Nonantibestrophin Anti-RPE Antibodies in Paraneoplastic Exudative Polymorphous Vitelliform Maculopathy

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Purpose: A previous report demonstrated antibodies to bestrophin in paraneoplastic exudative polymorphous vitelliform maculopathy (PEPVM). Other cases demonstrated antibodies to different proteins in the retinal pigment epithelium (RPE). In this report, serum was analyzed to determine whether a patient with PEPVM and a reduced Arden ratio had developed autoantibodies to human Bestrophin-1 (Best1).

Methods: Human embryonic kidney 293 cells (HEK293) were transfected with Best1 and stained with an antibody specific to Best1 (E6-6), or patient serum. Staining patterns were compared with those of untransfected cells stained with E6-6, patient serum, control serum, or secondary antibody alone. Western blots were performed using lysed RPE and stained with E6-6, patient serum, control serum, or secondary antibody alone.

Results: Immunofluorescence staining of HEK-293 cells or HEK-293 cells expressing Best1 did not differ between patient and control sera or show a staining pattern consistent with recognition of Best1. Immunoblotting of human RPE lysate with patient serum did not identify Best1 (68 kDa) but did recognize a band at approximately 48 kDa that was absent in blots using control serum.

Conclusions: To our knowledge, this is the first report of PEPVM with an autoantibody to an approximately 48-kDa RPE protein, but previous reports have demonstrated autoantibodies to other RPE proteins, suggesting that autoantibody formation is an important component of PEPVM.

Translational Relevance: This research emphasizes the role that autoantibodies play in PEPVM. The fact that different autoantibodies appear to cause similar patterns demonstrates the heterogeneity of causes of vitelliform lesions.

Introduction

Paraneoplastic exudative polymorphous vitelliform maculopathy (PEPVM) is a rare retinal disorder, originally described by Gass et al.\(^1\), that is characterized by multifocal yellow fundus deposits clinically resembling the lesions found in Best disease.\(^2\) Originally described in young males, PEPVM has recently been described in older patients and in females.\(^1,3\) Patients present with blurred vision, nyctalopia, and photopsias, sometimes following an upper respiratory infection, headache, or flu-like symptoms.\(^2,5\) Funduscopy typically reveals multiple yellow, orange, or white lesions at the level of the retinal pigment epithelium (RPE) in the posterior pole, which correspond to shallow serous retinal detachments on optical coherence tomography (OCT).\(^1,2,5,6,7\) Many patients already carry a diagnosis of malignancy at the time of presentation, with cutaneous and choroidal melanoma being the most common types; however, there has been a report of PEPVM preceding melanoma diagnosis as well as reports of PEPVM in association with carcinoma.\(^3,6,8-12\) Moreover, acute exudative polymorphous vitelliform maculopathy (AEPVM), which is clinically indistinguishable from PEPVM, has been described in association with trauma, acute viral illness, syphilis, and Lyme disease.\(^5,12-16\)

A previous study has demonstrated antibodies to
Bestrophin-1 (Best1) in a patient with PEPVM, and multiple other reports have demonstrated the presence of antibodies to various proteins in the RPE.\textsuperscript{3,5,8,11,17,18} Therefore, we hypothesized that Best1 autoantibody formation plays a role in the development of PEPVM. Specifically, we present a case of PEPVM associated with multiple myeloma with kappa light chain deposition disease and analyze the patient serum to determine whether the patient has autoantibodies to human Best1 or other autoantibodies to the RPE.

Case Report

A 58-year-old female presented to Mayo Clinic for a second opinion regarding a 7-year history of immunoglobulin G (IgG) monoclonal gammopathy of undetermined significance with no quantifiable M-spike and recent worsening of her chronic renal failure. Three years prior to presentation, she had undergone a reportedly negative bone marrow biopsy with 7% plasma cells and a bone survey that revealed no lytic lesions.

The patient underwent an extensive workup at Mayo. Repeat renal biopsy showed acute tubular necrosis and light chain deposition disease. A bone marrow biopsy was performed and revealed 50% plasma cells. Fluorescence in situ hybridization (FISH) showed 13q and translocation t(11;14). Positron emission tomography (PET) revealed a leukaemia lesion of the left acetabulum that was fluorodeoxyglucose avid. The patient was diagnosed with multiple myeloma with kappa light chain deposition disease, Salmon-Durie stage IIB, ISS stage III, and she was started on Velcade and dexamethasone for treatment. She underwent five cycles of treatment followed by an autologous peripheral blood stem cell transplant. One year after her transplant, her kappa-free light chain rose, and she was restarted on Velcade and dexamethasone weekly for 7 months. She was off treatment for 5 months, but was then restarted again for 8 months. Another 5 months later, she was seen again at Mayo. Her kappa light chain levels were stable at that visit, but she now complained of blurred vision, approximately 3 years after her multiple myeloma diagnosis.

