frontiers in Immunology

REVIEW
published: 08 January 2021
doi: 10.3389/fimmu.2020.609855

Immunological Insights in Equine Recurrent Uveitis

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Horses worldwide suffer from equine recurrent uveitis (ERU), an organ-specific, immune-mediated disease with painful, remitting-relapsing inflammatory attacks alternating with periods of quiescence, which ultimately leads to blindness. In course of disease, both eyes can eventually be affected and since blind horses pose a threat to themselves and their surroundings, these animals have to be killed. Therefore, this disease is highly relevant for veterinary medicine. Additionally, ERU shows strong clinical and pathological resemblance to autoimmune uveitis in man. The exact cause for the onset of ERU is unclear to date. T cells are believed to be the main effector cells in this disease, as they overcome the blood retinal barrier to invade the eye, an organ physiologically devoid of peripheral immune cells. These cells cause severe intraocular inflammation, especially in their primary target, the retina. With every inflammatory episode, retinal degeneration increases until eyesight is completely lost. In ERU, T cells show an activated phenotype, with enhanced deformability and migration ability, which is reflected in the composition of their proteome and downstream interaction pathways even in quiescent stage of disease. Besides the dysregulation of adaptive immune cells, emerging evidence suggests that cells of the innate immune system may also directly contribute to ERU pathogenesis. As investigations in both the target organ and the periphery have rapidly evolved in recent years, giving new insights on pathogenesis-associated processes on cellular and molecular level, this review summarizes latest developments in ERU research.

Keywords: retina, Mueller glia, vitreous, serum, lymphocyte, granulocyte, complement system, cytokines

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: *24 September 2020*
Accepted: *30 November 2020*
Published: *08 January 2021*

Citation:
Degroote RL and Deeg CA (2021) Immunological Insights in Equine Recurrent Uveitis. Front. Immunol. 11:609855. doi: 10.3389/fimmu.2020.609855

INTRODUCTION

Insights in the pathogenesis of equine recurrent uveitis (ERU) have grown in recent years, especially on cellular and molecular level in periphery and in the eye itself. ERU is an organ-specific, immune-mediated disease, which is predominantly driven by CD4+ T cells (1–3). These are somehow activated in periphery and then manage to overcome the blood retinal barrier (BRB), entering the eye and causing intraocular inflammation (4). Since ERU has a remitting-relapsing character (2), increasing numbers of immune cells invade and accumulate in the eye with every inflammatory attack, gradually destroying their main target, the retina. These acute phases alternate with periods of quiescence and increase in severity as the disease progresses (5). Factors associating to ERU onset are still discussed and an exact cause is elusive to date. A genome-wide association study suggested a correlation of genetic variants influencing the expression of IL-17A and IL-17F with the development of ERU (6). This study also substantiated previous reports linking ERU to MHC

class I haplotype ELA-A9 in warmblood horses (7). Direct evidence for a genetic component in ERU of warmblood horses, however, is still lacking. Disease prevalence among the horse population is high, ranging from 2%–25% in the US and 8%–10% in Europe (5, 8). ERU often affects both eyes and eventually leads to blindness if left untreated (9). These two factors, high prevalence and loss of sight, explain its importance for veterinary medicine, since blind horses have to be killed due to the threat they pose to their surroundings and themselves, which has a great personal but also economic impact on horse owners. Moreover, ERU has great potential to serve as a valuable spontaneous model for autoimmune uveitis in man, due to strong clinical and pathological similarities (3, 4, 10, 11).

In the past, several studies could show that the dysregulated immune response in ERU is directed against retinal autoantigens such as interphotoreceptor retinoid-binding protein (IRBP) and cellular retinaldehyde-binding protein (CRALBP) (4, 12), which, along with other factors, suggests presence of autoimmune reactions in ERU. These autoantigens not only remain stably expressed in advanced stages of ERU, when retinal architecture is drastically destructed (13), IRBP can also induce experimental uveitis in the horse itself, which shows close similarity to the spontaneous disease on clinical, cellular and molecular level (4, 14). Moreover, studies on ERU horses firstly provided CRALBP as novel autoantigen (12), which was subsequently proven to also have high prevalence in human patients with autoimmune uveitis (15). As shown in previous studies, the recognition of these autoantigens and the subsequent spreading of epitope recognition to a new determinant may be the cause for the relapsing character of ERU (12, 16). This process known as epitope spreading describes the reaction of immune cells against the initial epitope of an ERU autoantigen, which then redirects against a different, but structurally similar epitope, re-initializing an inflammatory response after the initial inflammation has subsided (17, 18).

