



Ophthalmic Res 2008;40:151–153 DOI: 10.1159/000119867 Published online: April 18, 2008

Equine Recurrent Uveitis – A Spontaneous Horse Model of Uveitis

Cornelia A. Deeg^a Stefanie M. Hauck^c Barbara Amann^a Dirk Pompetzki^a Frank Altmann^a Albert Raith^a Thomas Schmalzl^a Manfred Stangassinger^a Marius Ueffing^{b, c}

^aInstitute of Animal Physiology, LMU Munich, and ^bInstitute of Human Genetics, Technical University of Munich, Munich, and ^cInstitute of Human Genetics, Helmholtz Center for Environment and Health, Neuherberg, Germany

Key Words

Uveitis, spontaneous model, horse · Autoimmune · Target organ · Autoantigens · Proteomics · Regulated proteins

Abstract

Equine recurrent uveitis (ERU) is an autoimmune disease that occurs with a high prevalence (10%) in horses. ERU represents the only reliable spontaneous model for human autoimmune uveitis. We already identified and characterized novel autoantigens (malate dehydrogenase, recoverin, CRALBP) by analyzing the autoantibody-binding pattern of horses affected by spontaneous recurrent uveitis (ERU) to the retinal proteome. CRALBP also seems to be relevant to human autoimmune uveitis. Proteomic screening of vitreous and retinal samples from ERU diseased cases in comparison to healthy controls has led to the identification of a series of differentially regulated proteins, which are functionally linked to the immune system and the maintenance of the blood-retinal barrier. Copyright © 2008 S. Karger AG, Basel

Equine Uveitis, a Spontaneous Model of Recurrent Uveitis

Standard animal models for the investigation of autoimmune uveitis are experimentally induced uveitis in Lewis rats or in mice [1]. The only spontaneous model of recurrent uveitis is equine recurrent uveitis (ERU), a disease that occurs with high frequency (10%) in horses. ERU closely resembles human autoimmune uveitis regarding clinical and immune pathological aspects [2]. The spontaneous remitting-relapsing character of this disease discloses the value of the equine model in studying events initiating uveitis and uveitic relapses. Autoimmune uveitis is assigned to the organ-specific, T-cell-mediated autoimmune diseases. Infiltrating T cells are detectable in high proportions in the inner eyes of horses with ERU, forming even lymphoid follicles in the iris stroma [2]. Analyses of autoantigen and epitope specificity of these autoaggressive T cells revealed evidence for inter- and intramolecular epitope spreading [3, 4]. Immune reactions of horses were directed against the major retinal autoantigens S-antigen (S-Ag) [5, 6] and interphotoreceptor retinoid-binding protein (IRBP) [6, 7] but also against other proteins expressed in the retinal proteome [8]. The shifts in immunoreactivity could account for the remitting-relapsing character of the disease.

Cornelia A. Deeg Institute of Animal Physiology Veterinärstrasse 13, DE–80539 Munich (Germany) Tel. +49 892 180 1630, Fax +49 892 180 2554 E-Mail deeg@tiph.vetmed.uni-muenchen.de

KARGER

Fax +41 61 306 12 34 E-Mail karger@karger.ch www.karger.com © 2008 S. Karger AG, Basel 0030–3747/08/0404–0151\$24.50/0

Accessible online at: www.karger.com/ore

Detection of Novel Autoantigens Using the Equine Uveitis Model

Further examination of the autoimmune repertoire in ERU cases using the retinal proteome as autoantigenic source led to the identification of malate dehydrogenase (MDH), recoverin [8] and CRALBP [8] as potential novel ERU autoantigens. A further advantage of the horse model is that the horse itself can be used as an experimental animal model, thus allowing the direct verification of hypotheses created through investigating the spontaneous disease. The pathological potential of the candidate autoantigens MDH and CRALBP [8] was therefore directly tested in the horse as a last step of the full characterization of these autoantigens. CRALBP proved its uveitogenicity in the horse and we could directly compare the ensuing pathology in induced and spontaneous uveitis [8]. In contrast, MDH injections did not induce uveitis despite considerable autoantibody formation and autoaggressive, MDH-specific T cells. The relevance of CRALBP as a novel autoantigen also for human autoimmune uveitis was recently demonstrated in first experiments showing a high proportion of uveitis patients positive for anti-CRALBP autoantibody [9].

Interestingly, the validation of the retinal autoantigens IRBP and S-Ag pointed to a major role of IRBP as autoantigen in the horse model of uveitis [10]. Uveitis was inducible with an incidence of 100% in experimental horses and was also reinducible several times in a well-defined manner [10]. In contrast, S-Ag induced uveitis in 20% of the cases and uveitis was monophasic [11], as also seen in the Lewis rat [1]. Interestingly, all horses that were injected with S-Ag developed anti-S-Ag autoantibodies with high titer and also autoreactive T cells reacting to in vitro stimulation with S-Ag-derived peptides [11]. Nevertheless, these T cells did not overcome the blood-retinal barrier in the majority of the tested cases.

