Melanoma-Associated Retinopathy

A Paraneoplastic Autoimmune Complication

Ying Lu, MD, PhD; Lin Jia, MS; Shirley He, MD, MS; Mary C. Hurley, MS; Monique J. Leys, MD; Thiran Jayasundera, MD; John R. Heckenlively, MD

Objectives: To study 11 patients with melanoma-associated retinopathy (MAR) to clarify the reliability of various methods of diagnostic testing, to determine the underlying antigenic retinal proteins, and to study the clinical histories and types of associated melanomas.

Methods: Clinical data were obtained from patients with melanoma who developed marked visual problems. Testing included electroretinography, kinetic visual fields, comparative studies of Western blots, and indirect immunohistologic examination to detect antiretinal antibodies, as well as proteomic studies to identify underlying antigenic retinal proteins.

Results: Patients with MAR typically have rapid onset of photopsias, scotomata, and loss of central or paracentral vision. Ophthalmoscopy seldom shows significant changes early, but electroretinograms are abnormal. Results of Western blots and immunohistologic examination can show antiretinal antibodies but not always. Most patients (9 of 11) had a strong family history of autoimmune disorders. Any type of melanoma (cutaneous, choroidal, ciliary body, or choroidal nevi) may be associated with this paraneoplastic autoimmune reactivity. MAR may precede or follow the diagnosis of melanoma. Patients with MAR have the same antigenic retinal proteins that have been associated with cancer-associated retinopathy. In addition, 2 new antigenic retinal proteins, aldolase A and aldolase C, were found.

Conclusions: There was a high prevalence of positive family histories of autoimmune disease in patients with MAR. To confirm the disorder, multiple clinical and serum diagnostic techniques (Western blot or indirect immunohistologic examination) are needed. Two newly observed antigenic retinal proteins, aldolase A and aldolase C, are associated with MAR.


Autoimmune Retinopathy (AIR) was first recognized as a paraneoplastic disorder as early as 1976 in patients with carcinoma, and subsequent studies in the literature showed that AIR could occur with benign and malignant tumors. Melanoma-associated retinopathy (MAR) was initially reported by Gass in an atypical case with vitelliruptivelike yellow retinal lesions, but most MAR cases have diffuse retinal atrophy without pigment deposits. A broader view of 51 cases of MAR was published by Keltner et al summarizing many of the features and some of the controversies. Except for 2 case reports of choroidal melanoma, only MAR associated with cutaneous melanomas has been reported.

Paraneoplastic development of antiretinal antibodies with resulting retinal disease is an intriguing pathologic process and remains poorly understood. There have been reports of retinopathies associated with carcinomas, melanomas, tetratomas, and even lymphomas. Most cases of AIR occur in patients without a history of tumors, but occasionally a history of head trauma or preceding intraocular inflammatory disease may be found. Investigations of cancer-associated retinopathy (CAR) have found that the tumors in these patients aberrantly express proteins normally exclusive to retinal tissue, leading to the production of antibodies directed against these retinal antigens. There are no reports in which primary melanomas have been examined to investigate the presence of retinal proteins, although melanoma cell cultures have demonstrated their presence. The antiretinal antibodies in patients with MAR are associated with progressive panretinal degeneration that frequently results in legal blindness. Patients’ early symptoms typically manifest as sparkles and shimmers (photopsias) and blind spots in their vision. On clinical examination, early stages of retinopathy may
be difficult to see clinically, but an electroretinogram (ERG) (a standardized evoked response that measures photoreceptor and inner retinal responses) will clearly show retinal dysfunction and aids in diagnosis of this condition with otherwise minimal retinal changes.17

Conventional confirmatory evidence for MAR has included examination of serum for antiretinal antibodies by Western blot or measurement of antibody staining of donor retina, particularly bipolar cells, by indirect immunohistologic studies.18 However, there has been controversy about the best diagnostic tests, with data suggesting that Western blots may be unreliable for detecting antiretinal antibodies in patients with MAR.8 Milam et al9 recommended indirect immunohistologic investigations for retinal inner nuclear layer bipolar cell staining as a reliable technique for confirming MAR. To date, no studies have been performed in patients with MAR comparing Western blot with indirect immunohistologic detection of antiretinal antibodies. In addition, the specific antibodies (and their antigenic retinal proteins) involved in MAR have not been systematically examined. We investigated 11 patients with MAR who were initially seen with visual loss and photopsias and who underwent clinical examinations, electrophysiological and psychophysical testing, Western blots, indirect immunohistologic investigations, and proteomic analysis to identify the antigenic retinal proteins. Careful family histories were taken for autoimmune backgrounds to clarify the genetic autoimmune backgrounds of patients with MAR.

