Expression of LOX-1, an Oxidized Low-Density Lipoprotein Receptor, in Choroidal Neovascularization

Subfoveal choroidal neovascularization of various macular diseases is one of the causes of severe blindness, including age-related macular degeneration (AMD). Several environmental risk factors have been elucidated in the pathogenesis of AMD, including smoking, atherosclerosis, increased levels of plasma fibrinogen, and low levels of antioxidant vitamins. Recent observations support the hypothesis that antioxidant and/or vitamin treatment may delay progression of AMD and vision loss. However, the exact cause of AMD remains to be determined.

Recently, Ikeda et al showed that increased plasma oxidized low-density lipoprotein (oxLDL) levels may be involved in the pathogenesis of AMD. Oxidized LDL has been implicated as having a major role in atherosclerosis, and many of the pathologic and biochemical features seen in choroidal neovascularization are analogous to those seen in advanced atherosclerosis, such as the infiltration of monocytes and macrophages and the overexpression of adhesion molecules, monocyte chemotactic proteins, growth factors, and cytokines within lesions. Lectinlike oxidized low-density lipoprotein receptor type 1 (LOX-1) is a recently identified oxLDL receptor that is abundantly expressed in vascular endothelial cells. Its messenger RNA has been shown to be expressed in atheromatous lesions, and LOX-1 up-regulation has been observed in several vascular lesions, including hypertensive remodeling lesions, diabetic vascular lesions, and macrophages. The observation of LOX-1 up-regulation in vascular lesions, the potential roles of oxLDL in the pathogenesis of AMD, and the possible similarity between the pathogenesis of atherosclerosis and that of AMD prompted us to examine LOX-1 expression in choroidal neovascularization. In addition, we sought to measure plasma cholesterol levels to investigate the relationship between LOX-1 expression and hyperlipidemia.

We examined LOX-1 localization in 13 surgically excised neovascular membranes, including 10 from patients with AMD, 1 from a patient with idiopathic choroidal neovascularization, and 2 from patients with myopic choroidal neovascularization. The membranes were frozen in liquid nitrogen within 30 minutes of excision. Multiple 8-µm cryosections from each membrane were air-dried, fixed in acetone for 5 minutes, washed with phosphate-buffered saline, and blocked for 30 minutes with 2% bovine serum albumin in phosphate-buffered saline. They were then incubated with primary antibody and washed 3 times for 5 minutes with

Figure 1. Immunostaining (A) and Western blot analysis (B) results of human lectinlike oxidized low-density lipoprotein receptor type 1 (LOX-1) complementary DNA (hLOX-1-CHO). A, Immunostaining of hLOX-1-CHO was performed. The nonfixed CHO cells were incubated with the primary antibody JTX92, then the bound antibody was detected with fluorescein isothiocyanate conjugated–antihuman IgG. B, Western blot analysis was performed to determine the specificity of the antibody. M indicates molecular weight marker; W, wild-type CHO cells; and L, CHO cells expressing hLOX-1. The arrowhead points to the expected molecular weight.
**Figure 2.** Fundus photograph and angiographic image (A) and immunostaining of surgically excised choroidal neovascular (CNV) membrane (B). A, Representative color fundus photograph (left) and angiographic image (right) of the fundus of a 55-year-old man with age-related macular degeneration (patient 1). The area of serous retinal detachment is indicated by arrows. B, panels a through f, A CNV membrane from patient 1. Panels a and d, Lectinlike oxidized low-density lipoprotein receptor type 1 (LOX-1) staining is seen in red, some of which is indicated by arrows. Panels b and e, von Willebrand factor (vWF) staining for vascular endothelial cells is seen in green, some of which is indicated by small arrowheads. Panels c and f, Confocal image of double staining for LOX-1 and vWF. LOX-1 expression and vWF localization were associated, some of which is indicated by large arrowheads. Panel g, Control staining of a CNV membrane from patient 1. Panel h, image of double staining for LOX-1 and vWF for panel g. von Willebrand factor staining for vascular endothelial cells is seen in green, some of which is indicated by small arrowheads. Panel i, A CNV membrane from a 37-year-old woman with idiopathic CNV (patient 13). LOX-1 is faintly stained and seen in red, some of which is indicated by arrows. Panels a through c, original magnification ×200; d through i, original magnification ×600.
phosphate-buffered saline. Bound antibody was detected with Cy3-
biotin–conjugated secondary anti-
body. Polyclonal antibodies against
human von Willebrand factor
(DAKO Corp, Kyoto, Japan) were
used to identify vascular endothe-
cells. Antihuman LOX-1 mono-
clonal antibody (JTX92) was gen-
erated by immunizing Balb/Cc mice
with the CHO cell line that was
transfected human LOX-1 comple-
mentary DNA (HLOX-1-CHO). Hy-
bridomas from the splenocytes were
prepared with the use of standard
procedures and screened by means
of the immunostaining of HLOX-1-
CHO. The specificity of the anti-
body was determined by means of
Western blot analysis and the im-
munostaining of HLOX-1-CHO
(Figure 1). Immunochemical
staining was repeated on cryo-
sections of 10 choroidal neovasculari-
ization membranes, omitting the
anti–LOX-1 primary antibody as
controls. Additional control
samples included immunohisto-
chemical staining for LOX-1 of the
posterior sclera, choroid, choroi-
depapillaries, and retina of a normal
donor eye.