In addition to the retinal-autoantigen specific T cells driving ERU from periphery, further studies suggested that the retina itself promotes the intraocular inflammatory processes through active abundance downscaling of proteins which protect the maintenance of the blood-retina-barrier (19) and through secretion of the pro inflammatory cytokine interferon gamma (IFNγ) by retinal Mueller glial cells (20). Since then, research has advanced on the target organ of ERU as well as the cellular and molecular components in periphery. This review summarizes the latest immunological insights in ERU pathogenesis.

PERIPHERY

Adaptive Immune System

Although the concept of immune privilege is increasingly discussed since its initial description (21, 22), the eye is considered to be an immune privileged organ (23, 24), and is therefore usually devoid of immune cells form peripheral blood stream. In ERU, however, we find major cell infiltrates in the eye (2, 3, 25–27), which mainly consist of CD4+ lymphocytes (2, 27). To date, the exact mechanisms enabling these autoreactive cells to overcome the BRB in ERU are still elusive. Experimental autoimmune uveitis (EAU) is a valuable tool to investigate these mechanisms, not only in common rodent models (28–31), but to a certain extent also in the horse (4). Immunization of healthy horses with the retinal autoantigen IRBP mimics the spontaneous disease through the formation of auto-aggressive, IRBP specific T cells, which leave the peripheral blood stream to invade the eye directly prior to a uveitic attack (4). These cells show distinct protein changes before immunization, during the inflammatory phase and in quiescent episodes (14), which points to a highly dynamic functional phenotype depending on the stage of disease. During quiescence of this IRBP induced equine EAU, protein abundance changes in immune cells show many similarities to those observed in freshly obtained cells from horses in quiescent stage of ERU (14), underlining the close resemblance of the spontaneous and the induced disease in the horse. Although this type of induced horse model may allow more in depth investigations on ERU pathomechanisms, which is especially interesting for assessment of protein and cellular dynamics throughout the different stages of ERU, it is less suitable for large-scale experiments comparable to those performed on the several existing rodent models for autoimmune uveitis.

Analysis of primary T cells from horses with spontaneously occurring ERU revealed distinct proteome changes, mainly of proteins with a role in cell adhesion, cell migration and regulation of cell shape (32–34). In transmigration experiments, CD4+ T cells from ERU horses showed increased migration rates, which was linked to increased expression of the protein formin-like 1 (FMNL1) in the membranes of these cells (33). As shown in a new FMNL1 knock-out mouse model, FMNL1 deficiency impedes extravasation and trafficking of T cells to sites of inflammation (35), further undermining the importance of this protein and its functional effects in ERU pathogenesis. Furthermore, lymphocytes from ERU horses displayed significantly higher cell motility, cell speed and directness toward selected chemoattractants, especially IFNγ, and toward the ERU autoantigen CRALBP, in live-cell imaging experiments (36), which was associated to lower abundance of the protein septin 7 in these cells (32, 36). Previous studies on septin 7-depleted murine CD4+ T cells (clone D10.G4, as well as primary T cells from DO11.10 TCR transgenic mice), showed less rigidity and aberrant cell morphology in these depleted cells, resulting in the ability to migrate through narrow pores (37), which was also hypothesized for ERU (32), but never directly proven. Identification of interactors of septin 7, most importantly dedicator of cytokinesis 8 (DOCK8), which was decreased in ERU (34), pointed to impaired immunity and increased risk for recurrent inflammation, as suggested in studies on human cells from patients with DOCK8 deficiency (38). Furthermore, increased expression of DOCK8 interacting protein integrin-linked kinase (ILK) was functionally associated to a decreased apoptosis rate in ERU cells (34). Combined results from these *in vitro* experiments - increased life span, increased migratory reactivity toward chemokines and autoantigens and enhanced migration ability of

ERU lymphocytes - underlines the dysregulated nature of these cells in ERU. Although the transfer of these interpretations from *in vitro* experiments to *in vivo* mechanisms needs to be assessed with care, these insights further support the role of CD4+ T cells as key players in ERU pathogenesis.

