Cross-reactivity of antibodies against leptospiral recurrent uveitis-associated proteins A and B (LruA and LruB) with eye proteins

Ashutosh Verma  
*University of Kentucky*

Pawan Kumar  
*University of Kentucky*

Kelly Babb  
*University of Kentucky*

John F. Timoney  
*University of Kentucky*, jtimoney@uky.edu

Brian Stevenson  
*University of Kentucky*, brian.stevenson@uky.edu

Follow this and additional works at: [https://uknowledge.uky.edu/microbio_facpub](https://uknowledge.uky.edu/microbio_facpub)

Part of the Medical Immunology Commons, Medical Microbiology Commons, and the Molecular Genetics Commons

Right click to open a feedback form in a new tab to let us know how this document benefits you.

**Repository Citation**

Verma, Ashutosh; Kumar, Pawan; Babb, Kelly; Timoney, John F.; and Stevenson, Brian, "Cross-reactivity of antibodies against leptospiral recurrent uveitis-associated proteins A and B (LruA and LruB) with eye proteins" (2010). *Microbiology, Immunology, and Molecular Genetics Faculty Publications*. 10. [https://uknowledge.uky.edu/microbio_facpub/10](https://uknowledge.uky.edu/microbio_facpub/10)

This Article is brought to you for free and open access by the Microbiology, Immunology, and Molecular Genetics at UKnowledge. It has been accepted for inclusion in Microbiology, Immunology, and Molecular Genetics Faculty Publications by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.
Cross-reactivity of antibodies against leptospiral recurrent uveitis-associated proteins A and B (LruA and LruB) with eye proteins

Digital Object Identifier (DOI)
http://dx.doi.org/10.1371/journal.pntd.0000778

Notes/Citation Information
Published in PLoS Neglected Tropical Diseases, v. 4, no. 8, e778.

© 2010 Verma et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Cross-Reactivity of Antibodies against Leptospiral Recurrent Uveitis-Associated Proteins A and B (LruA and LruB) with Eye Proteins

Ashutosh Verma, Pawan Kumar, Kelly Babb, John F. Timoney, Brian Stevenson

Introduction

Infectious disease caused by spirochetes of the genus *Leptospira* is a veterinary and public health problem of global proportions [1,2]. Humans and other mammals are exposed to the organism when they contact groundwater contaminated with urine from carrier animals. The disease in humans varies from a mild flu-like form to a more severe syndrome involving multiorgan failure and death [3]. Uveitis is a common complication of systemic infection in humans affecting one or both eyes [4]. In equines, infection is mainly associated with spontaneous abortion in mares and recurrent uveitis [3]. After an initial infection, some horses develop a recurrent inflammation of the uveal tract of eye (iris, ciliary body and choroid), known as equine recurrent uveitis (ERU) or ‘moon blindness’. First described in 1819 by James Wardrop as a “specific inflammation” of uveal origin, it is the most common cause of blindness in horses worldwide [5,6] with a prevalence of approximately 8–10% in the United States [7]. Onset of the disease is usually acute with variable degrees of severity and duration. The acute phase is followed by a quiescent phase of no or low inflammation [8]. Subsequent recurrence of inflammation results in pronounced lesions with guarded prognosis for preservation of visual acuity [8,9,10,11]. The Appaloosa breed and horses with MHC class I haplotype ELA-A9 have been observed to be at increased risk of developing uveitis [12,13].

*Leptospira interrogans* serovar Pomona is the most common and well-documented infectious cause of ERU in the United States [14]. Its association with pathogenic leptospires has been well established by presence of high titers of leptospiral agglutinins in the blood and aqueous humor [15,16], by isolation of *Leptospira* from ocular fluids [17,18] and the detection of leptospiral DNA by polymerase chain reaction in vitreous humor of uveitic horses [17]. Initial evidence of the association was provided by Morter et al. [19] when they induced uveitis in ponies by subcutaneous injection of guinea pig blood containing live *L. interrogans* serovar Pomona. The resulting ocular pathology in experimental ponies was found to be similar to that of spontaneous cases of *Leptospira*-associated ERU.

