Published in final edited form as:

J Neuroimmunol. 2013 November 15; 264(0): . doi:10.1016/j.jneuroim.2013.08.010.

The Complement Anaphylatoxin Receptors Are Not Required for the Development of Experimental Autoimmune Uveitis

Russell W. Read^{a,b,c}, Susan D. Vogt^a, and Scott R. Barnum^c

^aDepartment of Ophthalmology, The University of Alabama at Birmingham, 1530 3rd Avenue South, Birmingham, AL 35294-1150, USA

^bDepartment of Pathology, The University of Alabama at Birmingham, 1530 3rd Avenue South, Birmingham, AL 35294-1150, USA

^cDepartment of Microbiology, The University of Alabama at Birmingham, 1530 3rd Avenue South, Birmingham, AL 35294-1150, USA

Abstract

To determine if complement anaphylatoxin-mediated inflammation contributes to the development and progression of experimental autoimmune uveitis (EAU), we induced disease in wild type and complement anaphylatoxin receptor-deficient mice (C3a receptor^{-/-}, C5a receptor^{-/-} and C3aR^{-/-}/C5aR^{-/-}) and evaluated eyes three weeks post-induction. No differences in disease severity or in disease incidence were seen between wild type controls and anaphylatoxin receptor-deficient mice. Our data indicate that C3a and C5a-mediated functions are not critical to the development of EAU.

Keywords

anaphylatoxins; complement; experimental autoimmune uveoretinitis

1. Introduction

Inflammation involving the uveal tract of the eye is termed uveitis and encompasses a wide range of conditions of importance to public health. Uveitis affects 52 of every 100,000 individuals per year in the United States (Gritz and Wong, 2004) and is estimated to be responsible for 5 to 20% of blindness (Durrani et al., 2004). Animal models of uveitis have revealed much about the immune response within the eye. In experimental autoimmune uveoretinitis (EAU), ocular inflammation is induced by peripheral immunization with retinal antigens or by adoptive transfer of retinal antigen-specific syngeneic CD4⁺ T-cells. Animals subsequently develop posterior segment inflammation, with evidence of retinal vasculitis, vitritis, and retinal and subretinal inflammatory infiltrates (Caspi et al., 1988). Activation of the complement system is involved in the pathogenesis of EAU (Bardenstein et al., 2001, Marak et al., 1979, Read et al., 2005, Sohn et al., 2000) as evidenced by the marked reduction in severity of disease when C3, the central component of the complement cascade,

© 2013 Elsevier B.V. All rights reserved.

Correspondence: Dr. Russell W. Read, University of Alabama at Birmingham, 700 18th Street South, Rm 601, Birmingham, AL 35233, 205-488-0756 (office), rwr@uab.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

is deleted or inhibited by treatment with the soluble regulatory protein sCrry (Read, Szalai, 2005). However, which of the complement anaphylatoxin receptors mediates the pro-inflammatory effects of complement during EAU remains unknown. To address the role of C3a and C5a mediated-inflammation in EAU, we induced disease in C3a receptor^{-/-} (C3aR^{-/-}), C5a receptor^{-/-} (C5aR^{-/-}) and C3aR^{-/-}/C5aR^{-/-} mice.

2. Material and methods

Mice

All experiments were carried out with approval of the University of Alabama at Birmingham Institutional Animal Care and Use Committee and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Generation of C3aR^{-/-} (Kildsgaard et al., 2000) and C5aR^{-/-} (Hopken et al., 1996) mice on the C57BL/6 background have been described previously. Inbred C57BL/6 mice, originally from The Jackson Laboratory (Bar Harbor, ME), were from our own colony. C3aR^{-/-}/C5aR^{-/-} mice were generated in our colony by crossing C3aR^{-/-} and C5aR^{-/-} mice. Eyes from all untreated mice appeared phenotypically normal with respect to ocular architecture and anatomy, both grossly and microscopically.

Induction and assessment of EAU

Human interphotoreceptor retinoid binding protein (IRBP) peptide 1–20 (GPTHLFQPSLVLDMAKVLLD) was synthesized using Fmoc chemistry and was >95% pure as determined by reversed phase-HPLC (Biosynthesis, Lewisville, TX). Mice were immunized subcutaneously in the right flank with 500 µg IRBP peptide emulsified in complete Freund's adjuvant (Difco, Detroit, MI) (1:1, vol/vol) supplemented with *Mycobacterium tuberculosis* strain H37RA (5 mg), and concurrently given 1.5 mg of purified Bordetella pertussis toxin (Sigma Chemical, St. Louis, MO) intraperitoneally (Avichezer et al., 2000). Eyes were collected from humanely euthanized mice on day 21 after immunization, the peak of disease in the IRBP EAU model (Avichezer, Silver, 2000). Freshly enucleated eyes were placed in OCT mounting medium and immediately snap-frozen at -80°C. Serial sections (10 µm) of each eye were then obtained and stained with hematoxylin-eosin. The incidence and severity of EAU was determined for each animal by grading histopathology on an ordinal 4-point scale (half-point increments) in a masked fashion as previously described (Chan et al., 1990). Twelve consecutive pairs of sections were graded for each animal (each pair comprised of one right-eye section and one left-eye section), with sections being evenly distributed across each globe. An EAU severity score was calculated for each animal by averaging the severity scores of all sections rated.