Recent studies showed that, although percentage of CD3+, CD8+, and CD4+ lymphocytes does not differ between healthy horses and those with ERU, only the disease-driving CD4+ T cells have an activated phenotype in ERU horses (39). These cells show significantly increased expression levels of IFNγ and decreased expression of IL-10, indicating Th1 immune response (39). This supports previous findings of increased levels of IFNγ as well as cytokine Interferon gamma-induced protein 10 (IP-10) in serum of ERU horses (40). A further known immune response in autoimmune uveitis in man as well as EAU in rodents is mediated through Th17 cells (41). In ERU, the exact role of Th17 cells has not been established to date, however, detection of cytokines IL-6, IL-17, and IL-23 *via* crossreactive anti-human antibodies in histological sections of the iris and the ciliary body of ERU horses provide first indications pointing toward a contribution of these cells to ERU pathogenesis (42). Similar conclusions could be drawn from *M. tuberculosis* (strain H37Ra) induced experimental uveitis in horses, where high levels of IL-17 were detected in aqueous humor and vitreous *via* Enzyme-linked Immunosorbent Assay (ELISA) (43).

Contrary to the Th1 and/or Th17 immune response inducing and maintaining inflammation in the uveitic eye, remission of each inflammatory bout in autoimmune uveitis and EAU can be associated to T regulatory cells (Treg) (44–46). During an acute inflammatory uveitic attack, these cells show decreased abundance in peripheral blood as opposed to phases of quiescence, suggesting a crucial role in the remitting-relapsing character of autoimmune uveitis (47, 48). These differences, however, could not be detected in peripheral blood of ERU horses compared to healthy controls (39). To date, no direct evidence for Tregs in the equine eye could be found. Nevertheless, the role of Treg in periodic remission of ERU is highly probable and thus merits further investigations.

Although an autoreactive Th1 response against retinal antigens is widely supported as the key process in ERU pathogenesis, one pivotal question remains: how can peripheral T cells be primed against tissue specific self-antigens which are "hidden" in an immune privileged organ? Emerging investigations on autoimmune uveitis suggest that commensal microbiota, if dysregulated (dysbiosis), may show sequence similarities with self-antigens (49, 50). This in turn may induce T cell cross-activation and priming against retina-specific antigens, triggering autoimmune reactions (49, 50). Other studies suggest, that a healthy gut microbiome is needed for prevention of autoimmunity through Treg homeostasis and that dysbiosis leads to changed or insufficiently effective Treg populations (51, 52). In ERU, the involvement of commensal microbiota has not been proven to date, however it is highly likely to play a possible role.

Equine T cell activation can be markedly reduced by co-incubation with adipose-derived equine mesenchymal stem cells (MSC) *in vitro*, resulting in downscaling of CD25 as well as intracellular IFNγ, IL-10, and FoxP3 (39). This feature of MSC might be useful for the development of new therapeutic approaches in ERU (53), as suggested by in equine immune-mediated keratitis, where subconjunctival injection of autologous MSCs improved clinical signs of disease (54). Although clinical application of this therapeutic strategy needs more in-depth validation, it might have the potential of broadening the currently applied methods of medical and surgical therapy, which classically comprise the systemical and topical application of immunosuppressive and anti-inflammatory medication, placement of a suprachoroidal cyclosporine sustainded-release device or pars plana vitrectomy (9, 55, 56).

Innate Immune System

The role of innate immune cells in autoimmune uveitis has been intensely investigated in the eyes of rodent EAU models (57–60) and infiltration of these cells in eyes of ERU horses, although to a lesser extent, has also been observed (3). The involvement of monocytes, which might promote pro-inflammatory behavior, as well as destruction of the targeted retinal cells in ERU could be shown by expression of CD68 on infiltrating peripheral immune cells in the diseased retina (61). Monocytes are major mediators of tissue damage in EAU (62, 63) and are potent antigen-presenting cells (64), which makes their role in ERU especially interesting, particularly in regard to their interaction with specific retinal cells (65). Further indication of participation of innate immune cells in ERU was proven by identification of decreased talin 1 abundance, a key integrin regulator in leukocytes, and its interacting proteins in low-density-neutrophils which were obtained as byproduct of lymphocyte isolation (25, 66). Targeted investigations of pure granulocyte fractions of healthy and ERU horses revealed significant proteome changes in diseased state, which associated to MHC-I-mediated antigen presentation, RAF/MAP kinase cascade, and neutrophil degranulation (67). Interestingly, increased neutrophil degranulation was also described in other T-cell driven autoimmune diseases, such as multiple sclerosis, where it is linked to a generally pre-activated state of these cells (68). Moreover, neutrophil degranulation relates to deviant equine granulocyte proteome after *in vitro* stimulation (69), underlining a latent state of activation of granulocytes in ERU, even in quiescent stage of disease (67). The fact that neutrophils from ERU horses more readily perform NETosis supports these findings (70). The exact role of innate immune cells and their timing in and impact on ERU pathogenesis, however, is still not completely understood to date and needs more in-depth investigation.

