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REVIEW

The HNK-1 Carbohydrate Epitope and the Human Eye in Health and Disease

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The HNK-1 carbohydrate epitope is part of many cell membrane and extracellular matrix molecules, several of which have been implicated in cell adhesion. It is a versatile tool in eye research. In the human eye this epitope is present in the retina, the optic and ciliary nerves, the ciliary and iris epithelia, the zonular lamella, and the sclera. It is phylogenetically conserved, but the positive cell types vary from species to species. In addition to revealing interspecies differences in the vertebrate retina, the HNK-1 epitope has been used to identify a novel cell type in the eye: the subepithelial matrix cells that reside in the inner connective tissue layer (ICTL) of the ciliary body. Although these cells resemble fibroblasts in ultrastructure, they form a distinct cell population that differs in antigenic profile from fibroblasts in other tissues. The HNK-1 epitope is also associated with the elastic fiber system of the ICTL, which may be produced by the subepithelial matrix cells. It may help to structurally stabilize the ciliary body and the retina. The HNK-1 epitope is also involved in many important eye diseases. The subepithelial matrix cells seem to be susceptible to irrreversible atrophy as a result of glaucoma, thermal injury, and tissue compression. On the other hand, the HNK-1 epitope is found in the extracellular matrix of secondary cataracts and may contribute to its pathogenesis. Finally, this epitope has proved to be useful in identifying deposits of exfoliation material, and in tracing neuroepithelial derivatives in developmental anomalies and tumors of the eye. (Pathology Oncology Research Vol 3, No 1, 8–14, 1997)

Key words: Cell adhesion, Ciliary body, Inner connective tissue layer, Natural killer cell, Retina, Subepithelial matrix cell

HNK-1 carbohydrate epitope

In 1981 Abo and Balch[1] raised monoclonal antibodies against a membrane fraction of the HSB-2 human T-lymphoblastoid cell line and selected one, designated HNK-1 (Human Natural Killer), because it specifically labelled a subset of lymphocytes enriched in natural killer and killer cells. Although initially aimed for use as a tool in leukocyte research, the same antibody was soon found to label many neuronal, glial, neuroectodermal and neuroendocrine cells.[2,7,30,32,52,53] The corresponding epitope common to lymphocytes and the nervous system is generally known as the HNK-1 epitope. It proved to be highly immunogenic,[16] and a number of other antibodies raised for different purposes recognize an antigen identical or closely related to the HNK-1 epitope.[5,28,58] In leukocyte research, the HNK-1 epitope was renamed first Leu-7 and then CD57.

The HNK-1 epitope is a 3-sulphoglucuronic acid-containing carbohydrate moiety, but its exact structure is still unknown.[4,8,34] It is part of several extracellular matrix and cell membrane glycoproteins, glycolipids and proteoglycans, such as neural cell adhesion molecule, myelin-associated glycoprotein, 11-integrin, tenascin and chondroitin sulphate proteoglycan.[27,28,34,37,43]

Functionally, the HNK-1 epitope has been linked to cell adhesion, and it has been proved vital in migration of fetal neural crest cells.[6] It has been shown to be the adhesive domain in at least the neuron-extracellular matrix interaction, and it is also involved in neuron-astrocyte and astro-

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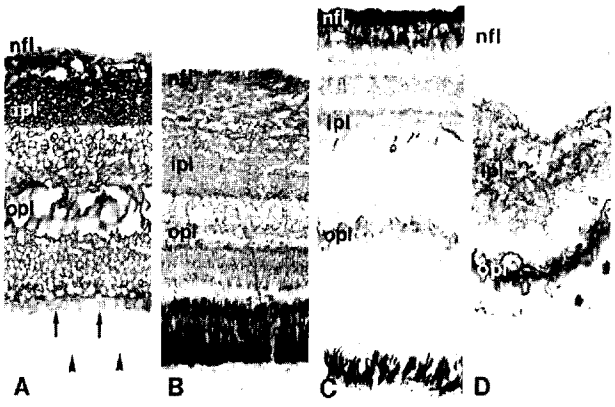

