Antigen-specificity of antiretinal antibodies in patients with noninfectious uveitis

Ebrima Gibbs, PhD,* Joanne Matsubara, PhD,† Sijia Cao, MD,† Jing Cui, MD,† Farzin Forooghian, MD, MSc, FRCSC†

ABSTRACT ●

Objective: Antiretinal antibodies (ARAs) have previously been described in noninfectious uveitis. However, the antigen specificity of these ARAs has not been investigated. The purpose of this study was to identify antigen-specific ARAs in noninfectious uveitis.

Methods: A total of 18 patients with noninfectious uveitis were enrolled. Surface plasmon resonance was used to measure binding responses of patient and control sera against several uveitogenic proteins: recoverin, S-antigen, interphotoreceptor retinoid binding (IRBP), retinal-pigment-epithelium-specific 65-kDa protein (RPE65), tyrosinase-related protein 1 (TRYP1), and tyrosinase-related protein 2 (TRYP2).

Results: The frequency of ARA positivity against S-antigen, IRBP, RPE65, TYRP1, and TYRP2 in patients with uveitis did not differ significantly from that of normal controls. However, ARA positivity for recoverin was more frequently observed in patients with uveitis ($p = 0.002$). A total of 10 patients in the uveitis cohort had birdshot chorioretinopathy, and all 10 were positive for anti-recoverin ARAs.

Conclusions: Patients with noninfectious uveitis have increased frequency of ARA positivity against recoverin. This ARA deserves further investigations as a potential biomarker and pathogenic agent in noninfectious uveitis, especially in birdshot chorioretinopathy.

Background

Uveitis is a heterogeneous group of inflammatory diseases involving the uvea and the retina. In the animal model of uveitis, experimental autoimmune uveitis, cellular immunity has been shown to play a major pathogenic role, while humoral immunity is believed to play little or no pathogenic role.[1] Despite this, there has been a significant amount of interest in measurement of antiretinal antibodies (ARAs) in patients with uveitis. Previous studies have reported elevated levels of ARAs in patients with noninfectious uveitis.[2–6] These studies either did not identify the antigen-specificity of these ARAs[2,3,6] or limited their investigation to S-antigen (arrestin).[4,5] Previous investigations in infectious uveitis have investigated S-antigen and interphotoreceptor retinoid binding (IRBP).[7,8] ARAs are known to occur in healthy individuals, and the pathogenicity of many ARAs has not been determined.[9–11] Hence, it is difficult to know which ARAs are a normal finding and which are potentially pathogenic. Knowledge of the antigen-specificity of ARAs in uveitis could be useful in determining the clinical and pathologic significance of these autoantibodies. ARAs targeting antigens that are known to be uveitogenic could be of more clinical relevance in the setting of uveitis. In addition to S-antigen and IRBP, proteins that have been shown to be uveitogenic in experimental autoimmune uveitis include recoverin,[12,13] retinal-pigment-epithelium-specific 65-kDa protein (RPE65),[14,15] tyrosinase-related protein 1 (TRYP1),[16] and tyrosinase-related protein 2 (TRYP2).[16]

Laboratory techniques for measurement of ARAs have included enzyme-linked immunosorbent assay (ELISA) and Western blot (WB).[9–11,17] Surface plasmon resonance (SPR) is a novel technique for measurement of antigen-antibody interaction that has some advantages over ELISA and WB, allowing for real-time kinetic and label-free analysis with high sensitivity.[18] The label-free approach means that a secondary antibody is not required for the assay, thereby eliminating the concern over false-positive ARA results that have been raised with respect to ELISA and WB assays.[11,19] To our knowledge, SPR for the measurement of ARAs has not previously been described. The purpose of this study was to use SPR to measure antigen-specific ARAs in patients with noninfectious uveitis.

Methods

Consecutive patients (nonrandomly selected) with noninfectious uveitis were asked to participate in this prospective study. Serum samples were collected for ARA analysis. Patient characteristics were obtained from review of clinical charts. Uveitis activity was graded on the basis of the Standardization of Uveitis Nomenclature (SUN) criteria.[20] For controls, we used commercially purchased pooled normal human serum (Complement Technology Inc., Tyler, Tex.), which is derived from a minimum of 13 healthy donors. In addition, we used serum samples from 5 unrelated age- and gender-matched healthy donors.

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The Research Ethics Board at the University of British Columbia approved this study.

All experiments were carried out by using a Molecular Affinity Screening System (MASS-1) (Sierra Sensors GmbH, Hamburg, Germany). MASS-1 is a SPR imaging analytical biosensor that employs high-intensity laser light and high-speed optical scanning to monitor binding interactions in real time. Ligand is immobilized onto a sensor surface, over which analyte is injected and binding monitored by SPR. Binding of molecules to the surface causes a change in the refractive index, resulting in a shift in the SPR angle, which is monitored via a sensorgram, a trace of binding versus time.