METHODS

CLINICAL EXAMINATIONS

Eleven patients with histories of melanoma and subsequent retinopathies of unknown origins and 8 patients with CAR and AIR (included for comparison) were examined at the retinal dystrophy clinic at the Kellogg Eye Center, University of Michigan, Ann Arbor. The patients were evaluated using standardized kinetic visual fields, electroretinographic testing, fundus photography, and clinical examinations. Serum samples were obtained after informed consent and were stored at −80°C until studied. As control subjects, 9 healthy volunteers with normal ERGs and no family history of autoimmune diseases were recruited. Informed consent was obtained from all patients and control subjects. The experimental protocol was approved by the University of Michigan institutional review board.

2-DIMENSIONAL ELECTROPHORESIS

WESTERN BLOT ANALYSIS

Reagents and materials were obtained (from GE Healthcare, Uppsala, Sweden, unless otherwise indicated) for 2-dimensional electrophoresis experiments. Recent postmortem normal human retina (Michigan Eye Bank, Ann Arbor) was lysed (Cel-Lytic MT, 5mM Tris[2-carboxyethyl]phosphine hydrochloride), 1% protease inhibitor cocktail [Sigma-Aldrich, St Louis, Missouri]) and centrifuged at 10 000g for 10 minutes at 4°C, and the supernatant was pooled. Protein (300 µg) was precipitated from the lysate (Amersham Clean-Up Kit, GE Healthcare). The pellets were incubated for 60 minutes at 30°C in 220 µL of isoelectric focusing rehydration buffer (7M urea, 2M thiourea, 1% amidoxylsulfo betaine 14, 1% Triton X-100, 1% [(3-cholamido propyl)dimethylammonio]-1-propanesulfonic acid, and 1% immobilized pH gradient buffer). The solubilized protein was loaded overnight onto 11-cm (pH 3-10) immobilized pH gradient strips using active rehydration at 50 V. Isoelectrofocusing was performed at 40 000 V/h using isoelectric focusing Cell (Biorad Industries, Hercules, California). The focused IPG strips were equilibrated in buffers containing sodium dodecyl sulfate (SDS)-Tris (2-carboxyethyl)phosphine hydrochloride and SDS-tetraacetamide. The second-dimension gels were run on 4% to 12% acrylamide Bis-Tris gels (BioRad Industries). After electrophoresis, duplicate gels were immunoblotted or stained with SYPRO Ruby (Molecular Probes, Eugene, Oregon).

Separated proteins were transferred onto nitrocellulose membranes and incubated with serum from patients having MAR and with horseradish peroxidase–conjugated mouse antihuman immunoglobulin as a secondary antibody. Immunoreactive spots on each membrane were compared visually and by using a computerized system. Each of the spots was matched to an equivalent spot on staining gels.

IN GEL ENZYME DIGESTION AND MASS SPECTROMETRY

Spots from 2-dimensional electrophoresis–stained gels were excised and in gel digested (Trypsin Gold; Promega, Madison, Wisconsin). Peptides were extracted from the gel plugs in 30 µL of 2% acetonitrile, 1% formic acid. Five microliters of alphan cyano-4-hydroxycinnamic acid (5 mg/mL in 50% acetonitrile, 0.1% trifuoroacetic acid [TFA], and 2mM ammonium citrate) matrix was added to the digested peptides. The extracts were evaporated to dryness and then dissolved in 5 µL of 60% acetonitrile, 0.1% TFA, A 0.5-µL volume of this solution was spotted on a 192-well matrix-assisted laser desorption ionization target and allowed to dry. Mass spectra were acquired using tandem time-of-flight mass spectrometry (4800 Proteomics Analyzer; Applied Biosystems, Foster City, California). Database searching was performed using commercially available software (GPS Explorer version 3.6, with Mascot version 2.1 against International Protein Index human version 3.32 using the following parameters: 30-ppm mass tolerance, 1 missed cleavage, carbamidomethyl (C) fixed modification, and variable modifications pyroglu (N-term Q) and oxidation (M). Spectra were acquired in tandem mass spectrometry 2-kV—positive mode.