By using ERU uveitic fluids to screen a lambda phage library of *L. interrogans*, we identified leptospiral lipoproteins, LruA and LruB,
Author Summary

*Leptospira* is the most common infectious cause of uveitis, a potentially debilitating inflammation of the eye. In our earlier work, we discovered that eye fluids of uveitic horses contain high levels of antibodies directed against novel leptospiral proteins, which we named LruA and LruB (*Leptospiral recurrent uveitis* associated proteins A and B). Significantly, antibodies raised against LruA and LruB also recognize lens and retinal tissue. We have now identified the cross-reactive eye proteins as alpha-crystallin B, vimentin and beta-crystallin B2. We also demonstrated that ocular fluids from uveitic horses contain high levels of antibodies recognizing alpha-crystallin B, vimentin and beta-crystallin B2. These data suggest that antibodies directed against leptospiral LruA and LruB during infection can also react with eye proteins, alpha-crystallin B, vimentin and beta-crystallin B2, potentially contributing to the severity of this eye disease.

Associated with recurrent uveitis in horses [20]. Uveitic equine eye fluids contained significantly higher levels of immunoglobulin A (IgA) and IgG specific for LruA and LruB than did companion sera, indicating strong local antibody responses. Moreover, monospecific antiserum to LruA and LruB reacted with extracts of equine ocular tissue. In the present study we have examined the reactivity of LruA- and LruB-antiserum with sections of lens and retinal tissue and identified the ocular proteins involved in the interaction. In addition, the significance of the identified autoantigens was assessed by measuring their immuno-reactivities in eye fluids of uveitic and healthy animals.

Materials and Methods

Ethics statement

All animals were handled in strict accordance with relevant national and international guidelines, and all animal work was approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC # 2009-0477).

Eye fluids and eye tissue extracts

Eye fluids and companion sera from horses of varied age, breed, and origin were obtained from a commercial horse slaughter plant in North America. Eyes with gross evidence of uveitis were enucleated after slaughter, and aqueous humor was removed with a 10-ml syringe and stored at −70°C; and later embedded in tissue freezing medium containing 3-aminopropytriethoxysilane in acetone. The sections were fixed in acetone at 20°C for 20 min followed by two washes with phosphate buffered saline (PBS, pH 7.4) for 5 min each. Blocking was performed using 2% bovine serum albumin (BSA; Sigma, St. Louis, MO) in PBS for 30 min. Sections were again washed thrice with PBS and incubated with 1:100 polyclonal rabbit antisera or pre-immune serum overnight at 4°C in a humidifying chamber. Sections were washed three times for 5 min each and subsequently incubated with 1:250 dilution of FITC conjugated goat anti-rabbit IgG (Invitrogen, Carlsbad, CA) for 1 h at room temperature in a humidifying chamber. Slides were mounted in a mounting medium containing anti-fading reagent Mowiol (EMD Chemicals, Gibbstown, NJ) and screened by epifluorescence microscopy (Axioskop-20; Zeiss, Thornwood, NY, USA) and image analysis was carried out using the QUIPS-XL and QUIPS-AKS system (Vysis, Downer’s Grove, IL, USA). The same IFA protocol as above was used for testing lens tissues obtained from two healthy sheep.

Two dimensional polyacrylamide gel electrophoresis

Lenticular and retinal aqueous extracts were separated by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) using MultiPhor-II system (GE Healthcare, Piscataway, NJ). Briefly, the aqueous extracts were subjected to isoelectric focusing using precast IPG strips (Bio-Rad, Hercules, CA) for 3000 V-h (500 V, 6 h, 10°C). Strips were then equilibrated and subjected to conventional sodium dodecylsulfate-12.5% polyacrylamide gel electrophoresis (SDS-PAGE). Gels were either stained with SYPRO Ruby (Invitrogen) or transferred to nitrocellulose membranes for immunoblot analysis with LruA- or LruB-directed antisera. Immunoblot positive protein spots were extracted from gels and analyzed by matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrometry (University of Louisville Mass Spectrometry Core Laboratory, Louisville, KY). Spectrometry outputs were compared with known sequences using Mascot (Matrix Science, Boston, MA).