Figure 1. Phylogenetic differences in the immunostaining for the HNK-1 epitope in the retina (immunoperoxidase staining). A. In the human retina all retinal layers except the photoreceptor layer (arrowheads) are labelled. Note that labelling is present in the basket fibers of Müller's cells between the photoreceptors (arrows). B. Equal immunoreactivity is seen in the retina of the bass, a fish. C. In the duck, both plexiform layers, some cells adjacent to them, and the nerve fiber layer are labelled. In B and C, note pigmented epithelial cells below the retina. D. In the rabbit, immunoreaction is limited to both plexiform layers. Original magnifications: A x340, B x160, C x340, and D x330; nfl – nerve fiber layer, ipl – inner plexiform layer, opl – outer plexiform layer.

cyte-astrocyte adhesion.[18,29,46] Circulating autoantibodies to the HNK-1 epitope can cross-react with myelin proteins and cause a peripheral neuropathy.[17,41]

HNK-1 epitope and the healthy eye

1. Posterior segment

In the eye, the first tissues found to contain the HNK-1 epitope were the optic nerve and the retina.[19,42,58,68] The oligodendrocytes and myelin sheaths of the optic nerve express the HNK-1 epitope.[19,24] It is additionally present in the ground substance between the collagen lamellae of the sclera, but it has not been detected in the choroid.[59,64]

The HNK-1 epitope appears in the neuroblastic layers and in the pigment epithelium of the developing retina before the 13th gestational week.[24,64] In the adult human eye all retinal layers except the photoreceptor cell layer express the epitope (*Fig.1A*). It is probably associated with neuronal cell membranes in both plexiform layers and in the nerve fiber layer. It is present on astrocytes of the inner retina and prominent in the radial fibers of Müller. The internal limiting membrane and the basket fibers of Müller's cells between the inner segments of photoreceptors are likewise immunoreactive. Due to its adhesive properties the HNK-1 epitope may well help to stabilize the retinal architecture.

The HNK-1 epitope in the retina is phylogenetically conserved as it is found in various fishes, birds, mammals, and primates (*Fig.1B-D*).[5,11,38,48,61,68] In fishes and primates this epitope is present throughout the retina, analogous to humans (*Fig.1A,B*). However, in other studied mammals only the plexiform layers contain the epitope, and in birds it is restricted to the plexiform and the nerve fiber layers (*Fig.1 C,D*).

2. The inner connective tissue layer of the ciliary body

An observation of major importance has been the detection of a novel cell type in the human ciliary body that is identified by its immunoreactivity for the HNK-1 epitope.

The stromal layer filling the space between the ciliary epithelia and the ciliary muscle in the ciliary body is known as the inner connective tissue layer (ICTL; *Fig.2*).[15] This layer has been regarded as a nondescript tissue with no special functional role. It traditionally consists of fibroblasts, melanocytes, lymphocytes, mast cells and macrophages together with blood vessels, nerves, collagen bundles and elastin fibers.[15,55] It merges imperceptibly with the stroma of the iris and choroid when studied by routine light microscopy. In contrast, immunoreaction for the HNK-1 epitope distinctly delineates the human ICTL from the base of the iris, from the ora serrata, and from the ciliary muscle (*Fig.3A*).[59,64]

The HNK-1 epitope in the ICTL first appears adjacent to the pigmented epithelium of the ciliary processes of the pars plicata by the 20th week of gestation (*Fig.4*).[24,64] This has raised the possibility that the latter induces and organises its development. The epitope gruadally spreads to the entire ICTL of the pars plicata and then to the pars plana by the first year of life (*Fig.4*). Thereafter the HNK-1 epitope remains apparently unaltered in the ICTL throughout life.

By light microscopy, immunoreaction for the HNK-1 epitope outlines in the ICTL a population of stellate cells with long processes related to coarse immunoreactive bundles and to a meshwork of fine fibers (*Fig.3A-C*).[60,64]

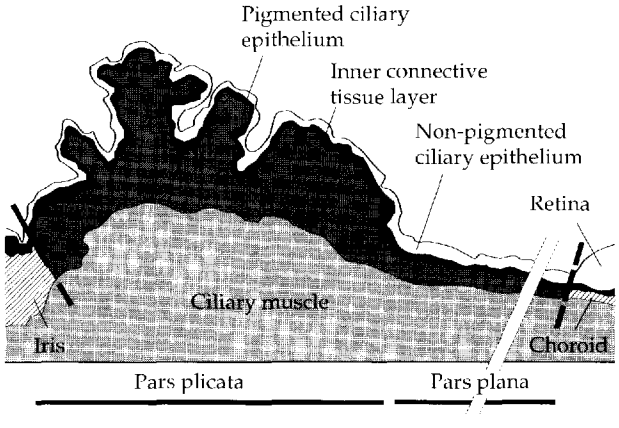

Figure 2. Diagrammatic cross-section through the ciliary body, defining the location of the inner connective tissue layer.