Implication of a role of the complement system was shown by the identification of several complement factors and split factors and their increased expression in ERU sera and eyes (19, 61, 71). Namely complement factor B, as well as C3 derived split products C3d and iC3b showed highly increased abundance, indicating strong activation of the complement system not only in the peripheral blood stream but also locally in the eye itself (61, 71). Although the exact source of these complement components in ERU eyes is still unclear, they might be

macrophage-derived, as suggested by studies on human retinas from patients with age-related macular degeneration (AMD) and *in vivo* retina analyses of rodent models for AMD (72). Other studies on C57BL/6 mice showed that complement factor B is constitutively expressed by RPE cells and this expression is positively regulated by inflammatory cytokines in the human RPE cell line ARPE19 (73), a process which might also apply to the increased occurrence in ERU eyes. Deviant regulation of the complement system is involved in the pathogenesis of various ocular diseases, including autoimmune uveitis (74, 75) and the severity of experimental autoimmune uveitis drastically decreases in complement-receptor deficient rodent models (76–78). Moreover, EAU can be effectively suppressed in C57BL/6 mice when activation of complement *via* the alternative pathway is blocked through complement receptor CRIg-Fc. Since complement factor B is exclusive to the alternative pathway, increased intraocular levels in EAU suggest a role of this pathway in disease pathogenesis (79). Despite the high abundance of complement factor B in uveitic horse eyes, indicating a possible contribution of the alternative pathway to ERU pathogenesis, this correlation has not yet been assessed.

Serum Proteins

In the last decade, proteomic studies on sera of ERU diseased horses revealed substantial differences compared to sera of healthy horses (80). Due to breakdown of the BRB in course of multiple uveitic attacks, several of these proteins can also be detected inside the eye (3, 71, 81, 82).

Among these differentially expressed proteins, kininogen was identified with decreased abundance in sera of ERU horses (71). Contrary to this, kininogen showed increased levels in vitreous and retina in ERU, whereas it was not detectable in healthy eyes (71). Kininogen promotes angiogenesis and neovascularization (83), a process that plays a significant role in the pathogenesis of autoimmune uveitis (71). It is also a part of the kallikrein-kinin system, which promotes integrity loss of barrier systems, such as the blood-brain-barrier in a mouse model of multiple sclerosis (84). Increased vitreal and retinal kininogen levels combined with a decrease in serum of ERU horses therefore points to disruption of the BRB in course of disease. IgM levels were also increased in ERU sera compared to healthy horses, suggesting a recent immune response pattern prior to blood sampling (71).

Decreased levels of pigment epithelium derived factor (PEDF) could be shown in sera of ERU horses (82), which was also observed in the retina and the vitreous of these animals in earlier studies (19, 20). PEDF was also decreased in plasma and retina from rats with endotoxin-induced uveitis, describing PEDF as a negative acute-phase protein (85). Furthermore, PEDF plays an important role in the viability of rat and mouse retinal cells (86, 87), the protection of human retinal pigment epithelium cells (ARPE-19 cell line) against oxidative stress (88) and the protection of tight junction proteins in rat eyes (85). Therefore, the decrease of PEDF in periphery as well as the target organ suggests increased permeability of the blood retinal-barrier.

TARGET ORGAN

Retinal Pigment Epithelium

Increased BRB permeability in ERU is supported by studies on the outer BRB, which physiologically maintains ocular immune privilege through disconnection of the inner eye from peripheral blood-derived immune cells (24). The outer BRB is formed by retinal pigment epithelium (RPE) cells, which play a crucial role in the mainly avascular retina of the horse, since they maintain homeostasis of retinal cells (89). This is also reflected in the RPE surface proteome, which strongly associates to transport processes (90). Furthermore, horses with ERU display a distinctly changed RPE membrane protein repertoire in ERU (91). A significant decrease of peripherin 2, a protein that is associated with membrane fusion processes, in RPE cells of ERU horses is thought to provoke disruption of the cell-to-cell junctions which are physiolocically maintained through these cells (92), which in turn points to increased permeability of the outer BRB. As also hypothesized for PEDF (19, 20, 82), this might promote integrity loss of the BRB. Although loss of barrier function of the BRB and leukocyte infiltration in course of ERU is evident (4, 27, 91, 92), the exact chronological order of these events is still not completely clear. Integrity loss of the BRB may occur either as an initial process which subsequently facilitates migration of peripheral immune cells into the eye, or as a secondary effect in response to leukocyte recruitment. The latter could be shown in an IRBP-induced B10.RIII mouse EAU model, where Evans blue dye, which was injected shortly before killing of the mice, could be observed leaking into the retinal parenchyma only after intravascular sticking and extravasation of blood-derived leukocytes from retinal venules took place (93). Further studies on murine EAU models implied that BRB breakdown might be a result of several consecutive steps, actively triggered by intravascular adherence of primed lymphocytes, followed by changed expression of adhesion molecules and chemokine receptors in the vascular epithelium and reduced shear stress in retinal veins prior to infiltration (93–97). Interestingly, adoptive transfer of EAU in lewis rats could show that antigen specificity for retinal antigens is not a prerequisite for migration of activated T cells through the BRB, however, for the induction of actual intraocular inflammation, it is indispensable (98).