Eye protein antibody assays

Recombinant human α-crystallin B (Abcam, Cambridge, MA), purified vimentin from bovine lens (Sigma) or human recombinant β-crystallin B2 (Abnova) were separated by SDS-PAGE, transferred to a nitrocellulose membrane and blocked with 5% nonfat dry milk in Tris-buffered saline (20 mM Tris, 150 mM NaCl, 0.05% Tween 20, pH 7.5). Membranes were incubated with LruA or LruB-antisera (1:400) followed by incubation with protein G conjugated to horseradish peroxidase (Zymed, San Francisco, CA). Membranes were developed with the SuperSignal West Pico enhanced chemiluminescence substrate (Pierce), and bands were visualized with BioMax Light film Kodak.

ELISA measured α-crystallin B, vimentin and β-crystallin B2 antibody levels in leptospiral uveitic and normal eye fluids as described previously [20,22]. Briefly, ELISAs were performed in Maxisorp 96-well plate wells (Nalge-Nunc, Rochester, NY) coated with 200 ng human recombinant alpha-crystallin B (Abcam),
purified vimentin from bovine lens (Sigma) or human recombinant β-crystallin B2 (Abnova) followed by blocking with 5% nonfat dry milk. Uveitic and normal eye fluids (1:100) were added and incubated for 1 h at 37°C. Bound antibodies were detected using HRP conjugated Protein G (1:4000; Zymed, San Francisco, CA). Plates were developed using ready-to-use 3,3',5,5'-tetramethyl benzidine substrate solution (1-Step Turbo TMB-ELISA, Thermo Scientific, Rockford, IL). Reactions were stopped by addition of 2N H2SO4, 50 µl/well. Absorbance was read at 450 nm in a Spectramax plate reader using SoftMax Pro (Molecular Devices, Sunnyvale, CA). Statistical analyses were performed using Student’s t-test assuming unequal variances.

Results

Cross-reactivity of LruA and LruB with equine lens and retina, respectively

In a previous study [20] LruA-antiserum was shown to recognize a ~22 kDa protein in lens extract and a ~65 kDa protein in ciliary body extract. In the same work, LruB-antiserum reacted with a ~30 kDa band in retinal extract. To further examine the observed cross-reactivity between equine ocular tissue and LruA and LruB specific antisera, immunofluorescent assays were performed. Frozen lenticular and retinal tissue sections (8–10 µ) were fixed, blocked and incubated with antiserum or preserum to LruA and LruB (diluted 1:100). The lens fibers showed uniform homogenous pattern of fluorescence when incubated with LruA-specific antiserum but not with normal rabbit serum [Figure 1A and B]. Similarly, fluorescence was observed when equine retinal tissue sections were incubated with LruB-specific antiserum but not with normal rabbit serum [Figure 1C and D]. The positive fluorescence seen in retinal tissue incubated with LruB-antiserum was restricted to one or more deeper retinal layers, which include the inner limiting membrane, layer of nerve fiber and may be the ganglion cell layer, in contrast to a diffused positive fluorescence seen in lens tissue sections incubated with LruA-antiserum. In addition, sclera and choroid were devoid of any fluorescence in the same tissue section [Figure 1].

Similar results were obtained with lenticular tissues from a healthy sheep. Sections of sheep lens incubated with LruA-antiserum showed positive fluorescence but not when these sections were incubated with pre-immune serum (not shown). The reactivities of equine and sheep lenticular tissue sections with LruA-antiserum indicated that the observed interaction is not unique to equines lens.