IMMUNOBLOTS AND SDS–POLYACRYLAMIDE GEL ELECTROPHORESIS

Retinal extract (20 µg of proteins), 0.1 µg of aldolase A, 0.1 µg of carbonic anhydrase II (CAII), 0.4 µg of aldolase C, 0.2 µg of recoverin, 0.2 µg of S-arrestin, 0.4 µg of S-transferase (GST) fusion α-enolase, and 0.2 µg of heat shock protein 60 (HSP60) were used. Samples were separated on 10% SDS–polyacrylamide gel electrophoresis gels. After electrophoretic run, immunoblot analysis was performed as described previously. We used GST fusion α-enolase (Abnova, Taipei, Taiwan) and aldolase A and HSP60 (Sigma-Aldrich). Bovine recombinant recoverin was purified as previously described.19 Human recombinant CAII was purified as previously described.20

IMMUNOHISTOCHEMISTRY

Frozen sections were obtained from recent postmortem normal human retina (Michigan Eye Bank) embedded in optimum cutting temperature compound (Tissue-Tek; Miles Inc, Elkhart, Indiana). Sections were blocked for nonspecific protein binding with 5% goat serum in a phosphate-buffered saline (PBS) solution at room temperature for 1 hour and then

©2009 American Medical Association. All rights reserved.

Downloaded From: on 07/20/2018
incubated with serum from patients with MAR (dilution, 1:500) overnight at 4°C. After washing with PBS plus 0.2% Triton X-100 (3 times at room temperature for 10 minutes each), the sections were incubated with Alexa Fluor 488–conjugated anti-goat IgG secondary antibody (dilution, 1:2000) (Molecular Probes). They were then counterstained with 4',6'-diamidino-2-phenylindole (0.3µM) before observation with a fluorescence microscope (Olympus, Tokyo, Japan).

Figure 1. Western blots of serum samples from patients with melanoma-associated retinopathy (patients 175, 317, 322, 471, 886, 1066, 1099, 2052, S49, and 3361) showing immunoreactivity of autoantibodies to (1) human, (2) mouse, and (3) bovine retinal extract. Bands found on human Western blots were isolated and analyzed by mass spectrometry, and results were confirmed by immunoblotting with the following candidate proteins: (4) aldolase A (39 kDa), (5) carbonic anhydrase II (29 kDa), (6) aldolase C (39 kDa), (7) recoverin (23 kDa), (8) S-arrestin (45 kDa), (9) glutathione S-transferase fusion/α-enolase (74 kDa), and (10) heat shock protein 60 (60 kDa). Molecular size standards are shown on the left.

RESULTS

IDENTIFICATION OF RETINAL AUTOANTIBODIES IN SERUM SAMPLES FROM PATIENTS WITH MAR

Proteomic analysis was performed of antigenic retinal proteins identified on Western blots. The identified MAR-
related antigenic retinal proteins are the same ones that have been identified in many CARs and AIRs cases, namely, antibodies against recoverin, \textit{H9251}-enolase, S-arrestin, CAII, and HSP60\textsuperscript{10,14,21} (Figure 1 and Table 1). We found 2 new antigenic retinal proteins on Western blots (aldolase C and aldolase A), which have not been reported in MAR, CAR, or AIR, to our knowledge. Immunoblot analysis of serum samples from patients with MAR using human, mouse, or bovine retinal extract, as well as purified proteins, found autoantibodies to recoverin (2 of 11), CAII (2 of 11), S-arrestin (3 of 11), \textit{H9251}-enolase (5 of 11), aldolase A (2 of 11), aldolase C (4 of 11), and HSP60 (11 of 11). Immunoblot analysis of 8 serum samples from patients with CAR, and 9 control serum samples using purified proteins was also performed for comparison. Antibodies to recoverin (1 of 8), S-arrestin (1 of 8), aldolase A (1 of 8), and HSP60 (7 of 8) were identified in serum samples from patients with CAR, while antibodies to CAII, \textit{H9251}-enolase, and aldolase C were not identified in serum samples from patients with CAR. Antibodies to CAII (1 of 9) and HSP60 (5 of 9) were identified in control serum samples, while antibodies to recoverin, S-arrestin, \textit{H9251}-enolase, aldolase A, and aldolase C were not identified in control serum samples.

