Immunoreactivity Against Tau, Amyloid Precursor Protein, and Beta-Amyloid in the Human Retina

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Purpose. Increased immunoreactivity (IR) of beta-amyloid and the amyloid-associated proteins tau and amyloid precursor protein (APP) in the brain have been linked to the pathogenesis of neurodegenerative disorders such as Alzheimer's disease. However, the expression of these proteins has not been investigated in the normal or diseased human retina.

Methods. Using immunohistochemical techniques, we examined the distribution and age-related changes of anti-tau-1, anti-tau-2, anti-APP, and anti-beta-amyloid IR in the human retina at various ages (n = 24), in retinitis pigmentosa (RP, n = 6), and in age-related macular degeneration (ARMD, n = 10).

Results. Tau-1 immunoreactivity was intense in the inner retinal layers and did not change with age or in RP. Eyes with ARMD showed less intense staining but exhibited a similar distribution. Tau-2 IR was faint and did not change with age but was mildly increased in the retinal pigment epithelium (RPE) of eyes with RP and in the retina of eyes with ARMD. APP IR was most prominent in the ganglion cell and nerve fiber layer, and it appeared to increase in ganglion cells of older persons and in RPE cells of eyes with RP and ARMD. Beta-amyloid IR was only detected focally in sub-RPE deposits in eyes from older persons.

Conclusions. The proteins investigated in this study are present in the human retina. The staining pattern of tau is different from the brain, but it shows no age-related changes. The increased immunoreactivity of APP in retinal ganglion cells of older eyes and in RPE cells of eyes with RP and ARMD, as well as the patchy staining of beta-amyloid within sub-RPE deposits, might indicate a relationship of these proteins to retinal aging and possibly to retinal degeneration in RP. Invest Ophthalmol Vis Sci. 1995;36:24-31.

Alzheimer's disease is a neurodegenerative disorder of the brain that is associated with an increase in amyloid deposition and the formation of extracellular senile plaques, neurofibrillary tangles, or both. The deposition of beta-amyloid is thought to be an important factor in the neuronal degeneration in Alzheimer's disease and was shown to be the major constituent of cerebrovascular amyloid and of senile plaques. It has been suggested that most probably beta-amyloid is derived from the membrane-spanning amyloid precursor protein (APP) by abnormal processing. Neurofibrillary tangles react with an antibody to a phosphatase-independent epitope of the microtubule-associated tau protein and have also been suggested to be due to abnormal phosphorylation. Increased immunoreactivity (IR) of tau, APP, and beta-amyloid is also a feature of aging, but the mechanism of amyloid formation and its further implication remain poorly understood.

The retina, an integral part of the central nervous system, undergoes age-related and hereditary degeneration, the pathogenetic mechanisms of which are unclear and puzzling. It is possible that the expression of neuron-related proteins, such as tau or APP, may be altered in the aging or degenerating retina as seen in other regions of the central nervous system. The presence and expression of the amyloid-related proteins have not yet been investigated in the normal or diseased human retina. Using immunohistochemical techniques, we examined the immunoreactivity, distribution, and age-related changes of anti-tau-1 (the antibody that reacts preferably with a nonphosphorylated epitope), of anti-tau-2 (the antibody that reacts with a phosphatase-independent epitope), of anti-APP and of anti-beta-amyloid in normal human retina at various ages, and in eyes with retinitis pigmentosa (RP) and age-related macular degeneration.

METHODS

To study age-related changes in the retina, we examined 24 cadaver eyes received from the Lions of Illinois...
Amyloid-Related Proteins in Human Retina

FIGURE 1. (A) Anti-tau-1 immunoreactivity in normal young human retina. Note intense labeling of outer plexiform layer (arrow) and inner nuclear layer. Inner plexiform and nerve fiber layers are stained less intensely. No labeling of photoreceptor elements or outer nuclear layer (ONL) can be seen (primary antibody 10 μg/ml, using NBT/BCIP as chromogen, no counterstain, original magnification ×33). (B) Anti-tau-1 immunoreactivity in normal older human retina. Note similar staining pattern as in (A) (primary antibody 10 μg/ml, using NBT/BCIP as chromogen, no counterstain, original magnification ×33). (C) Anti-tau-1 immunoreactivity in para-macular area of human ARMD retina (atrophic form). Less intense immunoreactivity is seen, but the location is similar when compared with (A). PhR = photoreceptor outer and inner segments; OPL = outer plexiform layer; IPL = inner plexiform layer (primary antibody 10 μg/ml, using DAB [aminobenzidine] as chromogen; ARMD = age-related macular degeneration; NBT/BCIP = nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate. No counterstain, original magnification ×132).