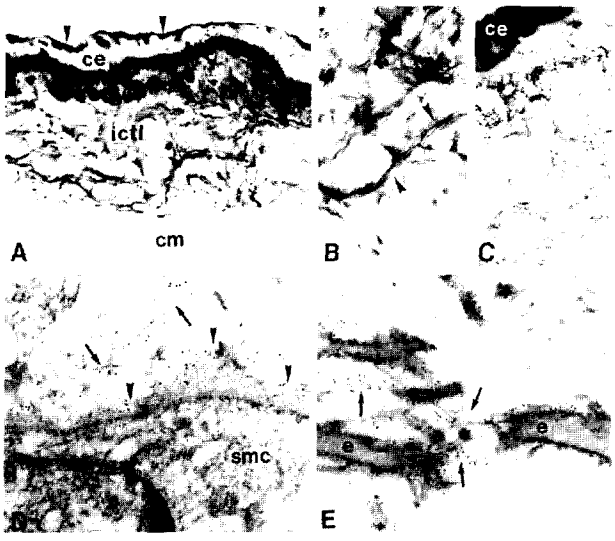

Figure 3. A novel cell type of the inner connective tissue layer (ICTL) of the human ciliary body identified by immunoreaction for the HNK-1 epitope (A-C light microscopic immunoperoxidase staining, D and E immunoelectron microscopy). A. Immunostaining distinctly delineatied the ICTL against the ciliary muscle (cm). Immunoreaction is also present at the basement membrane (arrowheads) of the non-pigmented ciliary epithelium (ce). The pigmented ciliary epithelium is dark due to its melanin content. B. With higher magnification, immunolabelling reveals in the ICTL long strands (arrowheads), and C. a meshwork of fine fibrils. D. Immunoelectron microscopy reveals that the epitope is situated on the surface of the subepithelial matrix cells (smc) at the level of their fragmented basement membrane (arrrowheads). Immunoreaction also highlights labelled microfibrils (arrows) that extend from the cell surface. No labelling is seen in the cytoplasm or the nucleus (n). E. Immunoreactive fibers (arrows) seem to connect elastic fibers to each other (e). Immunostaining is also present on the mantle of elastic fibers surrounding their amorphous core. Original magnifications: A x310, B x800, C x1000, D x37000, and E x36000.

Immunoelectron microscopy has revealed that the epitope resides on the surface of these cells (*Fig.3D*) that ultrastructurally resemble fibroblasts. It must be emphasized that these HNK-1 positive stromal cells form a distinct cell population that differs from fibroblasts of the iris, ciliary muscle and choroid. In particular, they do not react for vimentin, the intermediate filament type usually characteristic of fibroblasts.[64] We thus believe that it is justified to set this well defined population of cells aside and designate it subepithelial matrix cells based on their location in the ICTL.

In addition, the HNK-1 epitope is present along long microfibrillar bundles that consist of ca. 10 nm thick oxytalan microfibrils (*Fig.3E*).[67] These bundles come into close contact both with the subepithelial matrix cells and with elastic fibers (*Fig.3E*), which they seemingly connect to each other. Indeed, the HNK-1 epitope is also present in a mantle of microfibrils around the elastin core at the periphery of elastic fibers (*Fig.3E*). The fact that the epitope is a common denominator to the subepithelial matrix cells and to the elastic fiber system of the ciliary body suggests that it may have a role in structurally stabilizing the ICTL, for example in relation to accommodation or secretion of aqueous humor.

To date we lack a method to label these subepithelial matrix cells in species other than man, including primate eyes.[61] They lack the HNK-1 epitope in spite of the fact that it is present in other structures of the eye. The corresponding carbohydrate epitope or the molecule bearing it in the ICTL, if present, may be different in these species, or the HNK-1 epitope is somehow hidden so that antibodies cannot detect it.

