Tau-2 Immunoreactivity of Corpora Amylacea in the Human Retina and Optic Nerve

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Purpose. To characterize the constituents of corpora amylacea in the human retina and optic nerve.

Methods. Immunohistochemistry was performed on sections of retina, optic nerve, and brain tissue using antibodies against tau 1, tau-2, and amyloid precursor protein.

Results. Consistent anti-tau-2 immunoreactivity was noted in the corpora amylacea in the retina, optic nerve, and brain tissue, albeit with variations in pattern and intensity of staining. No immunoreactivity was observed with antibodies anti-tau 1 and anti-amyloid precursor protein.

Conclusion. Our findings suggest the accumulation of possibly abnormal tau-2 within the corpora amylacea, which may be either astrocytic or axonal in origin. Invest Ophthalmol Vis Sci. 1993;34:2600-2603.

Corpora amylacea are homogeneous or laminated bodies that are frequently found in the optic nerve and peripapillary retina of aging human eyes. They are also well recognized in the human brain and spinal cord. Electron microscopy of these bodies demonstrates a filamentous tangle with a whorl-like pattern and areas of loose ground substance containing scattered tubules and dense bodies.

Degenerated organelles suggestive of mitochondria and dense granular particles resembling glycogen, as well as a varying rim of surrounding axoplasm, were also described. Although corpora amylacea are frequently observed in the normal aging process and in a variety of neurodegenerative disorders, their pathologic significance is still undetermined. Because corpora amylacea were related to neurodegenerative diseases, we used antibodies against microtubule-associated proteins tau 1 and tau-2 and amyloid precursor protein to characterize further their constituents in the retina and optic nerve and to investigate their relationship to neurodegenerative processes, which are frequently associated with these antigens.

Materials and Methods. Ten human eyes with corpora amylacea in the optic nerve and peripapillary retina were examined in this study. The eyes that were received postmortem from the Lions of Illinois Eye Bank (Chicago, Illinois) were fixed in 4% buffered formaldehyde solution. Donors’ ages ranged from 35 to 84 yr. Tissues were then processed and embedded in paraffin for light microscopy. The presence of corpora amylacea was established by positive staining with periodic acid-Schiff of the typical spherical homogeneous or laminated bodies within the distal optic nerve and the peripapillary retina (Fig. 1, top left). The methods used in this study followed the tenets of the Declaration of Helsinki.

Immunohistochemistry was performed on 5-μm-thick paraffin sections from the donors’ eyes. We used monoclonal antibodies against the phosphatase-independent epitope of tau (tau-2, Sigma Chemical Co., St Louis, MO), the nonphosphorylated epitope of the microtubule-associated protein tau (tau 1, Clone PC1C6, Boehringer Mannheim Co., Indianapolis, IN), and against the amyloid precursor protein (Alzheimer precursor protein A4, Clone 22C11, Boehringer Mannheim Co., Indianapolis, IN) in dilutions of 3.9 μg/ml, 5 μg/ml, and 10 μg/ml, respectively.

An avidin-biotin-alkaline phosphatase complex was used to visualize the reaction product with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) as chromogen. Nonimmune mouse serum replaced the primary antibody in the negative controls. The sections were mounted with histologic mounting medium (Permount, Fisher Scientific, Fair Lawn, NJ) without counterstaining.

To compare ocular corpora amylacea with corpora amylacea in the brain, we immunostained sections of the hippocampus area from a formaldehyde-fixed brain of a 77-year-old patient with Alzheimer’s disease that showed numerous corpora amylacea as well as neurofibrillary tangles, and we examined these sections under identical conditions.

Results. The corpora amylacea reacted with varying intensity with anti-tau-2 (Fig. 1) in all 10 eyes as well as in the brain tissue. In most corpora amylacea, the staining pattern was intense and homogeneous with a slightly granular appearance, especially in large corpora amylacea (Fig. 1, top center). A few corpora amylacea showed only faint immunoreactivity, which was still clearly distinct from the surrounding tissue (Fig. 1, top right and bottom left). In some corpora amylacea, the immunoreactivity exhibited a laminated pattern. In those instances, prominent staining of the peripheral rim area was usually observed (Fig. 1, bottom center). Although the immunostaining in larger
corpora amylacea appeared to be more intense, we could not quantitatively correlate the diameter of corpora amylacea with the intensity of the immunoreactivity.