Identification of lenticular and retinal proteins cross-reactive with LruA and LruB

To identify the eye protein(s) recognized by LruA-directed antibodies, proteins in equine lenticular extract were separated on two-dimensional polyacrylamide gels, transferred to nitrocellulose membranes and probed with LruA-specific antiserum [Figure 2A and B]. LruA-antiserum recognized protein spots with apparent

Figure 1. LruA and LruB-antiserum reacts with lenticular and retinal tissues. (A) Photomicrographs showing uniform homogenous fluorescence in a section of equine lens incubated with LruA-antiserum (1:100) but not with preserum (B) (×100). (C) Photomicrographs showing a positive fluorescence in a frozen section of equine retina incubated with LruB-antiserum (1:100) but not with pre-immunization serum (D) (×100). Inset (×400). doi:10.1371/journal.pntd.0000778.g001
molecular masses of approximately 20 and 60 kDa. The immune
blot was aligned with the stained gel, to locate the corresponding
protein spots which were then subjected to mass spectrometric
analysis. The 20 and 60 kDa protein spots were identified as α-
crystallin B and vimentin, respectively (Table 1) with 66% and
44% coverage (not shown). Alpha-crystallin B and vimentin have
molecular masses of 20188 Daltons and 53727 Daltons, respective-
ly. Attempts to identify ciliary body protein(s) reactive to LruA-
specific antiserum by this method have not yet been successful.

Similarly, LruB-antiserum recognized three spots of retinal
proteins (Figure 2C and D), which were identified by mass
spectrometry to be β-crystallin B2 (Table 1).

LruA-directed antiserum recognizes human eye lens α-
crystallin B and vimentin

Mammalian α-crystallin B protein sequences are highly
conserved across species (Figure 3). Therefore, purified

| Protein Spot | Identified Protein | Accession Number | Predicted Mass* |
|--------------|-------------------|-----------------|-----------------|
| Spot 1       | Alpha-crystallin B| CYBOAB           | 20024           |
| Spot 2       | Vimentin          | VIM_BOVINE      | 53752           |
| Spot 3       | Beta-crystallin B2| Q2 LEC2_CANFA   | 23318           |
| Spot 4       | Beta-crystallin B2| Q2 LEC2_CANFA   | 23318           |
| Spot 5       | Beta-crystallin B2| Q2 LEC2_CANFA   | 23318           |

*Peptide masses were analyzed using the MASCOT Database search engine v1.9 (www.matrixscience.com) (Matrix Science Ltd.).

Figure 2. Two-dimensional electrophoretic analysis of proteins in equine lenticular and retinal tissue extracts. (A) Lens extract separated on a polyacrylamide gel stained with the fluorescent dye SYPRO-Ruby. (B) Lens proteins transferred from a second gel to nitrocellulose membrane and blotted with LruA-antiserum. The arrowheads indicate the protein spots excised from the stained gel for analysis by mass spectrometry. (C) Retinal extract separated on a polyacrylamide gel stained with SYPRO-Ruby. (D) Retinal proteins transferred from a second gel to nitrocellulose membrane and blotted with LruB-antiserum. Three protein spots (numbered 3, 4 and 5) were excised from the stained gel for analysis by mass spectrometry. Results of mass spectrometric analyses are tabulated in Table 1.

doi:10.1371/journal.pntd.0000778.g002

Table 1. Identification of ocular proteins cross-reactive with LruA and LruB antiserum (Figure 2) by mass spectrometry.
recombinant human α-crystallin B was used in immunoblot analyses to confirm α-crystallin B as a cross-reacting antigen. LruA-directed antiserum, but not the pre-immune serum, reacted with recombinant α-crystallin B, indicating that this lenticular protein is indeed the cross-reacting antigen (Figure 4A and B).

Figure 3. Multisequence alignment of alpha-crystallin B of horse (Equus caballus), man (Homo sapiens), cow (Bos taurus), sheep (Ovis aries) and mouse (Mus musculus) using T-COFFEE Version 5.05 [http://www.tcoffee.org].

doi:10.1371/journal.pntd.0000778.g003

Figure 4. LruA antiserum reacts with recombinant human alpha-crystallin B and purified vimentin. (A) Immunoblot showing reactivity of LruA-specific antiserum (1:400) with recombinant human alpha-crystallin B (1µg). (B) Pre-immune serum (1:400) did not react with this protein. (C) Immunoblots showing reactivity of LruA-antiserum (1:400), but not the pre-immunization serum (D), with purified vimentin (1µg). Molecular mass markers are indicated in kilodaltons.

doi:10.1371/journal.pntd.0000778.g004
The amino acid sequence identity between equine and bovine vimentin is 91% (not shown). So, purified vimentin from bovine lens was used in immunoblot to examine its reactivity to LruA-directed antisera. LruA-directed antisera but not the pre-immune serum reacted with purified vimentin in an immunoblot (Figure 4C and D).