The patient had been seen by an outside ophthalmologist who noted abnormalities on funduscopy and recommended referral to a retina specialist. The outside retina specialist noted visual acuity of 20/20 OU and full fields to confrontation. On exam, he noted multiple ¼- to ½-disc diameter discrete yellow subretinal and sub-RPE deposits surrounding the macula in both eyes as well as evidence of non-proliferative diabetic retinopathy and hypertensive retinopathy. Antiretinal autoantibody testing revealed a positive Western blot for antiretinal antibodies against 35-, 42-, and 48-kDa proteins.

Approximately 1 year after the onset of visual symptoms, the patient was evaluated in the Mayo Ophthalmology Department. Visual acuity was 20/20 oculus dexter (OD) and 20/25 oculus sinster (OS). Funduscopy of the right eye revealed a 500 × 500 μm discrete, slightly elevated yellowish-orange subretinal round deposit 3-mm superotemporal to the fovea with surrounding ovoid subretinal yellow-orange deposits. There was a soft cotton wool spot 2.5-mm inferotemporal to fovea along the inferotemporal arcade. Approximately 1-mm superior to the disc, there were more discrete round and ovoid subretinal yellow-orange deposits, with hemorrhage overlying one of the deposits. Finally, there was one ovoid subretinal lesion 3-mm nasal to the disc. There were a few dot-blot hemorrhages but no lesions outside of posterior pole (Fig. 1). Funduscopy of the left eye demonstrated scattered small subretinal yellow-orange discrete deposits temporal to the fovea, discrete grayish lesions with slightly hyperpigmented boarders superior to the disc, the largest of which was 250 × 250 um, and more yellowish-orange ovoid and irregularly shaped lesions along the superotemporal arcade (Fig. 1).

The patient had OCT examinations with the Spectralis Spectral-Domain OCT machine (Heidelberg Engineering Inc., Carlsbad, CA). OCT showed hyperreflective homogenous sub-RPE and subretinal deposits with normal macula and no subretinal fluid in both eyes (Fig. 2). Fluorescein angiography (FA) revealed early hyperfluorescence and late hypo-fluorescence with late leakage in the right eye (Fig. 3). FA showed early hyperfluorescence and late hypofluorescence with no leakage in the left eye. Indocyanine green (ICG) showed early and late hypofluorescence of the lesions in both eyes (Fig. 3). The deposits were hyperfluorescent on autofluorescence imaging.

The patient underwent an electrooculogram (EOG) that revealed significantly reduced Arden ratios of 1.3 and 1.2 in the right and left eyes, respectively. Outside testing showed that the patient’s serum tested negative for antioptic nerve antibodies with negative antiretinal antibodies on immunohistochemical testing (IHC), which is less sensitive than the previously positive Western blot. She also had an unremarkable paraneoplastic panel, including a negative CRMP-5. Genetic testing was negative for mutations in the genes...
BEST1, peripherin 2 (PRPH2), interphotoreceptor matrix proteoglycan 1 (IMPG1), interphotoreceptor matrix proteoglycan 2 (IMPG2), orthodenticle homeobox 2 (OTX2), ATP-binding cassette, sub-family A (ABC1), member 4 (Abca4), TIMP metallopetidase inhibitor 3 (TIMP3), C1q and tumor necrosis factor related protein 5 (C1QTNF5), and multidrug-resistance associated protein (MRP).

The patient agreed to provide a blood sample for research purposes, which was used to investigate whether autoantibodies to Best1 were present. The patient is currently being monitored at her home facility with serial visual fields every 4 months. She is not receiving any active treatment for multiple myeloma at the time of this report.

**Methods**

This research adheres to the tenets of the Declaration of Helsinki. Informed consent was obtained from the subject after explanation of the nature, risks, and benefits of the study. Patient serum was collected and used as described.

**Cell Culture and Transfections**

Human embryonic kidney 293 cells (HEK293, American Type Cell Culture Collection) were maintained in a 95% air 5%/CO₂ environment at 37°C. Cells were grown in Dulbecco’s Modified Eagle’s Medium (Cellgro) supplemented with 10% fetal bovine serum (Cellgro) and 1% penicillin/streptomycin solution (Cellgro). For transfections, HEK293 cells were grown on 22-mm glass coverslips and transfected with pAdlox-Best1 using Lipofectamine 2000 (Invitrogen) as before.19,20

