in the control and experimental animals: the number of macrophages and keratoblasts was notably higher in experimental animals.

Morphometric analysis of corneal sections was carried out on postburn day 14 (Table 1).

The number of fibroblasts and keratocytes in all layers of the cornea was 2-3 times higher in the experimental group in comparison with the control. In untreated animals, ulcers in the focus of burn formed 14 days after the injury. In experimental group necrosis involved only the surface layers in the burn focus; no ulcers formed. The most pronounced differences were observed 30 days after burn (Fig. 1): a greater number of fibroblasts was observed in experimental animals in comparison with the control, probably due to more intense migration and active proliferation [7].

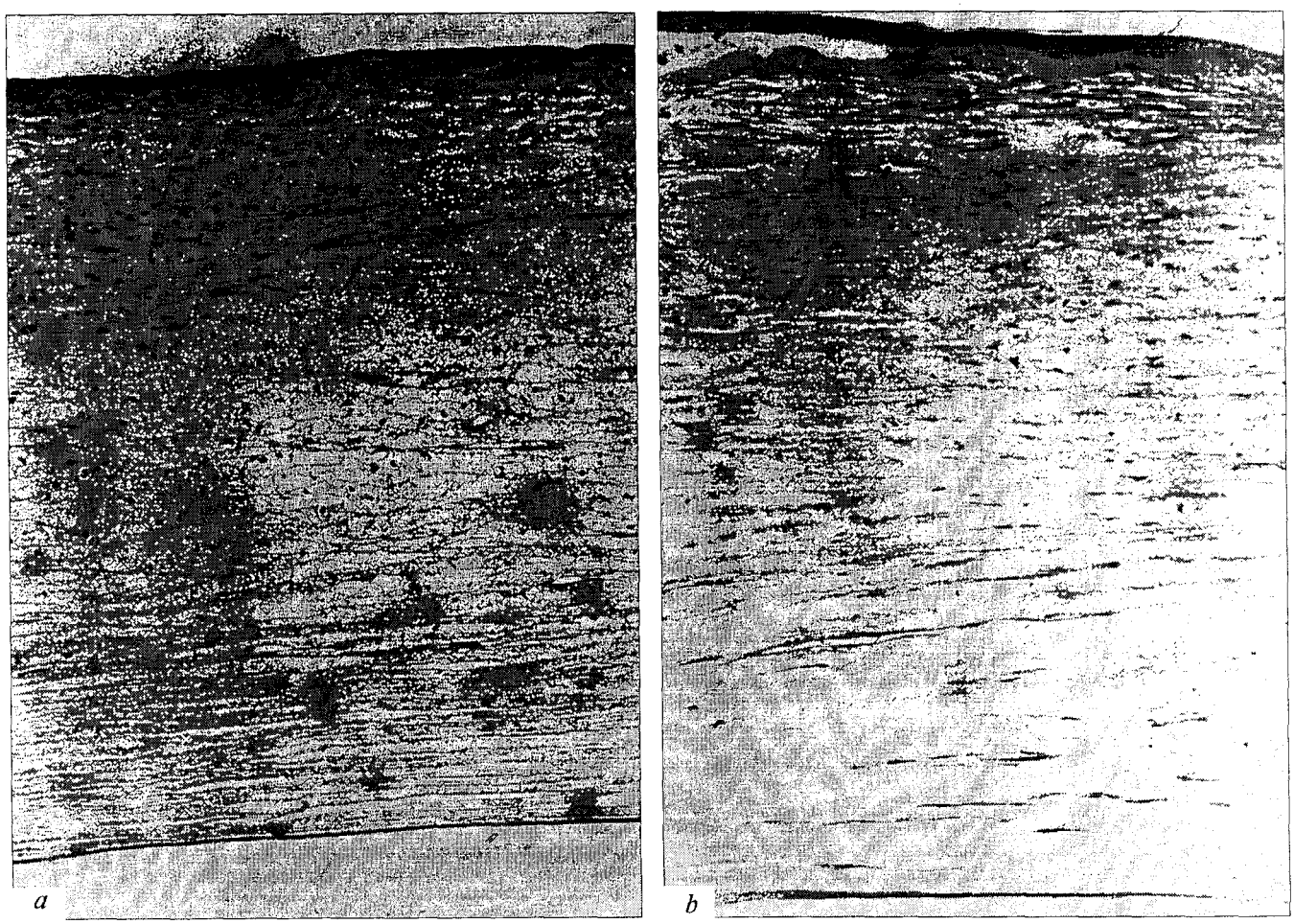

Fig. 1. Focus of corneal burn in the control (*a*) and after treatment with cytokine complex (*b*). *a*) active inflammatory neutrophilic lymphocytic infiltration in surface and middle layers; homogenization of corneal lamellae and the absence of cell elements in deep layers. *b*) recovery of corneal basal epithelium, replacement of surface corneal stromal layers with fibrous tissue, penetration of keratoblastic elements into deep layers of the stroma and recovery of its lamellar structure. Hematoxylin-eosin staining, ×160.

Histological studies and *in vitro* experiments showed that exogenous CC regulated postburn regeneration of the cornea at all stages. At the early stages (the first 7 days) CC primarily stimulated macrophage migration to the focus of injury and activated phagocytosis, generation of active oxygen forms, cytokine, interleukin-1 and TNF-α cascade [7], which determined their triggering role in the regulation of inflammation and regeneration.

At the later stages CC accelerated reparative processes in the cornea by stimulating active migration and proliferation of fibroblasts. GTF-β_1 is a key factor triggering healing process; it induces collagen and fibronectin synthesis by corneal stromal cells [9]. The concentrations of GTF-β_1 in the lacrimal fluid of rabbits treated with CC and controls were virtually the same on day 3 after the injury. However, long-term instillations of CC containing GTF-β_1 can contribute to the creation of its high local concentration in the anterior chamber of the eye. Cytokines in the complex can also regulate connective tissue remodeling in burn lesions at the later stages. This preserves partial lamellar structure of the cornea in the burn focus and limits the zone of cicatricial changes in comparison with the control. Such a structure of the cornea is essential for its optic characteristics forming less intensive postburn opacity.

The results open new possibilities for using CC in the treatment of corneal burns.

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