LruB-directed antisera recognizes human β-crystallin B2

Recombinant human β-crystallin B2 was used in immunoblot analyses to confirm β-crystallin B2 as a cross-reacting antigen. LruB-directed antisera, reacted with recombinant β-crystallin B2, indicating that this lenticular protein is indeed the cross-reacting antigen (Figure 5A). Pre-immunization serum did not react with β-crystallin B2 (Figure 5B).

Antibodies directed to alpha-crystallin B, vimentin and β-crystallin B2 in uveitic fluids

The biological significance of α-crystallin B, vimentin and β-crystallin B2 as a cross-reacting antigen was investigated by examining antibody levels against these lenticular or retinal proteins in eye fluids obtained from clinical cases of leptospiral uveitis and healthy controls. ELISA was performed using recombinant α-crystallin B, purified vimentin or recombinant β-crystallin B2 as coating antigens. Antibody levels to α-crystallin B, vimentin and β-crystallin B2 (Figure 6) were found to be significantly elevated in uveitic compared to normal eye fluids (p<0.001).

Discussion

The pathogenesis of leptospiral uveitis is currently under investigation and several possible mechanisms have been proposed [6,9,12,17,20,21,23,24,25,26,27,28]. How leptospires survive in the eye, causing breach of the ocular immune privilege and initiation of pro-inflammatory changes, is not understood. Although direct Leptospira-mediated injury to eye structures is possible, a growing body of evidence suggests that autoimmune responses to ocular tissue components play a significant role in pathogenesis [6,9,12,17,20,21,22,23,24,25,26,27,28]. Parma et al. [21] demonstrated reactivity of anti-equine cornea antibodies with Leptospira and binding of Leptospira and cornea specific antibodies to equine cornea [21]. Subsequently, an antigenic relationship between equine lens and leptospires was proposed by the same group [27]. Electron microscopic studies revealed that the antigenic protein of L. interrogans that shares epitopes with equine cornea and lens is not exposed on the outer surface of leptospires [28]. However, in those studies, specific leptosomal and/or ocular proteins involved in the antigenic relationship were not identified.

In this study, we have shown that the lenticular proteins, α-crystallin B and vimentin, cross-react with LruA and retinal protein, β-crystallin B2, cross-reacts with LruB confirming our previous observations of reactivity of LruA and LruB antibodies with equine lens and retina, respectively.

Alpha-crystallin B and vimentin are critical for maintaining lens clarity and thus visual acuity [29]. Alpha-crystallin is the principal constituent of the lens and acts as a molecular chaperone that keeps other lens proteins from precipitating [30]. Disruption of this function may lead to impairment of light refraction and potentially vision. Alpha-crystallin B is a 175-amino acid small heat shock protein and shares high interspecies sequence homology (Figure 3). Its involvement in several disease states including uveitis, Alexander disease, Alzheimer’s, Creutzfeldt-Jacob disease and multiple sclerosis are under investigation [31,32,33]. In addition to lens and central nervous system (CNS), it is also present in many other tissues including skeletal muscles and kidney epithelial cells.

Vimentin is an important structural determinant in the human lens cell and is mainly expressed in the epithelium of the lens. In a previous study, high expression of vimentin was negatively correlated with the normal differentiation of the lens fibers. In that study, animals developed pronounced cataract and extensive lens degeneration as a result of impairment of lens fiber cell differentiation [34]. A study on expression of vimentin in lens epithelium of age-related cataract suggested that damage to the lens epithelial cells might initiate a decrease in vimentin expression leading to degradation of the lens cytoskeleton [35]. Recently, small interfering RNA (siRNA) mediated downregulation of human pigment epithelium-derived factor (PEDF) expression in primary human lens epithelial cells was shown to result in a decrease in the expression of vimentin and increase of α-crystallin B expression [29]. Interestingly, serum and ocular levels of PEDF have been shown to decrease in uveitic horses, but not the normal horses [36,37].

