Immunohistochemical localization of chondroitin sulfate proteoglycan and tenascin in the human eye compared with the HNK-1 epitope

Abstract  • Background: A previous study revealed the HNK-1 epitope in the human ciliary body beneath the ciliary epithelium. The molecules bearing this 3-sulphoglucuronic acid-containing oligosaccharide epitope in the eye remain unknown. As chondroitin sulphate proteoglycan (CSPG) and tenascin are potential candidates as bearers of the HNK-1 epitope, their distribution in the human eye was compared with that of the HNK-1 epitope. • Methods: Fifty-five formalin-fixed, paraffin-embedded human eyes, including 20 normal eyes and 35 eyes with exfoliation syndrome or glaucoma, were studied immunohistochemically with monoclonal antibody (MAb) CS-56 to CSPG, MAb TN2 to tenascin, and MAbs HNK-1 and VCI-1 to the HNK-1 epitope. Additionally, four frozen lens capsules with exfoliation material were studied by indirect immunofluorescence. • Results: A population of dendritic cells in the inner connective tissue layer of the ciliary body and exfoliation material were immunoreactive with antibodies to the HNK-1 epitope, but no labelling for CSPG and tenascin was seen in them, including frozen sections. The inner surface of the nonpigmented ciliary epithelium was reactive for the HNK-1 epitope, and at the ora serrata also for CSPG. In some eyes with glaucoma, immunoreaction for CSPG and tenascin was seen beneath the epithelium and endothelium of the cornea. The nerve fibre layer of the retina was labelled for tenascin. In the sclera, all antibodies labelled the ground substance, and in some large blood vessels immunoreaction for CSPG and tenascin was seen subendothelially. • Conclusion: Apart from the sclera, the distribution of CSPG and tenascin was different from that of the HNK-1 epitope, suggesting that this carbohydrate epitope may not be borne by these molecules in the human ciliary body.

Introduction

A previous study revealed a strong and constant immunoreaction with monoclonal antibodies to the HNK-1 carbohydrate epitope in the human inner connective tissue layer, between the ciliary epithelium and the ciliary muscle [35]. This area has been regarded as a nondescript tissue, with no special functional role, containing fibroblasts, melanocytes, lymphocytes, mast cells and macrophages together with blood vessels, collagen fibrils and nerves [14]. None of these components can easily explain the extensive immunoreaction for the HNK-1 epitope in the ciliary body.

The HNK-1 epitope, a 3-sulphoglucuronic acid-containing oligosaccharide [8, 22], is situated on many extracellular matrix and integral membrane glycoproteins and glycolipids, such as neural cell adhesion molecule (N-CAM) [18], myelin-associated glycoprotein (MAG)
[18, 24], peripheral myelin glycoprotein P<sub>n</sub> [6], J1/cytotactin/tenascin [10, 11, 19], cytotactin-binding proteoglycan [13], chondroitin sulphate proteoglycan [22], acetylcholinesterase [7], and αβ1-integrin [20, 28]. However, the molecules which bear the HNK-1 epitope in the human eye and their function are so far unknown.

The immunoreaction for the HNK-1 epitope cannot be explained by ciliary nerves, and therefore the HNK-1 epitope in the ciliary body is probably not part of NCAM, MAG or P<sub>n</sub>. Chondroitin sulphate proteoglycans and tenascin are widely distributed extracellular matrix molecules, and bear the HNK-1 epitope in some organs [11, 13, 19, 22, 38]. Thus they are possible bearers of the HNK-1 epitope in the inner connective tissue layer, and I studied the distribution of these molecules in the human eye by immunohistochemistry.

**Materials and methods**

Histological specimens

Fifty-five formalin-fixed and paraffin-embedded adult human eyes were selected from the files of the Ophthalmic Pathology Laboratory, Department of Ophthalmology, Helsinki University Central Hospital. Twenty of these eyes with light microscopically normal anterior segments. They included eight eyes enucleated because of a posteriorly located malignant choroidal melanoma and six due to an orbital tumour; six were normal eye bank eyes. The remaining 35 eyes comprised two with exfoliation syndrome only, four with moderately advanced capsular glaucoma, 13 with absolute capsule glaucoma, seven with absolute capsular glaucoma complicated by neovascular glaucoma, three with absolute primary open-angle glaucoma, and six with absolute neovascular glaucoma. The patients' ages ranged from 23 to 88 years. Altogether, 44 eyes were surgically enucleated and 11 were obtained at autopsy.