Retina and Vitreous

After infiltrating the eye, peripheral immune cells accumulate in the iris, ciliary body and retina of horses with ERU (4, 27). Although the vast majority of the infiltrating cells were identified as CD4+ T cells (2, 27), pure granulocyte infiltrates can be observed in few spontaneous ERU and IRBP induced cases (3, 4). This is in stark contrast to the composition of the cellular infiltrate in IRBP-induced murine EAU models, where a more heterogeneous cell infiltrate can be observed, containing around 30% CD4+ T cells and a high percentage of macrophages (99, 100). This points to different molecular pathways and cellular processes in EAU and ERU, underlining the great variability of disease pathogenesis between species, which needs to be kept in

mind while interpreting associated data. In the base of the ciliary body and the iris of ERU horses, the infiltrated cells organize into lymphoid follicle nodules, which predominantly comprise CD3+ T cells (26, 27). In the retina, we mostly see scattered areas of T cell infiltration, especially near the ora ciliaris retinae and the optic disc, but occasional lymphoid follicle formation is also observed (27). Interestingly, the majority of infiltrated CD4+ lymphocytes in the ERU retina also show surface expression of CD166, a molecule associated with activation and transmigration of T cells into inflamed tissues (26). Presence of phosphorylated signal transducer and activator of transcription (STAT) proteins 1 and 5 in these cells underline the role of activated Th1 cells in ERU but also indirectly point toward inhibition of the Th17 pathway (26). Detection of IL-6, IL-17, and IL-23 in cell infiltrates of the ciliary body and iris, on the other hand, indirectly suggests that a Th17 mediated immune reaction might also present in ERU eyes (42). In rodent models, EAU can develop either as a Th1 or a Th17 response depending on the model and the antigen used for induction, showing the importance of both T cell subpopulations in disease pathogenesis (41, 101–104). In ERU, on the other hand, the important role of Th1 lymphocytes as effector cells is well known (2, 3, 36), the exact impact of Th17 cells on disease pathogenesis, however, merits further investigations.

Through membrane proteome analyses of equine retinae, numerous differentially expressed proteins were identified in ERU, several of which were associated to retinal Mueller glia cell derived proteins (105). Retinal Mueller glia cells, the main macroglial cells of the retina, are indispensable for the maintenance of retinal function and integrity (106). Apart from their proinflammatory role in ERU (20) and in murine EAU (65), these cells display a gliotic phenotype, which presents with morphological changes and impaired functionality in diseased retinae (12, 20, 105, 107). One of the important functions of these cells is the regulation of potassium and water homeostasis in the retina (108, 109), which shows severe misbalance in ERU (105, 107). This is mirrored in changed abundance and distribution pattern of potassium channels Kir4.1 and Kir2.1 as well as water channels AQP4, AQP5, and AQP11 which is hypothesized to contribute to impaired Mueller cell function and intracellular fluid regulation and, subsequently, retinal edema (105, 107, 110), a severe complication of ERU (27). Furthermore, in ERU, dysfunctional RMG are connected to decreased secretion of Wnt signaling inhibitors DKK3 and SFRP2, which point to a regulatory role of Wnt signaling in ERU (111), similar to other autoimmune diseases (112, 113). These insights on RMG support the role of these cells as prime responders to autoimmune triggers in ERU, promoting inflammatory processes and increasing severity of disease pathogenesis.