Beta-crystallin B2 is present in lens and non-lenticular tissues, including the retina. The appearance and accumulation of beta-crystallin B2 in neural retina coincides with its functional maturation [38]. Recently, antibodies against α-crystallin A, α-crystallin B and β-crystallin B1 were found to be significantly elevated in uveitis patients and seroreactivity was found to be significantly associated with cortical cataract [39]. In another study, Çelet and colleagues [31] reported an elevated humoral response to α-crystallin B in neuro-Behçet’s disease and Guillain-Barré syndrome. We recently demonstrated that LruA and LruB were recognized by antibodies from Behçet’s and Fuchs uveitis patients, without any evidence of those patients having been exposed to Leptospira [22]. Both of these diseases are believed to be autoimmune diseases [22,40,41,42,43,44,45]. In the same study,
we also observed an association in humans between high levels of antibodies recognizing LruA and LruB and the presence of cataract [22]. The high levels of antibodies cross-reactive with LruA and LruB in patients with Fuchs or Behçet’s uveitis, and the strong association of LruA and LruB antibodies with cataract could be due to increased levels of antibodies to the common autoantigens, α-crystallin B, vimentin and β-crystallin B2, in those diseases. Also, elevated levels of LruA- and LruB-antibodies in sera of human patients with leptospiral uveitis [22] and reactivity of LruA- and LruB-antiserum with human alpha-crystallin B and β-crystallin B2 suggest a similar phenomenon in human leptospiral uveitis. We are presently pursuing those hypotheses to determine the causes of leptospiral and non-leptospiral uveitis.

A linear amino acid similarity or a conformational homology between microbial and host proteins is a potential basis for molecular mimicry. The limited linear amino acid similarities between these leptosporal proteins and their respective cross-reacting ocular proteins (not shown) suggests similarities at the conformational level. Studies to identify the cross-reactive epitopes are underway.

In conclusion, we have identified two lens proteins and a retinal protein that react with antiserum directed against LruA and LruB, leptospiral proteins expressed in uveitic eyes. The presence of antibodies recognizing α-crystallin B, vimentin and β-crystallin B2 in uveitic, but not normal eye fluids, strongly suggests a role for these antibodies in Leptospira-associated recurrent uveitis. In the immune privileged ocular environment, it is likely that the early phase of leptospiral infection involves a non-inflammatory immune responses specific for LruA and LruB. Resulting antibodies may interact with cross-reacting proteins in lens and retinal tissues and may therefore initiate a process of desequestration of these ocular antigens, and possibly other components. How early after an initial infection this interaction results in development of the changes in eye, and what other pro-inflammatory changes, if any, are required remains to be determined.

Acknowledgments

We thank Claire Adams, Sergey Artiushin, Amy Bowman, Catherine Brissette, Logan Burns, Alicia Chernail, Brandon Jutras and Samir Shah for technical assistance and helpful comments.

We dedicate this work to the memory of Dr. George Allen.

Author Contributions

Conceived and designed the experiments: AV JFT BS. Performed the experiments: AV PK KB. Analyzed the data: AV PK KB JFT BS. Wrote the paper: AV.

References

1. Thiermann AB (1984) Leptospirosis: Current developments and trends. J Am Vet Med Assoc 184: 729–735.

2. Vinues JM (2001) Leptospirosis. Curr Opin Infect Dis 14: 527–538.

3. Faine S, Adler B, Bolin C, Perolat P (1999) Leptospirosis and leptospirosis. Melbourne, Australia: MedSci.

4. Rathnam SR (2002) Ocular leptospirosis. Curr Opin Ophthalmol 13: 361–386.

5. Errington BJ (1941) Ophthalmology in Equidae. J Am Vet Med Assoc 90: 115–123.

6. Hartshee RA, Goris MG, Brem S, Meyer P, Kopp H, et al. (2004) Characterization of leptospora from the eyes of horses suffering from recurrent uveitis. J Vet Med B 51: 110–115.