The choroidal neovascular mem-
branes ranged from moderately cell-
lar with prominent neovasculari-
zeation to paucicellular and fibrotic
with few vascular channels. LOX-1
expression was detected in all, and
most of the LOX-1 was localized to
the endothelial cells (Figure 2).
Staining for endothelial cells was
seen in the LOX-1–positive cells. The
LOX-1–positive profiles exceeded
the number of von Willebrand fac-
tor–positive profiles, suggesting that
LOX-1 was localized in non-
vascular cells or within the stroma
of neovascular membranes, as well
as in the endothelial cells. The find-
ing that the labeling of LOX-1 was
not restricted to vascular endothe-

cial cells is in line with recent ob-
servations in advanced atheroscle-
rosis that LOX-1 is extensively
expressed in the new blood vessels,
macrophages, and smooth muscle
cells of advanced atherosclerotic le-
sions.10-12

We did not find LOX-1 within the
posterior segment of normal eyes, in-
cluding the choriocapillaries (Table). As is further summarized in the Table, greater LOX-1 stain-
ing was found in the membranes of
the patients with AMD compared with
those with idiopathic or my-
opic choroidal neovascularization,
those with a relatively high plasma
total cholesterol level, and those of
patients with larger macular serous
detachment.

Our findings suggest that LOX-1
plays an active role in the pathogen-

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Table. Clinical and Histological Characteristics

<table>
<thead>
<tr>
<th>Patient No./Eye/ Age, y/Sex</th>
<th>Diagnosis</th>
<th>TC, mg/dL (TC Status)</th>
<th>SRD, DD*</th>
<th>Preoperative CNV Size, DD†</th>
<th>LOX-1*</th>
<th>vWF*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/L/55/M</td>
<td>AMD</td>
<td>229 (High)</td>
<td>3</td>
<td>1.0</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>2/R/57/M</td>
<td>AMD</td>
<td>202 (Normal)</td>
<td>2</td>
<td>0.8</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>3/L/73/F</td>
<td>AMD</td>
<td>282 (High)</td>
<td>1.5</td>
<td>0.8</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>4/L/80/M</td>
<td>AMD</td>
<td>291 (High)</td>
<td>1.2</td>
<td>0.7</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>5/R/89/M</td>
<td>AMD</td>
<td>226 (High)</td>
<td>1.5</td>
<td>0.5</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>6/R/89/F</td>
<td>AMD</td>
<td>242 (High)</td>
<td>1.0</td>
<td>0.4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7/R/89/F</td>
<td>AMD</td>
<td>225 (High)</td>
<td>1.5</td>
<td>0.7</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>8/L/63/F</td>
<td>AMD</td>
<td>208 (Normal)</td>
<td>1.0</td>
<td>0.3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9/R/93/F</td>
<td>AMD</td>
<td>158 (Normal)</td>
<td>0.8</td>
<td>0.2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10/L/71/M</td>
<td>AMD</td>
<td>218 (Normal)</td>
<td>±</td>
<td>0.2</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>11/R/49/M</td>
<td>Myopic CNV</td>
<td>276 (High)</td>
<td>±</td>
<td>0.7</td>
<td>+</td>
<td>+</td>
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<tr>
<td>12/L/65/F</td>
<td>Myopic CNV</td>
<td>256 (High)</td>
<td>±</td>
<td>0.6</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>13/R/57/F</td>
<td>Idiopathic CNV</td>
<td>177 (Normal)</td>
<td>1.5</td>
<td>1.0</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; CNV, choroidal neovascularization; DD, disk diameter; L, left; LOX-1, lectinlike oxidized low-density lipoprotein receptor type 1; R, right; SRD, serous retinal detachment; TC, total cholesterol level; vWF, von Willebrand factor.

*Measured on fluorescein angiography.
†Measured on fluororescein angiography.

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Dietary antioxidants and age-related macu-
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**Histopathology of Documented Growth in Small Melanocytic Choroidal Tumors**

Differentiation of a choroidal nevus from a small choroidal melanoma can be difficult. Choroidal nevi are generally asymptomatic lesions that are less than 6 mm in diameter and less than 1.5 mm in height. The presence of drusen or atrophy of the overlying retinal pigment epithelium generally indicate a chronic, inactive choroidal nevus. Orange pigment and subretinal fluid are more commonly present in choroidal melanomas. Echography usually demonstrates medium to high internal reflectivity in nevi and low reflectivity in melanomas. Documented growth is widely interpreted as evidence of malignancy.

We provide the histopathology of 2 small choroidal melanocytic tumors that became symptomatic, developed orange pigment, and showed documented growth. One lesion was an epithelioid malignant melanoma; the other was a benign nevus.

**Report of Cases. Case 1.** A 45-year-old man was examined in May 1996 at the Ocular Oncology Clinic of the University of Michigan Kellogg Eye Center, Ann Arbor, for an enlarging, small juxtapapillary choroidal lesion in his right eye. A photograph from 1994 (Figure 1) showed a flat, brown 3.0 × 1.8-mm choroidal lesion, superior to the disc, without drusen or orange pigment. The lesion had increased to 3.0 × 3.0 mm and developed extensive orange pigment over its surface. Echography revealed a maximal height of 1.5 mm with a medium internal acoustic pattern. The initial diagnosis was a probable melanoma, but a decision was made to observe for evidence of continued growth before initiating therapy. In July 1996, the patient complained of blurred vision in his right eye. Results of an examination showed that the base had further increased to 3.3 × 3.2 mm (Figure 2). Repeat echography revealed that tumor height had

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**Figure 1.** Fundus photograph of the right eye of case 1 shows a flat, pigmented choroidal lesion superior to the disc.

**Figure 2.** Fundus photograph of the right eye of case 1 shows a symptomatic, enlarged choroidal lesion 2 years after the photograph in Figure 1.