In the brain, the staining pattern of corpora amylacea with anti-tau-2 antibody was similar to that of corpora amylacea in the optic nerve and peripapillary retina. In most instances, the immunoreaction was intense, but occasionally only weak staining was observed (Fig. 1, bottom right).

In some ocular and brain sections, a small number of corpora amylacea did not exhibit any immunoreactivity with anti-tau-2. No immunolabeling was seen with antibodies against tau 1 or amyloid precursor

**FIGURE 1.** (top left) Optic nerve head and adjacent retina with several corpora amylacea (arrows) (case 1, periodic acid-Schiff, x63).

(top center) Intense anti-tau-2 immunoreactivity in corpora amylacea (arrows) of the optic nerve close to lamina cribrosa. Note slightly granular but homogeneous appearance (case 8, immunoreactivity visualized with NBT/BCIP without counterstain, x340).

(top right) Moderate anti-tau-2 immunoreactivity in corpora amylacea (arrows) of the optic nerve. Note less intense but distinct staining and granular appearance similar to Figure 1 (case 1, immunoreactivity visualized with NBT/BCIP without counterstain, x340).

(bottom left) Moderate-to-faint anti-tau-2 immunoreactivity in corpora amylacea (arrows) of the optic nerve. Note irregular staining pattern (case 5, immunoreactivity visualized with NBT/BCIP without counterstain, x340).

(bottom center) Laminar anti-tau-2 immunoreactivity in corpus amylaceum (arrow) of the retina. Note intense labeling of the outer rim area (case 10, immunoreactivity visualized with NBT/BCIP without counterstain, x340).

(bottom right) Intense anti-tau-2 immunoreactivity in corpora amylacea of the brain. Note some variation in immunolabeling, such as laminar staining (arrows) and less intense staining, especially of smaller corpora amylacea (arrowheads) (case 11, immunoreactivity visualized with NBT/BCIP without counterstain, x340).
protein in either ocular or cerebral corpora amylacea (not illustrated). Surrounding glial cells and axons, however, exhibited variable staining predominantly with anti-tau 1 and, to a lesser degree, with anti-amyloid precursor protein. In contrast to the distinct tau-2-immunoreactivity of corpora amylacea, immunolabeling of the adjacent retinal and optic nerve tissue with this antibody was extremely weak.

No staining reaction was observed when the primary antibody was replaced with nonimmune serum.

**Discussion.** We found marked immunoreactivity of corpora amylacea in the optic nerve, retina, and brain with antibody to tau-2, the phosphatase-independent (phosphorylated) epitope of the microtubule-associated protein tau. In previous studies, tau-2 was demonstrated intracellularly in normal axons, somatodendritic compartments, and astrocytes and was also shown to be one of the major constituents of paired helical filaments forming neurofibrillary tangles and senile plaques in various neurodegenerative disorders, where it is believed to be abnormally phosphorylated. The positive anti-tau-2 immunoreactivity in our study suggests an accumulation of either normal or possibly modified tau in corpora amylacea, which might, like neurofibrillary tangles, be related to cellular degeneration in neuronal tissue.

A previous ultrastructural study of ocular corpora amylacea demonstrated the presence of microtubules morphologically. This observation strongly suggested that the anti-tau-2 immunoreactivity in corpora amylacea resulted from an accumulation of microtubules and their associated proteins partaking in the formation of these structures. However, it was unclear whether the accumulation of microtubules observed morphologically resulted from an alteration such as abnormal phosphorylation in the tau protein, as seen in Alzheimer’s disease, or whether the accumulation of microtubules and the phosphorylated tau epitope tau-2 was secondary to other pathologic cellular events. It was also suggested that the formation of corpora amylacea was related to impaired axoplasmic flow, which explains their predominant location near the lamina cribrosa. This hypothesis was supported by experimental data using a slow axoplasmic transport inhibitor to create neurofilament tangles morphologically similar to corpora amylacea in the rat spinal cord. Because tau-2 was shown to be a major component of intracellular neurofilibrillary tangles in neurons, the tau-2 immunoreactivity might indeed be additional evidence for the significance of impaired axonal flow. The presence of corpora amylacea in apparently normal eyes from 35- and 57-yr-old individuals further indicates that the pathogenesis of their formation may not be related to age or disease only but may represent an aberrant manifestation of a physiologic process such as axoplasmic transport. Alternatively, cerebral astrocytes that have been shown to contain corpora amylacea may be a source of these ubiquitous structures.