Additionally, severe extracellular matrix (ECM) remodeling in ERU was suggested through the identification of the proteins fibronectin and osteopontin (OPN) (105, 114). Fibronectin is an important constituent of the vertebrate ECM, mediates cell-ECM interactions (115) and also plays a role in adhesion and migration of cultured rat Mueller cells (116). Since fibronectin expression in Mueller cell endfeet might contribute to the attachment of the retina to the vitreous body, the changed distribution of fibronectin in ERU retinae points to impaired Mueller cell adhesion (114). OPN is a multifunctional protein with pro-inflammatory as well as neuroprotective properties (117, 118). In EAU mice, an increased abundance of OPN correlates with severity of inflammation (119), whereas Mueller glia-derived OPN promotes photoreceptor survival in the Pde6brd1 mouse model of retinal degeneration (120). OPN also shows neuroprotective properties in porcine Mueller glia cells *in vitro* (121). Decreased expression of OPN in vitreous and retina might therefore point to reduced neuroprotection in ERU retinae (114). ERU-associated lack of neuroprotection in the retina was underlined by the identification of decreased levels and decreased activity of tissue inhibitor of metalloproteinases (TIMP)-2 in retina and vitreous (122). TIMP2 influences the activity of metalloproteinases (MMP) (123), which modulate cell-cell and cell-ECM interactions (124). It also has neuroprotective properties (125) and can inhibit migration of cells over physiological barriers (126). Hence, decrease in TIMP2 activity might promote the inflammatory responses in ERU. It has been shown that migrating immune cells, especially Th1 cells, increase their MMP2 and MMP9 expression to overcome blood-tissue barriers (127) and that inhibition of these MMPs ameliorates experimental autoimmune uveitis in rodent models (128, 129). Interestingly, infiltrating cells in ERU retinae stained highly positive for MMP9 (122).

Apart from high levels of IgG in eyes of ERU horses (3), ERU vitreous also contains various immunoglobulins of the subclass M (IgM), which show a broad reaction pattern against retinal proteins (81), whereas healthy eyes contain no IgM (or IgG). Since IgM is an activator of the complement system and complement has been proven to be highly present in the inner eye of ERU horses (61, 111), the presence of IgM in the ERU vitreous further implies involvement of innate immune system components in this T cell driven autoimmune disease. Further screening of retinal protein with vitreous from ERU horses provided potential retinal autoantigens which are targeted by IgM autoantibodies (81). One of these IgM targets was identified as neurofilament medium (NF-M) (81, 130). Interestingly, IgG response to NF-M was merely detectable, supporting the presence of a thymus-independent immune reaction with prolonged persistence of IgM response toward NF-M and lack of IgM/IgG switch (130). In the retina, on the other hand, NF-M showed decreased expression (130). The combination of active downscaling in damaged RMG and release of NF-M fragments into the adjacent vitreous might cause this combination of high vitreal occurrence and low retinal levels of NF-M (130). A further novel membrane-bound autoantigen, synaptotagmin-1, was identified with decreased expression in ERU (131), which might be a result of active downscaling of this protein in the retina through impaired neurotransmitter release in ERU. Since Synaptotagmin-1 is also expressed in the pineal gland, and pinealitis is known to concurrently develop in ERU horses (132), synaptotagmin-1 might play a role in pineal immunopathology in ERU (131). Whether these IgM-targeted retinal antigens qualify as autoantigen targets for ERU, however, remains to be proven in further studies.

CONCLUSIONS

Overall, these novel immunological insights in ERU pathogenesis point to a complex interplay of several dysregulated mechanisms, which, on the one hand, can be linked to changes in cellular and humoral components of the immune system, such as a deviant functional phenotype of T cells with increased migratory ability and a decreased apoptosis rate, latently activated granulocytes and involvement of the alternative pathway of the complement system. On the other hand, this dysregulation points to a pivotal pro-inflammatory role of retinal cells with critically impaired function in the target organ itself. Although these findings shed more light on disease mechanisms, the interaction of these dysfunctional molecular mechanisms driving ERU as well as their exact individual role and timing in ERU pathogenesis needs further assessment. Establishment of a Mueller glia cell line (133) and characterization of cultured RPE cells (90) provide valuable tools for more in depth functional examinations of ERU pathology. Additionally, the possibility of commensal microbiota involvement in ERU onset needs to be addressed.

AUTHOR CONTRIBUTIONS

CD conceived the outline of the manuscript. CD and RD wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

Work from research group Deeg mentioned in this review was supported by grants from the Münchener Universitätsgesellschaft 31697 (to RD) and the Deutsche Forschungsgemeinschaft DFG DE 719/4-3 (to CD).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.