7. Schwick KL (1979) Equine uveitis. Vet Clin North Am Equine Pract 5: 557–564.

8. Docter BS, Hargreaves DE (1983) Equine recurrent uveitis. Equine Vet J 2: 2–13.

9. Gильер BC, Malok E, Cutler KV, Stewart T, Horohov DW, et al. (1999) Characterization of T-lymphocytes in the anterior uvea of eyes with chronic equine recurrent uveitis. Vet Immunol Immunopathol 77: 17–28.

10. Gильер BC, Michau TM (2006) Equine recurrent uveitis: new methods of management. Vet Clin Equine 22: 417–427.

11. Rehman WC (1979) Diagnosis and treatment of equine uveitis. J Am Vet Med Assoc 173: 803–808.

12. Deyo AE, Gильер BC, Brem S, Kopp H, et al. (2004) Immunopathology of recurrent uveitis is strongly associated with MHC class I haplotype ELA-A9. Equine Vet J 36: 73–75.

13. Doey AE, Gильер BC (2005) Equine recurrent uveitis. In: Gильер BC, ed. Equine ophthalmology. Philadelphia, Pa: W. B. Saunders Co.

14. Hallwill RE, Brim TA, Hines MT, Wolf D, White FH (1985) Detection of Leptospira antigens in corneal tissues. Curr Eye Res 4: 1033–1040.

15. Deyo AE, Ehrenholzer M, Tharau SR, Reese S, Wäldner G, et al. (2002) Immunopathology of recurrent uveitis in spontaneously diseased horses. Exp Eye Res 75: 127–133.

16. Faber NA, Crawford M, LeFebvre RB, Bühkumnci NC, Madigan JE, et al. (2000) Detection of Leptospira in the aqueous humor of horses with naturally acquired recurrent uveitis. J Clin Microbiol 38: 2731–2733.

17. Brandes K, Wollanke B, Niedermaier G, Brem S, Gerhards H (2007) Recurrent uveitis in horses: vitreal examinations with ultrastructural detection of Leptospira. J Vet Med A Physiol Pathol Clin Med 54: 270–275.

18. Brem S, Gerhards H, Wollanke B, Meyer P, Kopp H (1999) 35 leptospora isolated from the vitreous body of 32 horses with recurrent uveitis. Berl Munch Tierarztl Wochenschr 112: 390–393.

19. Morter RL, Williams RD, Bode H, Freeman MJ (1969) Equine leptoconiosis. J Am Vet Med Assoc 153: 436–442.

20. Verma A, Artiushin S, Matsunaga J, Haake DA, Timoney JF (2005) LruA and LruB, novel lipoproteins of pathogenic Leptospira interrogans associated with recurrent uveitis. Infect Immun 73: 7259–7266.

21. Parma AE, Santisteban CG, Villalba JS, Bowden RA (1985) Experimental detection of an antigenic relationship between Leptospira and equine cornea. Vet Immunol Immunopathol 10: 215–224.

22. Verma A, Rathinam SR, Priya CG, Muthukkaruppan VR, Stevenson B, et al. (2008) LruA and LruB antibodies in sera of humans with leptospiral uveitis. Clin Vaccine Immunol 15: 1019–1023.

23. Deyo CA, Kaspers B, Gerhards H, Tharau SR, Wollanke B, et al. (2001) Immune responses to retinal autoantigens and peptides in equine recurrent uveitis. Invest Ophthalmol Vis Sci 42: 393–398.

24. Deyo CA, Tharau SR, Gerhards H, Ehrenholzer M, Wäldner G, et al. (2002) Uveitis in horses induced by interphotoreceptor retinoid binding protein is similar to the spontaneous disease. Eur J Immunol 32: 2580–2606.

25. Deyo CA, Pompezi D, Raith AJ, Hauke SM, Amanz B, et al. (2006) Identification and functional validation of novel autoantigens in equine uveitis. Mol Cell Proteomics 5: 1462–1470.

26. Deyo CA, Amanz B, Raith AJ, Kaspers B (2006) Intracellular and intramolecular epitope spreading in equine recurrent uveitis. Invest Ophthalmol Vis Sci 47: 632–636.

