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IMMUNOLOCALIZATION OF THE MYRISTOYLATED ALANINE-RICH C KINASE SUBSTRATE (MARCKS) IN HUMAN CORNEAL KERATOCYTES.

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Purpose. MARCKS is a ubiquitous protein kinase C substrate which interacts with calmodulin and cross-links the actin filaments. Its cellular function is probably related to the regulation of these two proteins. MARCKS is also a physiological substrate for proline-directed kinases like cyclin-dependent kinases (cdks) and MAP kinases. The expression of MARCKS is high in quiescent fibroblasts, with a rapid down-regulation occurring after serum stimulation. Altogether, these data suggest a function of the protein in the regulation of the cell cycle. MARCKS is localized at the plasma membrane and reversibly translocates to the cytosolic and lysosomal compartments after phosphorylation by PKC in mouse embryo fibroblasts. **Methods**. We have used cell fractionation, immunoblotting, and immunofluorescence on intact cells and nuclear preparations to investigate the expression and localization of MARCKS in cultured human corneal keratocytes before or after serum or PMA stimulation. **Results**. The expression of MARCKS in human keratocytes was followed by immunoblotting of crude cellular extracts. Maximal expression was observed in quiescent (serum-deprived) cells, and a clear decrease occurred after 16 hours of serum stimulation. This corresponds to the beginning of the S phase in this cell type. Immunoblotting of nuclear and non-nuclear (cytoplasm and membranes) extracts revealed the existence of a nuclear population of the protein both in 3T3 fibroblasts and keratocytes. The nuclear population seems to be constant after serum or PMA stimulation. Immunofluorescence of intact cells confirms the membrane localization in keratocytes, and the cytoplasmic redistribution after PMA stimulation. In nuclear preparations, the protein is clearly present in the nuclei, but with a different distribution in 3T3 fibroblasts and in keratocytes. In this cell type, a punctate distribution was observed, that is not changed after PMA stimulation. The nature of these nuclear structures remains to be established. **Conclusions**. We demonstrate the regulated expression of MARCKS during the cell cycle in keratocytes, and describe the existence of a nuclear population of the protein in specific nuclear structures not yet identified.

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DETECTION OF CELLULAR ADHESION MOLECULES (CAM) IN STROMA OF INFLAMED AND NON-INFLAMED CORNEAS.

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Purpose:CAM are surface glycoproteins that promote migration of inflammatory cells (IC) from vessels to inflammatory sites through vascular endothelium and activation of these IC by cell-cell and cell-matrix interactions.We studied the expression of CAM in corneal stroma,which is site of inflammatory processes
Methods:35 Corneas were divided into 4 groups based on histologic findings:G1(n=5):control;G2(n=7):non inflamed non neovascularized;G3(n=9):neovascularized non inflamed;G4(n=14):neovascularized and inflamed. Immuno alkaline phosphatase technique was performed with monoclonal antibodies (MoAb) including:VCAM-1, ELAM-1 LECAM-1,ICAM-1 ICAM-3,LFA-1,MAC-1,CD11c,LFA-3, VLA-4 CD51.Positive cells were counted and results were expressed with an average score for each MoAb and each group.
Results:All CAM were highly expressed in G4.ICAM-1 lymphocytic ligand LFA-1 and monocytic ligand MAC-1 were strongly expressed with similar pattern;LFA-3, binded on T-lymphocytic membrane,CD11c,binded on monocytic membrane VCAM-1 and his ligand VLA-4 were highly expressed too.Selectins (ELAM-1-LECAM-1) were expressed in the G4 only.
Conclusion:CAM are expressed at corneal stromal inflammatory sites and should be involved in the mechanisms of recruitment,transport of immune cells and in the regulation of immune reaction by interactions between immune cells and with extracellular matrix The involvement of CAM in corneal inflammatory diseases suggests a new therapeutic way by blocking or by modulation of their expression by McAb.

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SCANNING SLIT CONFOCAL MICROSCOPIC OBSERVATION OF NORMAL HUMAN CORNEAL INNERVATION AND REINNERVATION AFTER PERFORATING KERATOPLASTY

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So far descriptions of the structure of the corneal innervation were only possible on the basis of histological techniques. The confocal microscopy represents a new non-invasive method for the structural examination of the cornea in vivo. Through our examinations we first defined the control group and then proceeded to record the reinnervation of donor tissue after perforating keratoplasty.

Method: We used the confocal slit-scanning video-microscope 'Microphthal' to examine forty corneas from twenty normal patients as well as fifteen donor eyes before their preparation for a tissue culture. These results were compared to our findings from follow-up checks on fourteen patients after perforating keratoplasty over a period of one month to two years.

Results: With system used we were able to visualise nerves in the middle and superficial stroma and in the basal epithelium in normal patients as well as in donor eyes. Normal stromal nerves were relatively thick, somewhat radially orientated, and gently curved over long distances. As the anterior stromal nerves reached the interface between the stroma and Bowman's layer, they either sent branches directly into the epithelium through Bowman's layer or broke up into a network of branched nerves - the subepithelial nerve plexus. The nerves of this sparsely distributed plexus were of very low contrast and with a granular texture. The basal epithelial nerve plexus appeared as beaded nerves, mostly arranged in a parallel mode. They either branched twice or, unlike to the stromal nerves, three times. Occassionally, fibres seemed to fuse for a short distance.
The first stromal nerves after perforating keratoplasty were to be seen in the central corneal area after seven months. However, the first central reinnervation from these fibres in the region of Bowman's membrane as well as in the basal epithelium was not detected until fifteen months after operation.

Conclusions: With the confocal microscopy we have the unique potential to study the morphology of the corneal innervation in vivo and in fresh donor tissue. Furthermore, for the first time a non-invasive morphological examination of the reinnervation of the human cornea after surgical treatment can be performed.

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TITLE: CONFOCAL IMAGING OF CORNEAL ENDOTHELIAL AND STROMAL CELLS: A COMPARATIVE STUDY

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The apical characteristics of corneal endothelial cells have been investigated extensively in health and disease using specular (in vivo) and scanning electron (in vitro) microscopes. Micrographs must however be interpreted within the context of limitations inherent in both tissue preparation and imaging techniques. Images recorded through specular microscopes are of relatively low magnification (x30) and lack contrast whereas those observed in scanning electron microscopy are affected by fixative induced shrinkage, which in turn produces artifacts. Confocal technology, when applied to the imaging of the corneal endothelium, overcomes some of these problems in that unfixed tissue can be viewed at higher levels of axial and lateral resolution than are currently available through conventional light microscopes

In this study a Nikon laser scanning confocal microscope with a 40x water immersion lens (NA 0.55) was used to view corneal endothelial and stromal cells from a number of higher species. Fresh corneae, bathed in phosphate buffered saline (37 C), were flat mounted endothelial side up in 1.5% agar.

Results highlight the consistency of the corneal endothelial hexagonal mosiac observed in many species. Apical features previously noted on specular and scanning electron micrographs were identified in unfixed tissue. The generalised absence of multinucleate cells was reported in most species. Supravitally stained tissue (DASPMI) revealed cellular organelles within stromal fibroblasts. Typically elongated nuclei were seen in those cells immediately underlying Descemet's membrane. The relative appearance and density of stromal cells at varying depths throughout the corneal stroma were noted.

Confocal microscopy has been shown to be a useful tool in the comparative study of viable corneal endothelial and stromal cells in higher species.