3. Other anterior segment structures

The HNK-1 epitope appears in the neuroectodermally derived epithelia of the iris and ciliary body by the 22nd gestational week. In the anterior segment of the adult eye, it is found at the level of the basement membrane of the nonpigmented and the pigmented ciliary epithelium, as well as in the cytoplasm of the pigmented ciliary epithelium and of the posterior pigmented epithelium of the iris.[59,60] Schwann cells of ciliary nerves are also labelled.[19]

No clear immunoreaction is seen in the lens epithelial cells or the lens capsule by light microscopy, but the lamella to which the zonules attach is immunoreactive for the HNK-1 epitope.[65] Nevertheless, lesser amounts of the epitope have been detected in the outer two thirds of the lens capsule by immunoelectron microscopy.[44]

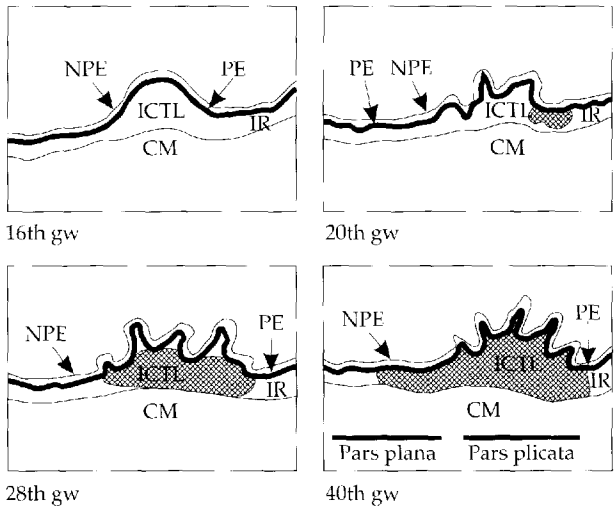

Figure 4. Appearance and development of the HNK-1 immunoreactivity (hatched area) in the human fetal ciliary body. ICTL – inner connective tissue layer, CM – ciliary muscle, NPE – non-pigmented and PE – pigmented ciliary epithelium, IR – iris, and gw – gestational week.

HNK-1 epitope and the diseased eye

Analysis of changes in the HNK-1 epitope under pathological conditions is necessary to find out both its physiologic role and its significance in ocular disease.

1. Glaucoma

Glaucoma is an important cause of blindness throughout the world. In eyes with glaucoma, the immunoreaction for the HNK-1 epitope diminishes and eventually disappears from the ICTL (*Fig.5A*).[66] In contrast, the expression of the epitope is unchanged in all other structures of the eye. This might be due to the elevated intraocular pressure, which for example by decreasing blood flow may cause atrophy and degeneration of the subepithelial matrix cells that probably are responsible for the production of the HNK-1 epitope in the ICTL. Alternatively, an as yet unidentified biochemical change or long-term effect of glaucoma medication may be responsible.

Also after cyclodestructive surgery, such as contact krypton laser cyclophotocoagulation, the HNK-1 immunoreaction is all but lost from the treated area of the ICTL but not from the ciliary epithelium (*Fig.5B*).[23] This also suggests that the subepithelial matrix cells are particularly prone to undergo irreversible atrophy, in this case due to thermal injury.

2. Cataract

During accommodation the eye focuses at near distance. The contraction of the ciliary muscle is transmitted to the lens via the zonules and the ICTL. After cataract surgery the accommodative status changes. In a pseudophakic eye with an artificial intraocular lens the zonules are intact, whereas in aphakic eyes the zonules have been digested and the lens is absent. Regardless of the surgical method, the immunoreaction for the HNK-1 epitope in the ICTL is equal to that seen in normal phakic eyes, even 16 years after cataract extraction.[63]

A common problem after cataract extraction is formation of a secondary cataract, which is thought to result from proliferation and migration of residual lens epithelial cells.[3] These metaplastic cells often acquire smooth muscle actin and produce abundant extracellular matrix that forms plaques on the lens capsule.[62] Granular immunoreaction for the HNK-1 epitope is present in this extracellular matrix of secondary cataract (*Fig.5C*).[63] The fact that the HNK-1 epitope is likewise present in anterior subcapsular cataracts (*Fig.5D*), in which abnormal extracellular matrix is produced under an intact lens capsule, suggests that it is produced by the metaplastic lens epithelial cells.[63] As the HNK-1 epitope is associated with cell adhesion and migration, it is tempting to suggest that it might contribute to the pathogenesis of secondary cataracts, for example by aiding the migration or adhesion of the metaplastic lens epithelial cells.