**Immunofluorescence**

Immunofluorescence was performed as before.20,21 Forty-eight hours after transfection, HEK293 cells on coverslips were washed with phosphate-buffered saline containing 0.13 mM CaCl₂ and 1.0 MgCl₂ (PBS-CM). Coverslips were then immersed in ice-cold methanol for 10 minutes on ice, and then washed again using PBS-CM. Cells were blocked in PBS-CM containing 3% bovine serum albumin for 30 minutes. Cells were then incubated with patient serum, control serum from a patient with no ocular disease, anti-Best1 monoclonal antibody E6-6, or secondary antibody only for 2 hours.19,20 Following the incubation, cells were washed with PBS-CM, and then stained with Alexa fluor 488 labelled goat anti-mouse IgG or goat anti-human IgG (1:1000; Life technologies) for 1 hour. Nuclei were then stained with 4',6-diamidino-2-phenylindole (DAPI; 1:5000; Life Technologies), and coverslips were mounted on microscope slides using Fluoromount G (Electron Microscopy Sciences). Images were obtained using a 4×40 oil immersion objective on a Nikon C2 confocal microscope.

**Western Blotting**

Western blotting was performed as previously described.20,21 Briefly, RPE cells isolated from human donor eyes were lysed in 150 µL of 1% Triton X-100, 20 mM Tris–HCl, pH 8.0, 150 mM NaCl, 5 mM EDTA containing 1% protease inhibitor cocktail (Millipore) and diluted in 4× sample.
buffer. Samples were resolved on 10% Mini-PRO-TEAN TGX gels (Bio-Rad) and transferred to PVDF membranes (GE Healthcare) overnight. Membranes were blocked in PBS-CM containing 1% milk and 0.1% TWEEN-20 and incubated for 2 hours with no primary antibody, E6-6, PEPVM patient serum, or control serum. Following several washes in PBS-CM, blots were incubated with alkaline phosphatase conjugated Goat anti-mouse IgG or Goat anti-human IgG (Rockland). Blots were developed using the substrates nitro-blue tetrazolium chloride and 5-bromo-4-chloro-3'-indolyphosphate P-toluidine salt (Promega).

Results

Immunofluorescence

In transfected HEK293 cells expressing Best1, E6-6 demonstrated specific recognition of Best1 protein (Fig. 4A). In untransfected cells not expressing Best1, secondary antibody only and E6-6 demonstrated no staining (Fig. 4B). Patient serum exhibited extensive nonspecific staining (Fig. 4A) similar to that observed in untransfected cells stained with serum from either the case patient or a control patient with no ocular pathology (Fig. 4B), indicating that both sera recognized antigens in HEK293 cells. Staining with patient or control serum resulted in an identical staining pattern in both transfected and untransfected
cells, indicating a lack of Best1-specific autoantibodies in the patient serum.

Western Blotting

Western blotting using antibody E6-6 of RPE lysates obtained from human donor eyes revealed a band corresponding to Best1 at approximately 68 kDa (Fig. 4C). Neither case patient nor control sera recognized a 68 kDa corresponding to Best1. However, patient serum, control serum, and secondary antibody alone each used anti-human IgG as a secondary and, therefore, recognized a 50-kDa band representing endogenous heavy-chain IgG present in the RPE lysate. While patient serum failed to detect Best1, it did detect an additional band of approxi-
mately 48 kDa that was not recognized by the control serum (Fig. 4C).

**Discussion**

PEPVM is a rare retinal disorder that clinically resembles multifocal Best disease with multifocal yellow-orange deposits in the posterior pole and serous retinal detachments on OCT.\(^1\)\(^-\)\(^3\)\(^,\)\(^6\)\(^-\)\(^7\) PEPVM has traditionally been viewed as a paraneoplastic syndrome with numerous reports, describing the condition in association with melanoma.\(^2\)\(^,\)\(^3\)\(^,\)\(^5\)\(^-\)\(^8\)\(^,\)\(^11\)\(^-\)\(^17\)\(^,\)\(^22\)\(^-\)\(^25\) It has also been described in association with carcinoma.\(^3\)\(^,\)\(^6\) However, the clinically indistinguishable entity, AEPVM, has been reported in association with trauma and various infectious agents, including hepatitis C, Coxsackie B virus, syphilis, and Lyme disease.\(^2\)\(^,\)\(^12\)\(^-\)\(^14\)\(^,\)\(^15\)\(^,\)\(^16\) Therefore, it has been suggested that infectious agents may precipitate autoantibody formation in patients without any neoplasm. To date, PEPVM has been associated with autoantibodies to recoverin at 23 kDa, peroxiredoxin 3 at 26 kDa, carbonic anhydrase 2 at 29 kDa, BEST1 at 68 kDa, a photoreceptor protein at 120 kDa, and interphotoreceptor retinoid-binding protein at 145 kDa.\(^3\)\(^,\)\(^5\)\(^,\)\(^8\)\(^,\)\(^11\)\(^,\)\(^17\)\(^,\)\(^18\) In this report, we presented a case of PEPVM associated with multiple myeloma with kappa light chain deposition disease.