Pure recombinant human recoverin, S-antigen, IRBP, RPE65, TRYP1, and TRYP2 were commercially obtained (OriGene Technologies Inc; Rockville, Md.). Under conditions of continuous flow of HBS-EP running buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% v/v P20; GE Healthcare, Little Chalfont, UK), the recombinant proteins were covalently immobilized on 6 separate flow cells of a High Amine Capacity sensor chip (Sierra Sensors GmbH, Hamburg, Germany), using standard amine-coupling chemistry. Briefly, a High Amine Capacity sensor chip was activated with equimolar amounts of [1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide] (EDC) and N hydroxysuccinimide (NHS). Recombinant proteins were diluted in 10 mM sodium acetate pH 4.5 and injected to covalently couple them by amide bonding to the activated surface of the flow cells, and unreacted sites were blocked with 1 M ethanolamine-HCl pH 8.5. Six adjacent flow cells were similarly immobilized with bovine serum albumin (BSA) as a reference control surface to account for nonspecific binding.

The individual surface activities of the immobilized recombinant proteins were further tested and verified by injecting the cognate antibodies (OriGene Technologies Inc, Rockville,Md.): mouse monoclonal anti-recoverin antibody, mouse monoclonal anti-S-antigen antibody, mouse monoclonal anti-IRBP antibody, mouse monoclonal anti- RPE65 antibody, rabbit polyclonal anti-TYRP1 antibody, and rabbit polyclonal anti-TRYP2 antibody. After this, patient and control sera were analyzed. Assay optimization was performed by testing serum at various dilutions to minimize background. For the final experiments, each serum sample was diluted 10-fold in running buffer and sequentially injected over all the immobilized recombinant proteins and BSA reference surfaces at a flow rate of 12.5 μL/min for a 4-minute association phase, followed by injection of running buffer only for a 3-minute dissociation phase. After every analytical cycle, a pulse of surface elution buffer regenerated the sensor chip surfaces. Sensorgrams were double-referenced by subtracting out binding from the BSA reference surfaces and blank running buffer injections, and binding response report points were measured at 20 seconds before the end of the dissociation in a 20 second window of data. The binding response was expressed as response units (RUs). The assay positivity cut-off was taken as mean + 3 SD of the normal human sera.

Because of the pilot nature of this study, a formal sample size calculation was not possible. A sample size of 18 was used based on study feasibility. Comparison of proportion of response positivity between patients and controls was performed using Fisher's exact test. For statistical significance, a p value of 0.008 was used (Bonferroni correction). All statistical analysis and graphing were performed by using Prism version 6.0 (GraphPad Software, La Jolla, Calif.).

Results

A total of 18 patients with noninfectious uveitis were recruited for this study. A summary of patient characteristics and type of ARA positivity is provided in Table 1. All patients were diagnosed within 1 to 5 years, and all had

Table 1—Patient characteristics and antiretinal antibody positivity

Patient	Age, Gender	Diagnosis	Treatment (Past/Current)	Antiretinal antibody
1	59, F	AZOOR*	None	None
2	57, M	Birdshot chorioretinopathy	Prednisone	Recoverin
3	63, F	Birdshot chorioretinopathy	None	Recoverin
4	73, F	Autoimmune retinopathy	None	Recoverin, IRBP
5	63, F	Birdshot chorioretinopathy	None	Recoverin, S-antigen, IRBP, RPE65, TYRP1
6	49, M	Birdshot chorioretinopathy	None	Recoverin, IRBP, RPE65
7	63, F	Birdshot chorioretinopathy	Mycophenolate mofetil	Recoverin
8	39, F	Punctate inner choroidopathy	Prednisone	RPE65
9	39, F	Vogt-Koyanagi-Harada disease	Prednisone	Recoverin
10	45, M	Sympathetic ophthalmia	Prednisone	Recoverin
11	55, M	Birdshot chorioretinopathy	Cyclosporine	Recoverin
12	55, F	Birdshot chorioretinopathy	Mycophenolate mofetil	Recoverin
13	52, F	Birdshot chorioretinopathy	Prednisone	Recoverin, TYRP1
14	55, F	Cancer-associated retinopathy	Rituximab	Recoverin, IRBP, RPE65
15	59, M	Multifocal choroiditis	Prednisone	S-antigen, RPE65, TYRP1
16	35, M	Birdshot chorioretinopathy	None	Recoverin
17	62, F	Birdshot chorioretinopathy	None	Recoverin
18	38, F	IU† (Sarcoidosis)	None	IRBP, RPE65

AZOOR, Acute zonal occult outer retinopathy; IU, intermediate uveitis; RPE65, retinal pigment epithelium-specific 65 kDa protein; IRBP, interphotoreceptor retinoid binding; TYRP1, tyrosinase-related protein 1; TYRP2, tyrosinase-related protein 2.