Some serum samples from patients with MAR did not show immunoreactivity on Western blots using solubilized normal retinal proteins, which led to development of the indirect immunohistologic method.\textsuperscript{8} Our investigations demonstrated immunoreactivity using both methods, with 9 of 11 patients having positive Western blots, 8 of 11 patients having positive immunohistologic reactivity, and 7 of 11 patients having both. Some patients with MAR have antibodies that are reactive with retinal cells other than bipolar cells, so the usefulness of bipolar cell staining as the sole basis for diagnosing MAR is limited to cases in which it occurs. Inner nuclear layer staining was common in our patients with MAR, but colocalization studies found bipolar cell staining in only 4 of 11 patients, while other inner nuclear layer retinal cells had focal immunoreactivity to patient serum (Figure 2). More detailed studies will be needed to elucidate the specificity of this immunoreactivity and to investigate other immunologic variables, as a few patients with MAR did not have reactive antiretinal antibodies by this method.

**Table 1. Mass Spectroscopy Results for Each Western Blot Spot and Prevalence of Autoantibodies Found Against Each Retinal Protein in Melanoma-Associated Retinopathy (MAR), Cancer-Associated Retinopathy (CAR), and Control Serum**

<table>
<thead>
<tr>
<th>Retinal Protein</th>
<th>Protein Molecular Weight, Da</th>
<th>Protein Isoelectric Point</th>
<th>Start-End Residue</th>
<th>Best Peptide Sequence</th>
<th>Calculated Mass, Da</th>
<th>Recurrence of Autoantibodies in Serum, No./Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldolase A</td>
<td>45 688.3</td>
<td>8.48</td>
<td>98-111</td>
<td>LDSG1TENTΕΕΝΗR</td>
<td>1646.81</td>
<td>2/11 (18) 1/8 (13) 0/9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>142-153</td>
<td>ADDGRPPODVK</td>
<td>1342.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>166-188</td>
<td>GVPVLATNGTTQGLDGLQER</td>
<td>2272.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>44-57</td>
<td>LSQIG1VENTΕΕΝΗR</td>
<td>1644.83</td>
<td>4/11 (36) 0/8 0/9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>88-96</td>
<td>DDNGVPFR</td>
<td>1652.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>112-134</td>
<td>GVPVLATGD1TQGLDGLQER</td>
<td>2273.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>158-173</td>
<td>TPSA1ILEAVNVLAR</td>
<td>1652.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>216-230</td>
<td>Y1TE1IA1M1AY1T1LAR</td>
<td>1681.85</td>
<td></td>
</tr>
<tr>
<td>Aldolase C</td>
<td>36 614.8</td>
<td>7.60</td>
<td>44-57</td>
<td>LSQIG1VENTΕΕΝΗR</td>
<td>1644.83</td>
<td>4/11 (36) 0/8 0/9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>88-96</td>
<td>DDNGVPFR</td>
<td>1652.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>112-134</td>
<td>GVPVLATGD1TQGLDGLQER</td>
<td>2273.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>158-173</td>
<td>TPSA1ILEAVNVLAR</td>
<td>1652.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>216-230</td>
<td>Y1TE1IA1M1AY1T1LAR</td>
<td>1681.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>158-173</td>
<td>TPSA1ILEAVNVLAR</td>
<td>1652.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>216-230</td>
<td>Y1TE1IA1M1AY1T1LAR</td>
<td>1681.85</td>
<td></td>
</tr>
<tr>
<td>Carbonic anhydrase II</td>
<td>29 400</td>
<td>6.63</td>
<td>97-121</td>
<td>L1VQDV1AN1TΝΕΕΑ1G1D1G1T1T1AT1L1AR</td>
<td>2560.25</td>
<td>11/11 (100) 7/8 (88) 5/9 (56)</td>
</tr>
<tr>
<td>α-Enolase</td>
<td>47 481.4</td>
<td>7.01</td>
<td>33-50</td>
<td>AAAVP1GAST1G1YE1ALE1L1R</td>
<td>1804.94</td>
<td>5/11 (45) 0/8 0/9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>163-179</td>
<td>LAMQ1EM1FL1M1P1G1A1AN1FR</td>
<td>1939.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>184-193</td>
<td>IGAEVYHN1L1K</td>
<td>1143.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>240-253</td>
<td>VVIG1M1DVA1A1E1F1FR</td>
<td>1556.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>270-281</td>
<td>Y1SPD1QL1AD1LY1K</td>
<td>1425.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>359-372</td>
<td>LAGA1NW1G1W1MV1S1H1R</td>
<td>1541.76</td>
<td></td>
</tr>
<tr>
<td>Heat shock protein 60</td>
<td>61 187.4</td>
<td>5.70</td>
<td>97-121</td>
<td>L1VQDV1AN1TΝΕΕΑ1G1D1G1T1T1AT1L1AR</td>
<td>2560.25</td>
<td>11/11 (100) 7/8 (88) 5/9 (56)</td>
</tr>
<tr>
<td>S-arrestin</td>
<td>45 262.5</td>
<td>6.14</td>
<td>25-33</td>
<td>SV1TI1YL1G1N1R</td>
<td>1022.56</td>
<td>3/11 (27) 1/8 (13) 0/9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>61-70</td>
<td>VYVT1L1TC1AF1R</td>
<td>1229.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>71-84</td>
<td>Y1G1E1D1I1D1V1G1L1T1FR</td>
<td>1625.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>180-193</td>
<td>KV1Q1H1A1P1L1M1G1P1D1PR</td>
<td>1603.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>281-292</td>
<td>T1L1TL1P1L1A1N1NR</td>
<td>1338.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>337-358</td>
<td>LT1V1S1G1F1L1G1E1L1T1S1E1V1A1E1T1V1P1FR</td>
<td>2339.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>370-384</td>
<td>E1SY1Q1D1A1N1L1V1F1E1F1ER</td>
<td>1817.83</td>
<td></td>
</tr>
<tr>
<td>Recoverin</td>
<td>23 229.7</td>
<td>5.06</td>
<td>44-55</td>
<td>IT1Q1Q1O1F1O1S1I1Y1K</td>
<td>1454.77</td>
<td>2/11 (18) 1/8 (13) 0/9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>140-151</td>
<td>LL1PD1D1E1NT1PE1KR</td>
<td>1426.72</td>
<td></td>
</tr>
</tbody>
</table>

