Advanced Glycation End Products in Age-related Macular Degeneration

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Objective: To investigate the localization of N\(^{\epsilon}\)-(carboxymethyl)lysine (CML), a component and major immunologic epitope of advanced glycation end products, in aged eyes and choroidal neovascular membranes (CNVMs) surgically excised from eyes with age-related macular degeneration.

Methods: Immunohistochemistry for CML was performed using 8 snap-frozen, surgically excised CNVMs. Twelve eyes from patients aged 69 to 82 years and 2 donor eyes, 1 each from a 23-week-old fetus and 21-year-old patient, without age-related macular degeneration or diabetic retinopathy were also examined. To determine if retinal pigment epithelial cells in CNVMs accumulate advanced glycation end products, cytokeratin and CML were stained in paired serial sections.

Results: Soft, macular drusen and/or basal laminar and basal linear deposits were observed in 8 of 12 aged eyes. Each case showed CML accumulation, while overlying retinal pigment epithelial cells showed no accumulation in all 12 eyes. In CNVMs, however, retinal pigment epithelial cells showed CML accumulation in their cytoplasm.

Conclusion: The additional accumulation of advanced glycation end products in soft, macular drusen and/or retinal pigment epithelial cells may play a role in the pathogenesis of CNVM formation in age-related macular degeneration.

Clinical Relevance: Recently, advanced glycation end products have been found to play a role both in aging changes and neovascularization. Localization of advanced glycation end products in the above-mentioned tissue may lead to a better understanding of the pathogenesis of age-related macular degeneration.

IMMUNOHISTOCHEMISTRY FOR CML

Thawed tissue sections were incubated for 30 minutes with 1% bovine serum albumin and 0.3% hydrogen peroxide. The specimens were incubated for 1 hour with anti–CML antibody (6D12, Wako Bio-Products, Richmond, Va), then washed for 15 minutes with phosphate-buffered saline solution. A recent study revealed that 6D12 monoclonal antibody specifically recognizes CML-protein adduct. Immunoperoxidase detection was performed using avidin-biotin-complex (ABC Elite kit, Vector Laboratories, Burlingame, Calif) with aminoethylcarbazole as the red chromogen. Negative controls included substituting mouse nonimmune IgG for the primary antibody; omitting the primary antibody in the staining protocol; and using anti–CML antibody (2 µg/mL) adsorbed with an excess of CML–bovine serum albumin (100 µg/mL).

IMMUNOHISTOCHEMISTRY FOR CYTOKERATIN

To investigate if RPE cells accumulate CML in CNVMs, immunohistochemistry for cytokeratin (Dako Inc, Carpinteria, Calif) was performed in the serial section adjacent to that used for CML staining. Expression of cytokeratin is limited to RPE cells in retinochoroidal tissue.

COMMENT

N-(carboxymethyl)lysine is one of the AGEs detected in vivo and is a major immunologic epitope for anti–AGE antibodies. Handa et al reported pentosidine deposition, another component of AGEs, in the Bruch membrane in aged eyes (patients aged 82 and 92 years), as well as in the RPE cells, and in the choroid, but not in a young eye (patient aged 20 months). The young eyes in our study (23-week-old fetus and 21-year-old patient) showed no evidence of CML accumulation in the retinochoroidal tissue (data not shown). In 8 of 12 control eyes (patients aged 69-82 years), CML deposition was detected in Bruch membrane and accentuated in deposits such as basal laminar and basal linear ones and in soft, macular drusen. As opposed to pentosidine, no apparent CML deposition was observed in the intact RPE layer or the choroid.

In contrast, CML accumulation in RPE cells was suggested in CNVMs, a feature not found in any of the control eyes not even in those RPE cells adjacent to the CML-positive soft, macular drusen. The cells positive for CML showed a distribution pattern similar to that of the cells positive for cytokeratin in CNVMs. Since cytokeratin is expressed only by RPE cells in the retinochoroidal tissue, this result suggests that RPE cells in CNVM have CML accumulation in their cytoplasm.

We and other investigators have reported the localization of vascular endothelial growth factor (VEGF) in CNVMs. In our previous study, smooth muscle actin-positive or fibroblastic (transdifferentiated) RPE cells were commonly found in the highly vascularized regions in CNVMs obtained from patients with AMD. These transdifferentiated RPE cells expressed VEGF, suggesting an angiogenic role of these cells and this growth factor in AMD-related CNV. The factors that trigger increased VEGF expression in RPE cells of CNVMs are unknown. In diabetic retinopathy, hypoxia is the major stimulus that induces retinal cells to express VEGF. Relative ischemia...
of the outer retina that may be caused by atherosclerosis and atrophy of choriocapillaris has been suggested to be involved in the development of exudative AMD. Other than ischemia, various agents including AGEs have been reported to have a promoting effect on VEGF expression in various cell types. Advanced glycation end products are of particular interest since they stimulate VEGF expression in both cultured RPE cells and the in vivo RPE layer. They also stimulate endothelial cells to secrete VEGF and have an in vitro angiogenic effect. This suggests that the additional accumulation of AGE in RPE cells may lead to VEGF overexpression in older patients’ eyes, resulting in the initiation and development of CNV.

In addition, CML could play a role in the fibrous membrane formation that accompanies CNV. The association between AGE accumulation and fibrosis has been reported. Advanced glycation end products induce overexpression of growth factors such as transforming growth factor β and platelet-derived growth factor in RPE cells, that may promote fibrosis.

The formation of CML has been shown to occur at sites of oxidant stress with hydroxyl radical formation. The finding of CML accumulation in soft, macular drusen, basal laminar and basal linear deposits, and RPE cells suggests that similar oxidant stress may also show up at these sites, where it could result in damage to Bruch membrane and surrounding tissue. This damage may contribute to disruption in Bruch membrane. This disruption causes CNV to extend from the choroid into the subretinal space and is another critical process for formation of CNV. Advanced glycation end products induce the increased expression of cytokines known to occur in CNV and in surrounding fibrous membrane, where these end products could play a pathologic role. Our study provides a basis for the hypothesis that abnormal accumulation of AGE in soft, macular drusen, basal laminar and linear deposits, and RPE cells may be involved in the pathogenesis of AMD.

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REFERENCES


From the Archives of the ARCHIVES

A look at the past . . .

B and keratopathy and calcification of the conjunctiva is reported in 18 cases of hypercalcemia and in another case in which the calcium level of the blood may be presumed to have been elevated previously. The hypercalcemia was due to hyperparathyroidism in 4 cases, to vitamin D poisoning in 5 cases and to sarcoidosis in 2 cases. In the remaining 8 cases it was associated with severe renal damage, owing, in some of the cases, to a high calcium and high alkali intake. The corneal opacity consisted of paralimbal opacification of the cornea extending 2 to 3 mm axialward in the palpebral fissure. Many of the patients had nephrocalcinosis of nephrolithiasis, and the process occurring in the kidneys was thought to be analogous to that occurring in the cornea.