Additionally, four central anterior lens capsules with exfoliation material were obtained for indirect immunofluorescence from extracapsular cataract surgery of patients with clinically diagnosed exfoliation syndrome.

Antibodies

Primary mouse monoclonal antibodies (MAbs) were used in the following optimal dilutions, as determined by preliminary stainings: MAb HNK-1 (Leu-7), IgM, Lot N1222, Becton Dickinson, San Jose, Calif., USA, 1:40, by definition reacting with the HNK-1 epitope [1]; MAb VC1.1, IgM, Lot 071H4828, Sigma, St Louis, Mo., USA, 1:16 000, which also detects the HNK-1 epitope [3]; MAb CS-56, IgM, Lot 081H4845, Sigma, 1:300, reacting with chondroitin sulphate of both types A and C, but not for type B (dermatan sulphate) [4]; and MAb TN2, IgG<sub>1</sub>, Lot 0121, Dakopatts, Burlingame, Calif., USA, 1:200, were used to induce fading and examined immediately with a Leitz Diaplan epi-fluorescence microscope. Sections (5 μm thick) were cut from the specimens. Routine histological staining was with haematoxylin-eosin. Retina present in all specimens was used as a positive internal control for antibodies to the HNK-1 epitope [16, 26].

Sections from all specimens were immunostained using a commercial version of the avidin-biotinylated peroxidase method (Vectastain ABC Elite Kit for Mouse IgG, Vector Laboratories, Burlingame, Calif., USA), as described in detail earlier [35]. Specimens immunostained with MAb TN2 were pretreated with 0.4% pepsin (2000 FIP-U/g; E. Merck, Darmstadt, Germany) in 0.01 N hydrochloric acid at 37°C for 10 min to reduce background and to enhance the intensity of specific immunostaining. Pretreatment with pepsin did not have any significant effect on the positive immunoreaction with MAb HNK-1 and VC1.1 recognizing the HNK-1 epitope, MAb CS-56 to chondroitin sulphate proteoglycan, and MAb D33 to desmin [16, 35].

Indirect immunofluorescence microscopy

The lens capsules were embedded and frozen in OCT compound (Miles, USA) and 6-μm sections were cut, air dried, rehydrated in phosphate-buffered saline (PBS, pH 7.4) and incubated in 2% bovine serum albumin (BSA, E. Merck) in PBS for 30 min at room temperature in a moist chamber to block nonimmunological binding. After a 45-min incubation in a moist chamber at 37°C with the primary antibody diluted in PBS-BSA (HNK-1 1:20, VC1.1 1:8000, CS-56 1:150, TN2 1:10) and three washes in PBS, the sections were incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG secondary antibody (Dakopatts) (1:30 in PBS-BSA) in a moist chamber at 37°C. After three washes in PBS, the sections were mounted with PBS-glycerol containing 1,4-diazobicyclo-(2,2,2)-octane (DABCO) to reduce fading and examined immediately with a Leitz Diaplan epi-fluorescence microscope equipped with filters for FITC fluorescence.

**Results**

**Light microscopy**

The clinical diagnoses of the eyes studied were confirmed by histopathological examination. Of the 35 eyes, exfoliation material was present in all eyes with exfoliation syndrome only or capsular glaucoma, and absent from other eyes by light microscopy.

**Immunohistochemistry**

**Monoclonal antibodies HNK-1 and VC1.1 to the HNK-1 epitope**

In all human eyes with a normal ciliary body, MAbs HNK-1 and VC1.1 to the HNK-1 epitope strongly immunolabelled the inner connective tissue layer between the ciliary epithelia and the ciliary muscle (Fig. 1A). As previously reported in more detail, this immunoreaction seemed to delineate cell membranes of a population of dendritic cells [35]. In eyes with exfoliation syndrome