To date, except for 2 case reports of choroidal melanoma,\textsuperscript{6,7} only MAR associated with cutaneous melanomas has been reported.\textsuperscript{3} We were surprised to find that any form of melanoma may potentially autosensitize the patient and lead to MAR. We examined 11 patients having MAR with the following types of melanoma: cutaneous (6 patients), choroidal tumors (2 patients), flat choroidal nevi (2 patients), and ciliary body (1 patient). Flat choroidal nevi usually are regarded as benign with little malignant potential unless there is demonstrated growth.\textsuperscript{22}
However, the carcinogenic potential of flat choroidal melanomas seems to differ from their ability to serve as antigenic foci in autoimmune-susceptible individuals. Both of our patients with choroidal nevi (patients 1066 and 3361 in Table 2) had central scotomata, antiretinal antibodies, and severe ERG changes similar to the other patients with MAR. These 2 patients also had obvious degeneration of the retina at the margins of the nevi, and patient 1066 showed asymmetry of disease, which was worse in the eye with the large nevus (Figure 3). Four individuals (patients 322, 1066, 2052, and S49 in Table 2) with different forms of melanoma are shown as representative examples in Figure 3.

Clinically, the patients with MAR have similar findings, with rapid onset that included photopsias and loss of vision. Electroretinograms show retinal dysfunction (Figure 3) and aid in diagnosis of this condition with otherwise minimal retinal changes.17 Patients often have negative electroretinographic waveforms (a-waves and poor b-waves not reaching the isoelectric point) on dark-adapted maximal stimulation and central scotomata on visual field testing (Figure 3). In more advanced cases, patients develop retinal vessel attenuation and areas of diffuse atrophy. Retinal pigmentary changes are absent to minimal. We also found that patients with MAR commonly have personal and family histories of autoimmune disease, suggesting a special susceptibility for the putative retinal antigens released by their melanomas (Table 2). The latency from melanoma diagnosis to recognition of MAR ranged from 6 months to 14 years (median, 2 years) preceding the diagnosis. The trigger that set off the MAR response in the patients with longer latencies is unknown. One particularly intriguing individual with MAR (patient 175) with malignant melanoma of an amputated toe did not manifest MAR until after she had knee surgery in the same leg 5 years after amputation of the toe.