Similar to previously reported cases, the patient described in this report presented with blurred vision.\(^2\)\(^-\)\(^5\) To our knowledge, there was no viral prodrome to the symptoms, and she had no history of melanoma or carcinoma. Fundus exam demonstrated multifocal hyperreflective yellow-orange deposits, consistent with the appearance of PEPVM. However, OCT demonstrated a distinct lack of subretinal fluid, which was commonly reported in many previous cases of PEPVM in the literature.\(^2\)\(^,\)\(^3\)\(^,\)\(^6\)\(^,\)\(^16\) OCT did, however, demonstrate the characteristic multiple hyperreflective sub-RPE deposits in both eyes. This difference could potentially demonstrate the capture of a different stage of the disease process, especially given the fact that the patient in this report had very good visual acuity compared with some of the previously reported cases that demonstrated subretinal fluid.

Of interest, this patient had an EOG demonstrating a markedly reduced Arden ratio of less than 1.3 in both eyes, which has been previously reported in association with PEPVM, including the case in the literature with reportedly positive antibodies to bestrophin.\(^5\)\(^,\)\(^17\) The patient tested negative for antiretinal antibodies on IHC but positive for antiretinal antibodies against 35-, 42-, and 48-kDa proteins on Western blotting. She had an unremarkable paraneoplastic panel, including a negative CRMP-5. These test results and the clinical similarity to Best disease prompted further analysis of the patient’s serum in the laboratory.

Patient serum for this case did not demonstrate autoantibodies to Best1. With IHC, there were no detectable differences between patient serum and control serum staining of untransfected cells, nor were there differences between patient serum staining of transfected or untransfected cells. Western blotting further confirmed the lack of Best1 antibodies by the distinct lack of a band near 68 kDa, while E6-6 both specifically stained transfected cells for Best1 and demonstrated the expected 68-kDa band on Western blot. Patient serum did, however, demonstrate a unique band at approximately 48 kDa that was not present when the Western blot was run with control serum. This is consistent with the positive send-out testing for antiretinal antibodies that revealed an antibody against a 48-kDa protein.

A previous report of PEPVM in the literature reported the presence of autoantibodies against Best1.\(^,\)\(^17\) Interestingly, the Western blotting reported in that study demonstrated a band of significantly less than 67 kDa in both the patient serum and antiBest1 lanes, while Best1 has a molecular mass of 68 kDa; therefore, further studies have to be done to confirm their findings.\(^19\) Our testing demonstrated the detection of Best1 at approximately 68 kDa by E6-6 as well as a lack of this antibody in the case patient. Our patient also demonstrated a lack of other previously-reported antibodies demonstrated in association with PEPVM, including antibodies against proteins sized 23, 26, 29, 120, or 145.\(^3\)\(^,\)\(^5\)\(^,\)\(^8\)\(^,\)\(^11\)\(^,\)\(^17\)\(^,\)\(^18\) To our knowledge, this is the first report of PEPVM in association with an autoantibody to a protein at approximately 48 kDa.

This case also appears to be the first report to have specifically named PEPVM to be associated with multiple myeloma with kappa light chain deposition disease. However, a previous report described a case of light chain deposition disease with vitelliform macular detachments that could also be described as PEPVM.\(^26\) This previous report, in contrast to our case, described a patient presenting with ocular findings prior to myeloma diagnosis. Following bone marrow transplant, the vitelliform detachments re-
solved; whereas in our case, the eye findings were not discovered until after the patient had already received a peripheral blood stem cell transplant. Another contrasting feature between this case and ours is that our patient had a reduced Arden ratio, while the EOG was normal in the previously published report. However, it seems that an EOG was not performed prior to bone marrow transplant, making it possible that the EOG was reduced prior to resolution of the vitelliform detachments. In cases of AEPVM, the EOG has been shown to improve with resolution of the disease, and it has even been suggested that EOG could be useful in monitoring disease progress. It the disease, and it has even been suggested that EOG has been shown to improve with resolution of vitelliform detachments. In cases of AEPVM, the EOG was reduced prior to resolution of the disease, and it has even been suggested that EOG could be useful in monitoring disease progress. It would be interesting to see if the EOG significantly changes over time in our case.

While the underlying pathophysiology of PEPVM still remains poorly understood, it seems that autoantibody formation is a consistent component. Interestingly, there is not yet consistency in the literature with what specific antibody or group of antibodies this might be. However, it seems likely that various antibodies against components of the RPE contribute to this clinical picture. Future studies are needed to better elucidate the underlying pathophysiological mechanisms of PEPVM and further the understanding of this disease.

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