Eye Bank (Chicago, IL) with no known history or obvious histologic evidence of previous ocular disease. Donor ages ranged from 7 days to 93 years (1 day to 35 years, 8 specimens; 36 years to 65 years, 9 specimens; 66 to 95 years, 7 specimens). Twenty-two specimens were fixed for 4 to 7 hours in 10% neutral-buffered formaldehyde, and two specimens were fixed in 1% glutaraldehyde-4% formaldehyde. In addition, nine eyes with age-related macular degeneration (disciform type) (Armed Forces Institute of Pathology) and one eye with atrophic age-related macular degeneration and six eyes with RP with varying stages of advanced degeneration (Georgia Theobald Ophthalmic Laboratory, Chicago) were also studied. Specimens from the Armed Forces Institute of Pathology had all been fixed overnight in 10% neutral-buffered formalin. Brain tissue removed after death from a patient with Alzheimer's disease, also fixed in 10% neutral-buffered formalin, served as a positive control.

All tissues were processed routinely and were embedded in paraffin for light microscopy. Sections including the pupil and optic nerve were stained with hematoxylin and eosin and with periodic acid-Schiff stain. Most specimens were also stained with Congo red. Immunohistochemistry was performed on adjacent 5-μm thick paraffin sections.

We used monoclonal antibodies against the non-phosphorylated epitope of the microtubule associated protein tau (tau-1; dilution of 5 to 10 μg/ml; Clone PC1C6, Boehringer Mannheim, Indianapolis, IN); the phosphatase-independent epitope of tau (tau-2; dilution of 4 μg/ml; Sigma Chemical, St Louis, MO); the amyloid precursor protein (Alzheimer precursor protein A4; dilution of 2 μg/ml; Clone 22C11, Boehringer). These antibodies were characterized previously. The antibody against beta-amyloid protein was a polyclonal human-antirabbit antibody (dilution of 15 μg/ml; Boehringer). An avidin–biotin–alkaline phosphatase or peroxidase complex was used to visualize the reaction product with nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP), ethoamino-carbazole, or diaminobenzidine as chromogen, respectively. For anti-APP and anti-beta-amyloid immunohistochemistry, some sections were pretreated with 15% formic acid for 30 minutes at room temperature. Nonimmune serum replaced the primary antibody in the negative controls. The sections were mounted with histologic mounting medium (Permoun; Fisher Scientific, Fair Lawn, NJ).
FIGURE 2. Anti-tau-1 immunoreactivity in human retina with retinitis pigmentosa. Note intense labeling of inner retina, which appears condensed because of atrophy of the outer retina. Nerve fiber layer is artifactiously missing centrally. Primary antibody 10 μg/ml; using NBT/BCIP as chromogen. IPL = inner plexiform layer; RPE = retinal pigment epithelium; NBT/BCIP = nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate. No counterstain, original magnification X33.

without counterstaining. Sections from the brain of a patient with Alzheimer’s disease were immunostained at the same time and examined under identical conditions.

RESULTS

Light microscopic examination of the cadaver eyes of various ages obtained from the eye bank did not reveal any significant pathologic features. The eyes with RP all showed advanced retinal degeneration with migration of retinal pigment epithelial (RPE) cells into the retina, degeneration, loss of the photoreceptor layer, and atrophy of the nerve fiber layer. Similarly, eyes with age-related macular degeneration (ARMD) showed end-stage disease with either extensive subretinal hemorrhage or advanced subretinal scarring in the macular area. Only one eye with atrophic ARMD, showing circumscribed loss of RPE and photoreceptors in the macula, was included. Therefore, our immunohistochemical findings in ARMD mainly refer to those eyes with disciform macular degeneration.

Congo Red

None of the retinal sections studied showed positive staining with Congo red. Patchy congophilia was noted in some sections, but no birefringence or dichroism could be demonstrated.

Tau-1

Intense retinal immunoreactivity was observed in the inner nuclear layer (a somatodendritic compartment of retinal neurons) and inner plexiform layer, and to degrees of intensity in the outer plexiform layer, ganglion cell layer, and nerve fiber layer (Fig. 1). No staining of photoreceptors or the RPE was seen. Although there was some variation between individual specimens, the overall pattern did not change with increasing age: The most prominent labeling was consistently observed in the region of the inner nuclear and inner plexiform layers (see Figs. 1A and 1B for comparison). The eye with atrophic ARMD also showed a similar, albeit slightly less intense, staining pattern (Fig. 1C). In eyes with RP, intense immunoreactivity was also restricted to the inner retina in areas of mild or severe degeneration and was comparable to that observed in normal retina (Fig. 2). Eyes with disciform ARMD exhibited markedly less intense immunoreactivity in all retinal areas (Fig. 3). There was no apparent difference in immunolabeling between the area of disciform degeneration and the adjacent retina.

Sections of the brain with Alzheimer’s disease showed positive immunolabeling in the cytoplasm of the axons (not shown).