27. Parma AE, Fernandez AS, Santisteban CG, Bowden RA, Cerone SI (1987) Tears and aqueous humor from horses inoculated with Leptospira contains antibodies which bind to cornea. Vet Immunol Immunopathol 14: 181–185.

28. Parma AE, Sanz ME, Lucchesi PM, Mazzonelli J, Petruccelli MA (1997) Detection of an antigenic protein of Leptospira interrogans which shares epitopes with the equine cornea and lens. Vet Immunol Immunopathol 59: 133–136.

29. Yang J, Luo L, Liu X, Rosenblatt MI, Qu B, et al. (2010) Down regulation of the PEDF gene in human lens epithelium cells changed the expression of proteins vimentin and alpha B-crystallin. Mol Vis 16: 105–112.

30. Horwitz J (1992) Alpha-crystallin can function as a molecular chaperone. Proc Natl Acad Sci USA 89: 10449–10453.

31. Celet B, Akman-Demir G, Serdaroglu P, Yentur SP, Tasci B, et al. (2000) Anti-alpha B-crystallin immunoreactivity in inflammatory nervous system diseases. J Immunol 164: 1987–1994.

32. Mann E, McDermott MJ, Goldman J, Chiesa R, Spector A (1991) Leptospiral antigens are strongly associated with the MHC class I haplotype ELA-A9. Equine Vet J 36: 439–442.
33. van Noort JM, van Sechel AC, Bajramovic JJ, el Ouagmiri M, Polman CH, et al. (1995) The small heat-shock protein alpha B-crystallin as candidate autoantigen in multiple sclerosis. Nature 375: 786-801.
34. Capetanaki Y, Smith S, Heath JP (1989) Overexpression of the vimentin gene in transgenic mice inhibits normal lens cell differentiation. J Cell Biol 109: 1653-1664.
35. Zhou J, Hui Y, Li Y (2001) Expression of vimentin in lens epithelial cells of age-related cataract. Zhonghua Yan Ke Za Zhi 37: 342-345.
36. Zipplies JK, Hauck SM, Schoeffmann S, Amann B, Stangassinger M, et al. (2009) Serum PEDF levels are decreased in a spontaneous animal model for human autoimmune uveitis. J Proteome Res 8: 992-998.
37. Deeg CA, Altmann F, Hauck SM, Schoeffmann S, Amann B, et al. (2007) Downregulation of PEDF in uveitic lesions associates with focal VEGF expression and breakdown of the blood retinal barrier. Proteomics 7: 1540-1548.
38. Head MW, Sedowofia K, Clayton RM (1995) Beta B2-crystallin in the mammalian retina. Exp Eye Res 61: 423–428.
39. Chen L, Holland GN, Yu F, Levinson RD, Lampi KJ, et al. (2008) Associations of seroreactivity against crystallin proteins with disease activity and cataract in patients with uveitis. Invest Ophthalmol Vis Sci 49: 4476-4481.
40. Al-Otaibi LM, Porter SR, Poate TVJ (2003) Behçet’s disease: a review. J Dent Res 84: 209–222.
41. Chowers I, Zamir E, Banin E, Merin S (2000) Retinitis pigmentosa associated with Fuch’s heterochromic uveitis. Arch Ophthalmol 118: 800–802.
42. Delunardo F, Conti F, Margutti P, Alessandrini C, Priori R, et al. (2006) Identification and characterization of the carboxy-terminal region of Sip-1, a novel autoantigen in Behçet’s disease. Arthritis Res Ther 8: R71.
43. La Hey E, Broersma L, van der Gaag R, Baarsma GS, Rothova A, et al. (1993) Does autoimmunity to S-antigen play a role in Fuch’s heterochromic cyclitis? Br J Ophthalmol 77: 436–439.
44. Lu Y, Ye P, Chen S, Tan EM, Chan EKL (2005) Identification of kinectin as a novel Behçet’s disease autoantigen. Arthritis Res Ther 7: R1133–R1139.
45. van der Gaag R, Broersma L, Rothova A, Baarsma GS, Klijstra A (1989) Immunity to a corneal antigen in Fuch’s heterochromic cyclitis patients. Invest Ophthalmol Vis Sci 30: 443–448.