Differentially Regulated Proteins in the Uveitis Target Organs

Therefore, further insight into the disease mechanisms caused by the target organ itself is needed. Systematic exploration of the intraocular proteomes of spontaneous uveitis cases and healthy controls enabled the identification of several differentially regulated proteins that belong to pathways involved in immune response [12, 13]. The respective upregulated proteins are complement component C3, carboxylesterase D1 and histone deacetylase complex subunit SAP18, which merit further exploration in the context of spontaneous uveitis. C3 is a member of the complement cascade that can be activated in 3 different pathways: the classical, alternate and lectin pathways. All three pathways merge at a common intersection, complement component C3. C3 split products have been reported to be deposited in large quantities under pathological conditions such as diabetic retinopathy [14]. Furthermore, intraocular complement activation, specifically the involvement of complement receptor 3, had a significant impact on disease activity in experimental autoimmune uveitis in rats [15, 16]. The second candidate, carboxylesterase D1, is a serine esterase that is highly similar to human monocyte/macrophage serine esterase 1 [17]. Esterase activity in human monocytes has been demonstrated to correlate with chemotaxis [18]. Histone deacetylase complex subunit SAP18 is a transcription factor. SAP18 is expressed during murine hematopoietic precursor differentiation, as well as in bipotent erythroid/megakaryocytic precursors and in maturing monocytes and megakaryocytes [19]. Since macrophages are detectable in considerable amounts in uveitic retinas [2], the expression of a transcription factor leading to macrophage differentiation in diseased retina also merits further investigation from our point of view.

Stable Expression of Autoantigens in all Stages of Uveitis

In both diseases, ERU and autoimmune recurrent uveitis of man, uveitic relapses are notable even in phthisical and blind eyes [20]. Histopathological examination of ERU eyes and eyes from experimentally induced uveitides revealed an almost complete loss of the photoreceptor outer segments in advanced stages of disease [1, 2]. Since the photoreceptor outer segments host 2 major autoantigens, retinal S-Ag (arrestin) and IRBP [21], the ongoing immune pathology after destruction of the physiological expression sites of these proteins is difficult to explain. In other autoimmune diseases, such as Hashimoto's thyroiditis, a termination of autoimmune attacks is notable after depletion of autoantigens [22, 23]. We therefore examined the autoantigen expression levels of IRBP, S-Ag and CRALBP in uveitic retinas of several clinical stages in comparison to healthy control retinas. Major retinal autoantigens, IRBP, S-Ag and CRALBP, were clearly detectable in retinal proteomes of normal equine retina and

in retinas with different stages of uveitis. Although the composition of retinal proteomes differs considerably between healthy and diseased states [12], the IRBP, S-Ag and CRALBP amounts remained unchanged [24]. Substantial photoreceptor damage in uveitic retinas tested in this study was evident by measuring rhodopsin expression levels that were reduced to 27% of the original expression. The unchanged total amount of 3 major retinal autoantigens indicates that the autoantigenic target protein is present despite considerable destruction of their hosting structures and can thus trigger unabated relapses in patients with blind eyes.

References

- 1 Caspi RR, Roberge FG, Chan CC, Wiggert B, Chader GJ, Rozenszajn LA, Lando Z, Nussenblatt RB: A new model of autoimmune disease: experimental autoimmune uveoretinitis induced in mice with two different retinal antigens. J Immunol 1988;140:1490– 1495.
- 2 Deeg CA, Ehrenhofer M, Thurau SR, Reese S, Wildner G, Kaspers B: Immunopathology of recurrent uveitis in spontaneously diseased horses. Exp Eye Res 2002;75:127–133.
- 3 De Smet MD, Bitar G, Mainigi S, Nussenblatt RB: Human S-antigen determinant recognition in uveitis. Invest Ophthalmol Vis Sci 2001;42:3233–3238.
- 4 Deeg CA, Amann B, Raith AJ, Kaspers B: Inter- and intramolecular epitope spreading in equine recurrent uveitis. Invest Ophthalmol Vis Sci 2006;47:652–656.
- 5 Wacker WB, Donoso LA, Kalsow CM, Yankeelov JA Jr, Organisciak DT: Experimental allergic uveitis: isolation, characterization, and localization of a soluble uveitopathogenic antigen from bovine retina. J Immunol 1977;119:1949–1958.
- 6 Caspi RR, Roberge FG, Chan CC, Wiggert B, Chader GJ, Rozenszajn LA, Lando Z, Nussenblatt R: Experimental autoimmune uveoretinitis induced in mice with two different retinal antigens. J Immunol 1988;140:1490– 1495.
- 7 Gery I, Wiggert B, Redmond TM, Kuwabara T, Crawford MA, Vistica BP, Chader GJ: Uveoretinitis and pinealitis induced by immunization with interphotoreceptor retinoid-binding protein. Invest Ophthalmol Vis Sci 1986;27:1296–1300.
- 8 Deeg CA, Pompetzki D, Raith AJ, Hauck SM, Amann B, Suppmann S, Goebel TW, Olazabal U, Gerhards H, Reese S, Stangassinger M, Kaspers B, Ueffing M: Identification and functional validation of novel autoantigens in equine uveitis. Mol Cell Proteomics 2006; 5:1462–1470.