**COMMENT**

Typically, malignant melanoma is a highly invasive tumor derived from neuroectodermal melanocytes, which share a lineage with retinal cells.23 The molecular mechanisms underlying the development of MAR are unknown. Investigations of patients with MAR, CAR, and AIR frequently show positive family histories for autoimmune diseases, and many patients have autoimmune genetic templates that increase their susceptibility to autoimmune diseases.11 Examination of tumors in pa-
Table 2. Findings in 11 Patients With Melanoma-Associated Retinopathy (MAR)

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Tumor Type</th>
<th>Time From Malignant Melanoma Diagnosis to Recognition of MAR</th>
<th>Personal or Family History of Autoimmune Disease</th>
<th>Visual Acuity</th>
<th>Retinal Appearance</th>
<th>Goldmann Visual Fields</th>
<th>Rod-Isoleted Response</th>
<th>Mixed Response (Ratio of b-Waves to a-Waves)</th>
<th>Photopic Response</th>
<th>Carbonic Anhydrase II</th>
<th>α-Enolase</th>
<th>Aldolase C</th>
<th>Heat Shock Protein 60</th>
<th>Staining Location With Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>175/F/75</td>
<td>Malignant melanoma, R toe amputated 1998, R knee surgery 2007</td>
<td>MAR 2004</td>
<td>DM (sister), lupus, fibromyalgia (daughter)</td>
<td>CF OU</td>
<td>Granular pigmentation of macular OU</td>
<td>5-Degree central scotoma with enlarged blind spots OU</td>
<td>NR OU</td>
<td>0.5, negative, 46 OD; 0.5, negative, 46 OD</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>INL inner plexiform layer</td>
<td></td>
</tr>
<tr>
<td>317/F/85</td>
<td>Choroidal</td>
<td>14 y Unknown</td>
<td>Unknown</td>
<td>20/25 OD, enucleated OS</td>
<td>Diffuse retinal and retinal pigment epithelial atrophy OD</td>
<td>Constricted peripheral fields (i.e., isopter constricted to 15 degrees)</td>
<td>NR OD</td>
<td>0.5, negative, 40 OD</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>INL</td>
<td></td>
</tr>
<tr>
<td>322/M/58 Ciliary body</td>
<td>Concurrent</td>
<td>None</td>
<td>None</td>
<td>20/20 OU</td>
<td>Generalized depigmented fundus with focal pigment deposits OU</td>
<td>Paracentral scotoma OD, partial ring scotoma OS</td>
<td>13 OD, 10 DS</td>
<td>1.3, negative, 29 OD; 1.0, negative, 44 DS</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>No staining</td>
<td></td>
</tr>
<tr>
<td>471/M/50 Metastatic</td>
<td>6 mo Before melanoma diagnosis</td>
<td>20/40 OU</td>
<td>Ulcerative colitis and arthritis (patient), asthma (mother)</td>
<td>20/80 OD, 20/100 OD</td>
<td>Retinal atrophy at macular and posterior pole RPE mottling OU</td>
<td>Central scotoma OU (5-degree segment 10-degree field)</td>
<td>31 OD, 41 DS</td>
<td>1.2, negative, 36 OD; 1.3, negative, 44 OD</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>Cone inner segments INL</td>
<td></td>
</tr>
<tr>
<td>886/F/72</td>
<td>Cutaneous</td>
<td>3 y RA (patient)</td>
<td>RA (patient)</td>
<td>20/80 OD, 20/100 OD</td>
<td>Retinal atrophy at macular and posterior pole RPE mottling OU</td>
<td>Central scotoma OU (5-degree segment 10-degree field)</td>
<td>31 OD, 41 DS</td>
<td>1.2, negative, 36 OD; 1.3, negative, 44 OD</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>INL GCL</td>
<td></td>
</tr>
<tr>
<td>1066/M/88 Choroidal nceus</td>
<td>Concurrent</td>
<td>2/2000 OD, 20/2000 OD</td>
<td>RA (mother, sister), DM (father), thyroid disease (daughter)</td>
<td>11 OD, 34 DS</td>
<td>Temporal island of vision only OD, ring scotoma with constriction of peripheral field to 50 degrees (4-V-4-e)</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>... ... ... ... ... ...</td>
<td>INL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(continued)
Table 2. Findings in 11 Patients With Melanoma-Associated Retinopathy (MAR) (continued)