No difference in immunoreactivity was observed between cerebral or ocular corpora amylacea, but the staining pattern of anti-tau-2 showed some variability. This variation may result from changing immunoreactivity in different stages of formation and corresponds well to the spectrum of ultrastructural features of corpora amylacea, where a filamentous core has been described as being associated with various normal or degenerated cell organelles.

Recently, an immunohistochemical study demonstrated anti-heat shock protein HSP72 immunoreactivity in corpora amylacea. The expression of heat shock proteins, however, is closely related to the cell response to stress, and the authors speculate that in evolving corpora amylacea, the presence of heat shock protein might represent a protective response of astrocytes to a range of metabolic or toxic insults in neurons. In heat shocked animals, the presence of altered phosphorylation of tau (tau-2) was also observed, thus indicating that proteins associated with cellular stress are indeed related to the formation of corpora amylacea.

The extracytoplasmic domain of the amyloid precursor protein was also demonstrated in corpora amylacea of the brain using immunohistochemical techniques. In our study, however, application of an antibody that reacts with different amino acid residues at the N-terminus of the amyloid precursor protein did not produce positive immunoreactivity.

In summary, we have demonstrated tau-2 immunoreactivity in most corpora amylacea of the optic nerve and retina, as well as in corpora amylacea in the brain of a patient with Alzheimer’s disease. Our findings indicate that corpora amylacea represent individual neuronal cell degeneration not necessarily associated with a general neurodegenerative disease. It is possible that the formation of corpora amylacea results from impaired axonal flow, as previously suggested, or as a response to cellular stress.

**Key Words**
corpora amylacea, optic nerve, retina, immunohistochemistry, ultrastructure

**Acknowledgment**
The authors thank Dr. Elizabeth Cochran, M.D., Pathology Department, Rush-Presbyterian-St. Luke’s Hospital, Chicago, Illinois, for providing the brain tissue.

**References**

Immunohistochemistry of Infiltrating Lymphocytes in Uveal Malignant Melanoma
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Purpose. To investigate using immunohistochemistry the unusual finding that tumor infiltrating lymphocytes (TIL) in uveal melanomas are associated with a higher mortality rate.

Methods. We performed immunohistochemistry for B and T lymphocytes on 80 uveal malignant melanomas, which previously had been reported to contain more than 100 TIL per 20 high-powered fields. In a second study of 90 patients, we counted the number of immunohistochemically stained T lymphocytes per 20 high-powered fields in uveal melanomas from 30 patients who survived at least 15 years after enucleation, from 30 patients who died with metastasis within 2 years, and from 30 patients who died with metastasis more than 10 years after enucleation.

Results. T cells predominated in 73.8% of the 80 patients, and B cells were more prevalent in 13.8%. T cells were usually scattered, and B cells were usually in clumps. Post-enucleation 18-yr mortality from metastasis was 73% for patients with either T- or B-cell predominance of their TIL. The mortality rate was 32% for patients with few TIL. The patients who survived at least 15 yrs after enucleation had fewer T lymphocytes infiltrating their uveal melanomas than the two groups of patients who died with metastasis.

Conclusions. The pattern of the TIL was different for T and B cells in uveal melanomas. T-lymphocytic infiltration is associated with death due to metastasis. Invest Ophthalmol Vis Sci. 1993;34:2603-2606.

A previous study of uveal malignant melanomas from the Armed Forces Institute of Pathology indicated that the presence of more than 100 tumor-infiltrating lymphocytes (TIL) per 20 high-powered fields (hpf) was associated with a decreased survival rate. In this study, we attempt to characterize the immunohistochemical profile of TIL in uveal melanomas and to determine the prognostic implications of T-lymphocytic infiltration.

MATERIALS AND METHODS. Paraffin blocks were obtained in 80 of the 129 previously studied patients in whom the tumors contained more than 100 lymphocytes per 20 high-powered (45X) fields. Glued sections were prepared and stained using the standard avidin/biotin system with 3-aminoh-carbazole as the chromogen. The immunohistochemical antibodies were L-26 (Dako) for B cells and UCHL-1 (Dako) for T cells. Using a prospective design, the slides were reviewed without knowledge of the patients' outcomes, and the cases were divided into two groups depending on whether the TIL were predominantly B or T cells. Cases were deleted if none of the lymphocytes stained with either L-26 or UCHL-1. Kaplan-Meier survival and statistical analyses were performed, based on deaths from metastatic melanoma. For comparison,