Conclusions

Our research exploits the advantages of this spontaneous animal model, enabling us to characterize the pathomechanisms, triggering disease from the perspective of the target organ itself as well as the immune reaction.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft SFB 571 and DE 719/2-1, EU grant EVI-GENORET LSHG-CT-2005-512036 and the German Federal Ministry of Education and Research: BMBF-QuantPro 0313865A.

- 9 Deeg CA, Raith AJ, Amann B, Crabb JW, Thurau SR, Hauck SM, Ueffing M, Wildner G, Stangassinger M: CRALBP is a highly prevalent autoantigen for human autoimmune uveitis. Clin Dev Immunol 2007; 2007:39245.
- 10 Deeg CA, Thurau SR, Gerhards H, Ehrenhofer M, Wildner G, Kaspers B: Uveitis in horses induced by interphotoreceptor retinoid-binding protein is similar to the spontaneous disease. Eur J Immunol 2002;32: 2598–2606.
- 11 Deeg CA, Reese S, Gerhards H, Wildner G, Kaspers B: The uveitogenic potential of retinal S-antigen in horses. Invest Ophthalmol Vis Sci 2004;45:2286–2292.
- 12 Deeg CA, Altmann F, Hauck SM, Schoeffmann S, Amann B, Stangassinger M, Ueffing M: Downregulation of PEDF in uveitic lesions associates with focal VEGF expression and breakdown of the blood retinal barrier. Proteomics 2007;7:1540–1548.
- 13 Hauck SM, Schoeffmann S, Amann B, Stangassinger M, Gerhards H, Ueffing M, Deeg CA: Retinal Müller glial cells trigger the hallmark inflammatory process in autoimmune uveitis. J Prot Res 2007;6:2121–2131.
- 14 Gerl VB, Bohl J, Pitz S, Stoffelns B, Pfeiffer N, Bhakdi S: Extensive deposits of complement c3d and c5b-9 in the choriocapillaris of eyes of patients with diabetic retinopathy. Invest Ophthalmol Vis Sci 2002;43:1104– 1108.
- 15 Jha P, Sohn JH, Xu Q, Nishihori H, Wang Y, Nishihori S, Manickam B, Kaplan HJ, Bora PS, Bora NS: The complement system plays a critical role in the development of experimental autoimmune anterior uveitis. Invest Ophthalmol Vis Sci 2006;47:1030–1038.
- 16 Jha P, Sohn JH, Xu Q, Wang Y, Kaplan HJ, Bora PS, Bora NS: Suppression of complement regulatory proteins (CRPs) exacerbates experimental autoimmune anterior uveitis (EAAU). J Immunol 2006;176:7221–7231.

- 17 Satoh T, Hosokawa M: The mammalian carboxylesterases: from molecules to functions. Annu Rev Pharmacol Toxicol 1998;38:257– 288.
- 18 Bozdech MJ, Bainton DF: Identification of alpha-naphthyl butyrate esterase as a plasma membrane ectoenzyme of monocytes and as a discrete intracellular membrane-bounded organelle in lymphocytes. J Exp Med 1981; 153:182–195.
- 19 Boehmelt G, Antonio L, Iscove NN: Cloning of the murine transcriptional corepressor component SAP18 and differential expression of its mRNA in the hematopoietic hierarchy. Gene 1998;207:267–275.
- 20 Werry H, Gerhards H: The surgical therapy of equine recurrent uveitis. Tierarztl Prax 1992;20:178–186.
- 21 De Smet MD, Chan CC: Regulation of ocular inflammation – what experimental and human studies have taught us. Prog Retin Eye Res 2001;20:761–797.
- 22 Pontes de Carvalho LC, Templeman J, Wick G, Roitt IM: The role of self-antigen in the development of autoimmunity in obese strain chickens with spontaneous autoallergic thyroiditis. J Exp Med 1982;155:1255– 1266.
- 23 Chiovato L, Latrofa F, Braverman LE, Pacini F, Capezzone M, Masserini L, Grasso L, Pinchera A: Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens. Ann Intern Med 2003; 139:346–351.
- 24 Deeg CA, Hauck SM, Amann B, Kremmer E, Stangassinger M, Ueffing M: Major retinal autoantigens remain stably expressed during all stages of spontaneous uveitis. Mol Immunol 2007;44:3291–3296.