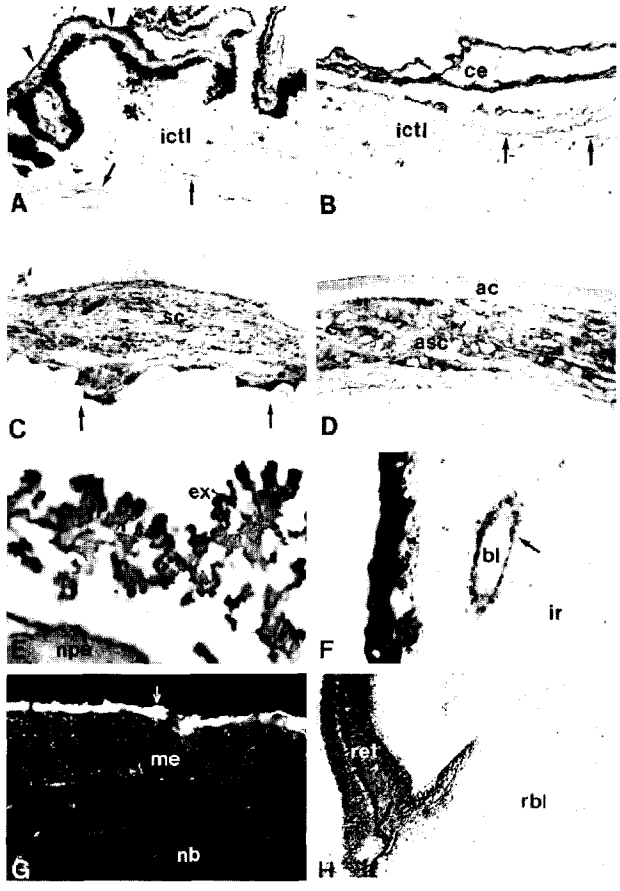

Figure 5. *The HNK-1 epitope in the diseased eye (A-F,H immunoperoxidase staining, G immunofluorescence).* ***A.*** *The immunoreaction (arrows) is greatly diminished in the ICTL of an eye with absolute glaucoma as compared to normal amount in Fig.3A. Note that immunostaining persists in the non-pigmented ciliary epithelium (arrowheads).* ***B.*** *Likewise, in an eye after krypton laser cyclophotocoagulation, immunolabelling (arrows) is faint in the ICTL, but strong in the ciliary epithelia (ce).* ***C.*** *Granular immunoreaction for the HNK-1 epitope is seen in extracellular matrix of a plaque of secondary cataract (sc). The posterior lens capsule (arrows) is not labelled.* ***D.*** *Equal immunostaining is seen in an anterior subcapsular cataract (asc), beneath an intact anterior lens capsule (ac).* ***E.*** *Exfoliation material (ex) on the non-pigmented ciliary epithelium (npe) is immunoreactive for the HNK-1 epitope.* ***F.*** *Immunostaining is also found around some blood vessels (bl) of the iris (ir) in an eye with exfoliation syndrome.* ***G.*** *As in the early developing retina, the inner aspect (arrow) of medullary epithelial cords (me) and radial fibers react for the HNK-1 epitope in an intraocular medulloepithelioma. Note negative neuroblastic cells (nb).* ***H.*** *No labelling is seen within a retinoblastoma (rbl) that originates from the labelled retina (ret). Original magnifications: A x175, B x200, C x220, D x340, E x330, F x340, G x310, and H x70.*

3. Exfoliation syndrome

Exfoliation syndrome is an intriquing disease in which whitish fibrillogranular material deposits throughout the anterior segment of the eye.[40] It is a major risk factor in cataract surgery. The origin of exfoliation material is obscure. Material resembling the ultrastructure of exfoliation fibers has been reported in various extrabulbar sites, pointing to the possibility that exfoliation syndrome may be a systemic condition.[10,51,54,56,57] Latest evidence points toward abnormal metabolism of extracellular matrix components, in particular of basement membranes and elastic fibers.[26,31,49,51] As mentioned, the HNK-1 epitope is associated with both elements. Indeed, intraocular deposits of exfoliation material are intensely labelled for the HNK-1 epitope (*Fig.5E*).[65] In exfoliation syndrome, the HNK-1 immunoreactivity in the ICTL of the ciliary body is as strong as in normal eyes, but it appears somewhat more granular.[66] The reason for this was not evident by immunoelectron microscopy.[67]