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Tumor Type</th>
<th>Time From Malignant Melanoma Diagnosis to Recognition of MAR</th>
<th>Personal or Family History of Autoimmune Disease</th>
<th>Visual Acuity</th>
<th>Retinal Appearance</th>
<th>Visual Fields</th>
<th>Mixed Response (Ratio of b-Waves to a-Waves)</th>
<th>Photopic Response</th>
<th>Carbonic Anhydrase II</th>
<th>S-Arrestin</th>
<th>α-Enolase</th>
<th>Aldolase A</th>
<th>Heat Shock Protein 60</th>
<th>Staining Location With Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1066/M/88 Choroidal nevus</td>
<td>Concurrent RA (mother, sister), DM (father), thyroid disease (daughter)</td>
<td>2/200/00 OD, 20/200 OS</td>
<td>Large choroidal nevus with surrounding choroidal atrophy OD, peripheral retina/RPE atrophy OS</td>
<td>11 OD, 34 DS</td>
<td>NR OD</td>
<td>NR OD, 16 DS</td>
<td>+ + + + + +</td>
<td>+ +</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>No staining</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1099/F/78 Cutaneous 2 y</td>
<td>Treated with hydroxychloroquine sulfate (patient)</td>
<td>20/400 OU</td>
<td>Scalloped retinal, RPE atrophy posterior pole with peripheral pigment OU</td>
<td>Midperipheral islands of vision to IV-4-e isopter OD, midperipheral inferonasal island of vision to IV-4-e isopter OS</td>
<td>NR OU</td>
<td>NR OU</td>
<td>NR OU</td>
<td>+ + + +</td>
<td>+</td>
<td>+</td>
<td>No staining</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002/F/83 Cutaneous 11 y</td>
<td>RA (patient)</td>
<td>20/200 OU</td>
<td>Crescentic degeneration temporal to macular and peripheral retinal atrophy OU</td>
<td>Paracentral scotoma OU</td>
<td>20 OD, 12 DS</td>
<td>53 OD</td>
<td>42 DS</td>
<td>+ + + +</td>
<td>+ +</td>
<td>+ +</td>
<td>+</td>
<td>No staining</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2052/F/44 Cutaneous 3 y</td>
<td>RA (patient)</td>
<td>20/80 OD, 20/50 OS</td>
<td>Macular retinal atrophy OU</td>
<td>Paracentral scotoma OU</td>
<td>NR OU</td>
<td>0.8</td>
<td>Negative, 6 OD; 0.8, negative, 8 OS</td>
<td>+ + + +</td>
<td>+ +</td>
<td>+ +</td>
<td>+</td>
<td>INL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3361/F/82 Choroidal nevus</td>
<td>Concurrent RA, Sjögren syndrome (patient); DM (mother, father, brother)</td>
<td>20/60 OD, 20/50 OS</td>
<td>Interperipheral nevus with surrounding choroidal atrophy OS, generalized retinal/RPE atrophy OU</td>
<td>Paracentral scotoma OD, central scotoma OS, moderate constriction of peripheral field OU</td>
<td>NR OU</td>
<td>NR OU</td>
<td>NR OU</td>
<td>+ + + +</td>
<td>+ +</td>
<td>+ +</td>
<td>+</td>
<td>+</td>
<td>INL GCL</td>
<td></td>
</tr>
<tr>
<td>549/M/73 Choroidal nevus</td>
<td>Arthritis treated with prednisone (patient)</td>
<td>Enucleated OD, 20/125 OS</td>
<td>Generalized retinal atrophy OS</td>
<td>Central 20-degree scotoma OS</td>
<td>40 OS</td>
<td>33 DS</td>
<td>+ + + +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>Cone INL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CF, counting fingers; DM, diabetes mellitus; GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; NR, not recordable; RA, rheumatoid arthritis; RPE, retinal pigment epithelium; ellipsis, not applicable.

a Percentage of mean normal (age 20-60 years) was used as a reference to grade the severity of dysfunction. Negative indicates a negative waveform in which the b-wave does not return to the isoelectric baseline, and near negative indicates that the b-wave generated is marginally above the isoelectric baseline.

b Plus sign indicates presence.