Tau-2

Only weak retinal immunoreactivity was seen (Fig. 4) and was mainly localized to the inner plexiform layer, the ganglion cell layer, the nerve fiber layer, and along the inner limiting membrane (presumably Müller cell foot plates). Occasionally, the RPE showed patchy immunolabeling, particularly at the posterior pole of older eyes. No convincing changes in the staining pattern were observed with increasing age. In some normal retinas, scant and variable staining was also noted in the inner nuclear layer, the outer plexiform layer, the outer nuclear layer, and...
in cone and rod inner segments (Fig. 4A). Again, the eye with atrophic ARMD exhibited a staining pattern similar to normal (Fig. 4B). In eyes with RP, retinal immunoreactivity was also faint (Fig. 5). However, focally increased immunoreactivity was present in the apices of the RPE cells in areas of moderate to advanced degeneration. The retina in eyes with disciform ARMD in all areas revealed essentially the same staining pattern as the normal retina throughout the section, but the immunoreactivity appeared to be slightly increased in intensity (Fig. 6) when compared with the normal retina.

The sections from the brain with Alzheimer's disease revealed numerous intracellular neurofibrillary tangles that were labeled intensely.

**APP**

With anti-APP, retinal immunoreactivity was observed in a distribution similar to the anti-tau-2 staining but

![Image](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933181/)
was much more intense. Again, immunolabeling was seen mainly in the inner retina (ganglion cell and nerve fiber layers) (Fig. 7) and to a variable degree in the inner plexiform, inner nuclear, outer plexiform, and outer nuclear layers. As with anti-tau-2, a subpopulation of photoreceptor (predominantly cone) inner segments were distinctly immunoreactive (Fig. 7A). The location of immunoreactivity did not change with increasing age, but labeling of ganglion cells appeared to increase in older eyes and in atrophic ARMD (see Figs. 7B and 7C). The RPE exhibited patchy immunoreactivity mainly in older eyes. This was particularly noteworthy in eyes with RP (Fig. 8), but no labeling of the migrated RPE cells around retinal blood vessels was seen. In most eyes with disciform ARMD, a staining pattern comparable to the older age group was noted; there was no dramatic increase in ganglion cell labeling, but immunolabeling of the RPE at the posterior pole appeared more intense and extensive, particularly at its basal aspect. In the area of disciform degeneration, immunolabeling of metaplastic RPE cells also appeared more intense (Fig. 9). No staining of sub-RPE deposits, such as drusen or basal linear deposit, was seen. In the brain, positively labeled axons and few extracellular plaques were identified (not illustrated).

**Beta-Amyloid**

No immunoreactivity was observed in the neural retina of normal specimens. In a few older retinas, however, patchy labeling of sub-RPE deposits in the posterior pole was noted (Fig. 10). By light microscopy, these deposits corresponded to either soft drusen or basal linear deposit, whereas the overlying retina still appeared unremarkable. The pattern of immunoreactivity in the adjacent retina overlying these deposits remained unchanged. No beta-amyloid staining was detected in RP eyes. No positive retinal or RPE immunoreactivity was seen in eyes with ARMD, although some basal linear deposits were identified.

Several senile plaques in the brain sections from Alzheimer's disease demonstrated positive labeling with the anti-beta amyloid antibody (not shown). Formic acid treatment of the brain sections increased the intensity and number of positively staining plaques but did not alter the immunoreactivity in the normal human retina.

No difference was noted in location or intensity

![Figure 7](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933181/ on 06/25/2017)
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FIGURE 8. Anti-APP immunoreactivity in human retina with retinitis pigmentosa. Intense labeling of the IPL and RPE is present. Primary antibody 10 µg/ml, using NBT/BCIP as chromogen. No counterstain, original magnification ×33.

For comparison with other antibodies, see Figures 2 and 5. IPL = inner plexiform layer; RPE = retinal pigment epithelium; NBT/BCIP = nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate.

of the immunoreaction when different visualization methods were used. The use of a different fixative in two of the normal eyes did not alter the degree or pattern of immunoreactivity. A summary of the immunoreactivity of the normal retina against the four antibodies studied is presented in Table 1.

DISCUSSION

We demonstrated the presence of tau proteins and amyloid precursor protein in the inner layers of the human retina at various ages and in eyes with RP and age-related macular degeneration. Beta-amyloid was only detected in small amounts in the sub-RPE space.