Another intriguing finding is that, in all eyes with exfoliation syndrome, a granular immunoreaction for the HNK-1 epitope is present in the iris at the level of the thick laminated basement membrane of a subset of blood vessels (*Fig.5F*).[65] Exfoliation material has previously been detected in this location by electron microscopy,[26,45] and the vasculopathy seems to have a definite association with exfoliation syndrome. In fact, the iris blood vessels become abnormal even before exfoliation deposits can be detected clinically or histopathologically in the eye.[20] In contrast, we have not detected immunoreactivity at the light microscopic level in the conjunctiva, an extraocular tissue in which exfoliation fibers have been claimed to be present.[14]

Because the HNK-1 epitope is associated with cell adhesion, it might bind together this intraocular multicomponent material and be responsible for its characteristic adhesiveness to intraocular surfaces. The HNK-1 epitope is an easy and reliable marker for exfoliation syndrome even in its early phase when the diagnosis is not immediately evident. It has been used to demonstrate that exfoliation syndrome is never truly monocular, since early changes are also present in the clinically healthy fellow eye.[20]

4. Developmental anomalies

The glial and neuroepithelial cells that bear the HNK-1 epitope in the eye retain it under many pathologic conditions. The epitope has been successfully used to trace the derivatives of these cells in developmental ocular anomalies. It identifies gliotic retinal remnants in intraocular teratoma and optic nerve coloboma,[22,24] a variable population of glial and neuroblastic cells in intraocular glioneuroma, medulloepithelioma and colobomatous cysts of the orbit,[21,24] and the embryonal ciliary epithelium in medulloepithelioma (*Fig.5G*) and glioneuroma of the ciliary body.[21,25]

5. Intraocular tumors

Even though the HNK-1 epitope is present throughout the normal adult and fetal retina, it is absent from retinoblastoma (*Fig.5H*), a tumor of the developing retina.[19,42] Either this tumor originates from cells that do not express the epitope or it is lost during malignant transformation. Interestingly, retinoblastoma cells are loose and easily detatch to the vitreous, indicating poor adhesiveness.

The HNK-1 epitope is reportedly present in one fifth of primary uveal melanomas, mostly in large ones, and in about one fourth of their metastases.[39] In eyes with a melanoma of the ciliary body, the HNK-1 immunoreaction in the ICTL almost disappears near the tumor.[60] This locally decreased immunoreaction may be due to atrophy of the subepithelial matrix cells near the tumor, caused either by direct compression, decreased blood flow, or a combination of factors.

Future directions

Antibodies to the HNK-1 epitope are emerging as a very versatile tool in eye research. They have already revealed a new element in the human eye, the subepithelial matrix cells, which probably produce the epitope throughout life. They have also revealed phylogenetic changes in retinal antigenic profile not visible with other antibodies. Moreover, the HNK-1 epitope may be of pathogenetic significance in exfoliation syndrome and secondary cataract that are frequent and clinically important eye diseases.

In the future, we have to find out the physiologic role and function for the subepithelial matrix cells of the ICTL, as well as for the HNK-1 epitope in the ICTL, ciliary epithelia, and retina. In this regard, the species differences already mentioned may prove to be informative.

The most important step is to find out which molecules bear the HNK-1 epitope in various tissues, and which molecules may bind to it. In the retina, neural cell adhesion molecules may be responsible.[28] In the ICTL, tenascin and chondroitin sulphate proteoglycans have been ruled out.[59] Other possible candidates might be collagen types I and III, fibronectin and fibrillin that are present in this region.[35,36,47,50] So far none of these molecules is known to bear this epitope, however. The epitope might also be part of integrins, which sometimes carry it.[43] Laminin and amyloid P-component are known to have a binding site for the HNK-1 epitope,[13,33] and the former is suggested to be one important extracellular component through which this epitope may act.[9,12,13] Obviously, many questions remain to be answered before the secrets of the HNK-1 epitope in the eye have been solved.

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