Aldolase B in liver,35 and aldolase C together with aldolase A in brain.34,36 Because aldolase A and aldolase C are of brain origin, the presence of circulating anti–aldolase A and anti–aldolase C autoantibody should serve as an indicator of blood–retinal barrier damage in patients with MAR. Aldolase C is expressed in the ganglion cell layer and inner nuclear layer in retina37 and in melanoma tissue.38 The role of aldolase A and aldolase C in retina is unknown at this time. Anti–aldolase A antibodies have been found in other diseases such as rheumatoid arthritis34 and Alzheimer disease,39 while anti–aldolase C antibodies that cross-reacted with aldolase A have been found in diabetic retinopathy.40 In the present study, we found a high prevalence of antialdolase antibody in patients with MAR. Cancer or mela-

©2009 American Medical Association. All rights reserved.
or absence of these markers with clinical data. The diversity of autoantibodies produced in MAR may be the consequence of varied overexpression among retinal proteins involved in melanoma tumor development, as well as among these patients’ propensity to develop autoimmune autoantibodies.11,18 In general, AIR is treatable with immunosuppression therapies,11,18 but no standardized protocols have been established. In addition, it is unknown whether the antibodies that develop in patients with MAR may have a therapeutic role in retarding tumor growth.

**Submitted for Publication:** April 7, 2009; final revision received May 18, 2009; accepted June 19, 2009.

**Correspondence:** John R. Heckenlively, MD, Department of Ophthalmology and Visual Sciences, Kellogg Eye Center, University of Michigan, 1000 Wall St, Ann Arbor, MI 48105 (jheck@umich.edu).

---

**Figure 3.** Clinical features of melanoma-associated retinopathy (MAR). A, A 44-year-old woman (patient 2052 in Table 2) had a cutaneous melanoma excised 2 years before developing pericentral scotomata. She was initially seen with generalized retinal atrophy demonstrating no pigment deposits and with rod-cone dysfunction on an electroretinogram (ERG) showing a negative waveform. B, A 73-year-old man (patient S49) had a choroidal melanoma in the right eye requiring enucleation. His remaining eye developed a scotoma 15 months later. Examination showed generalized atrophy of the retina, cone-rod dysfunction on an ERG, and an almost negative waveform. C, A 58-year-old man (patient 322) with a 2-month history of scotomata was found to have a ciliary body mass in the right eye and an overlying conjunctival sentinel vessel. He had generalized depigmented fundus with focal pigment deposits, central scotomata in the right eye, and partial ring scotomata in both eyes. His ERG showed severe cone-rod dysfunction and negative waveforms. The ciliary mass completely regressed over 4 months without treatment, and visual function stabilized in both eyes. D, An 88-year-old man (patient 1066) had progressive deterioration of central and right vision over 10 months, with a large choroidal nevus (7.5-mm wide and 1-mm thick) and surrounding choriotinal atrophy in the right eye. He had preexisting dry age-related macular degeneration in both eyes with central visual field loss far greater than the area of macular degeneration. Visual fields were asymmetric, with severe loss in the eye with the nevus. The ERG was nonrecordable in the right eye, and his left eye had cone-rod dysfunction with a negative waveform. E, A 40-year-old woman with no ophthalmic disease and with normal retinal appearance underwent visual field tests and an ERG.
Financial Disclosure: None reported.

Funding/Support: This study was supported by Foundation Fighting Blindness and by grant RO1 EY007758 from the National Institutes of Health.

Additional Contributions: Scott Grant, MD; Frank Barber, MD; Mark Johnson, MD; Thomas Mehelas, MD; Alan Ruby, MD; David Cooke, MD; and David Kaufman, MD; referred patients. James B. Hurley, PhD, provided recoverin plasmid. Carol Fiereke, PhD, provided carbonic anhydrase II plasmid. Vsevolod V. Gurevich, PhD, provided enolase plasmid. Jill Oversier, BS, coordinated the study.