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

a Institute of Animal Physiology, LMU Munich, and b Institute of Human Genetics, Technical University of Munich, Munich, and c Institute 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.

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.
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 comple-

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.

**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-S12036 and the German Federal Ministry of Education and Research: BMBF-QuantPro 0313865A.

**References**