Tau proteins are associated with the arrangement of microtubules, and an increase in these proteins has been demonstrated in specific lesions of degenerative diseases of the brain. Without prior phosphatase treatment, phosphorylated tau-1 mainly localizes to axons in the normal brain, whereas in the normal retina, we found strong immunoreactivity with anti-tau-1 not only in the nerve fiber layer but also in the somatodendritic compartment of cells in the inner nuclear layer. We could not detect any obvious changes in the immunoreactivity pattern or intensity of tau-1 in the aging retina. Furthermore, in eyes with RP, the pattern of immunolabeling was similar to that seen in the normal and aging retina. This suggests that tau-1 immunoreactivity remains unaltered with age and in the degenerating retina with RP. However,
TABLE 1. Immunoreactivity Against Tau, APP, and Beta-Amyloid in the Normal Human Retina

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RPE = Retinal pigment epithelium, PHR = photoreceptor outer and inner segments, ONL = outer nuclear layer, OPL = outer plexiform layer, INL = inner nuclear layer, IPL = inner plexiform layer, GCL = ganglion cell layer, NFL = nerve fiber layer, ILM = internal limiting membrane.

• = Weak immunoreactivity (IR), ++ = moderate IR, +++ = intense IR, ++++ = most intense IR.

(•+) = moderate IR, but patchy, [•+] = weak and patchy IR.

— = no immunoreactivity (IR).

the phosphorylation pattern in the retina appears to be different from the brain.

In contrast with the normal brain in which prominent anti-tau-2 immunoreactivity was demonstrated, there was only faint immunoreactivity observed in normal retinal structures with the phosphatase-independent epitope tau-2. Tau-2 immunoreactivity in the retina did not change significantly with age. In the brain, tau-2 has also been shown to be a major constituent of abnormal intracellular and extracellular neuronal deposits, and its abnormal phosphorylation has been suggested to play a role in Alzheimer’s disease. The pattern or intensity of tau-2 immunoreactivity in the degenerating retina with RP or in the ARMD eyes did not differ from that of the normal retina, nor did we find any abnormal intracellular or extracellular deposits that showed tau-2 immunoreactivity in either RP or ARMD eyes. However, mildly increased immunoreactivity was noted in the RPE of eyes with RP, and in eyes with ARMD a slightly more intense staining reaction throughout the inner retina was seen. The lack of specific alterations in anti-tau-2 immunoreactivity in the aging retina and in eyes with RP, though, suggests that tau-2, or its abnormal processing, may not be an important factor in either aging of the retina or degeneration of the retina in RP.

Amyloid precursor protein, the precursor of beta-amyloid, has been demonstrated in a variety of neurons and glia, as well as in rabbit retinal ganglion cells. It is thought to have a growth-regulating activity, and it appears to increase in neurons with age and in neurodegenerative diseases. Its precise functions, however, are still unclear. The increase of APP immunoreactivity in aging retinal ganglion cells might suggest a role of this protein in age-related loss of ganglion cells, and the increased APP immunoreactivity of RPE cells in RP and ARMD might indicate a possible involvement in degeneration and aberrant proliferation of the RPE. However, it is thought that the deposition of beta-amyloid in senile plaques is not necessarily related to an increase of APP-RNA or to a defect on the APP gene, but to abnormal processing of the protein. This corresponds to our findings in that we did not detect beta-amyloid deposits associated within the inner retina and especially not in the ganglion cells. The lack of beta-amyloid staining in the retina suggests that, although there may be increased APP synthesis as suggested by the increased retinal immunoreactivity in the aging retina, the processing of APP was unlikely to be abnormal as seen in Alzheimer’s disease. Furthermore, our study of RP and ARMD concentrated on eyes with advanced degeneration. It is possible that the immunoreactivity of these proteins in early disease may be different or that the changes noted in our study are secondary to other undefined factors associated with advanced disease. Thus, the exact implication of increased APP immunoreactivity in the diseased retina needs to be further clarified.

Beta-amyloid, a 4-kd protein, is the principal component of amyloid in Alzheimer’s disease and cerebrovascular amyloid and is derived from the amyloid precursor protein discussed above. In the brain, it is regarded as evidence of neuronal degeneration, although it also occurs in the absence of the latter. In the retina, only scant immunoreactivity was seen within soft drusen-like sub-RPE deposits, indicating that the deposition of beta-amyloid is not the major source of retinal degeneration. The complex nature of extracellular sub-RPE deposits, such as drusen or basal linear deposit, is still under investigation but has been presumed to be at least in part actively secreted. Thus, the formation and subsequent deposition of a protein similar, but not identical, to beta-amyloid may play a role in the degenerative process in age-related (macular) disease.

In summary, we present the immunoreactivity pat-
tern of anti-tau, anti-APP, and beta-amyloid in the human retina. We did not find any abnormal deposits or marked changes in immunoreactivity in the outer neural retina (outer nuclear and photoreceptor layers), the proposed site of early pathologic events in ARMD and RP, but increased anti-APP immunoreactivity in ganglion cells of older persons and in the RPE of eyes with RP and ARMD. Beta-amyloid was detected focally in sub-RPE deposits. Further studies are necessary to evaluate the particular role of these proteins in ocular disease.

Key Words
retinal immunoreactivity, amyloid, Alzheimer’s disease, retinal aging, neurodegenerative disease

References