Statistics

Statistical analyses were carried out to determine the significance of differences in severity scores (Mann-Whitney test) and incidence of disease (Fisher's exact test) between wild type and C3aR^{-/-}, C5aR^{-/-} and C3aR^{-/-}/C5aR^{-/-} mice (Graphpad Prism 5.0). Results were considered significant when the *p* value was < 0.05.

3. Results

EAU in C3aR^{-/-}, C5aR^{-/-} and C3aR^{-/-}/C5aR^{-/-} mice is comparable to wild type mice

EAU was induced in wild type and complement anaphylatoxin-deficient mice and three weeks later eyes were removed for histopathological analysis. We found no difference in disease severity nor incidence between C3aR^{-/-} or C5aR^{-/-} and wild type mice (Table 1 and Fig. 1A and B, *p*>0.05 for both groups of mice, Mann-Whitney test). Because of the possibility of compensatory mechanisms occurring in the single anaphylatoxin-deficient

mice during EAU, we generated double anaphylatoxin receptor-deficient mice and performed EAU. Similar to C3aR^{-/-} and C5aR^{-/-} mice, we observed no difference in disease onset and severity in C3aR^{-/-}/C5aR^{-/-} mice compared to wild type mice (Table 1 and Fig. 1C, $p>0.05$, Mann-Whitney test).

4. Discussion

Complement mediates its host defense functions through multiple mechanisms: anaphylatoxin production (C3a and C5a), opsonization (C3b/iC3b), and the membrane attack complex (C5b-9). The anaphylatoxins serve to enhance the immune response by promoting degranulation of neutrophils, eosinophils, and mast cells, generation of reactive oxygen species, induction of proinflammatory cytokine production, and adhesion molecule expression. In addition, C3a and C5a are potent chemoattractants for numerous myeloid cell types and lymphocytes (reviewed in (Woodruff et al., 2011)). We thus theorized that a loss of anaphylatoxin production in C3^{-/-} mice and a reduced anaphylatoxin production in sCrry/GFAP transgenic mice were, in part, responsible for disease reduction observed in those studies (Read, Szalai, 2005). In this report, we examined the effect that deletion of one or both of the complement anaphylatoxin receptors had on the development and progression of EAU. No significant differences were seen between animals deficient in either anaphylatoxin receptor and wild type control mice. Unexpectedly, mice deficient in both receptors developed worse disease than wild type mice, but the difference was not statistically significant. The mechanism for more severe disease in the double-receptor-deficient mice is not clear, but may suggest that the anaphylatoxin receptors play a minor protective role in disease development as opposed to disease exacerbation. We cannot completely rule out a contribution of C5L2, a second more recently characterized C5aR (Ohno et al., 2000) to the phenotypes we report here, however, it seems unlikely given that this receptor lacks the ability to interact with G-coupled proteins and mobilize Ca⁺⁺ (Li et al., 2013, Sarma and Ward, 2012). The functional role for C5L2 in inflammatory diseases of all types remains controversial and unresolved (Li, Coulthard, 2013). Nevertheless, our data indicate a redundant role for the complement anaphylatoxins in EAU chemotactic-mediated mechanisms and point to IL-17, GRO- α , KC and MIP-2 as the dominant chemoattractants and TNF- α , IL-6, IFN- γ and other cytokines as more dominant pro-inflammatory mediators in EAU (Horai and Caspi, 2011, Kerr et al., 2008).

Our previous studies examining the role of C3 in EAU combined with the data we report here on the anaphylatoxin receptors suggest that the major contribution of complement to EAU pathophysiology is not from anaphylatoxin activity but rather occurs either at the level of C3 (or earlier) in the cascade or involves the membrane attack complex (MAC). Data on the role of MAC is contradictory, with our group finding that mice deficient in C5 (and thus unable to form the MAC) developed EAU comparable to that of wild type mice (data not shown). In contrast, Copland and coworkers found that anti-C5 antibodies resulted in a reduction in disease severity (Copland et al., 2010). Taken together, our data indicate inhibition of complement at the level of the C3 convertase may offer a new therapeutic avenue for EAU. Several well-characterized complement inhibitors act at the level of the C3 convertase and have been shown to be effective in several animal disease models and are now in the clinical pipeline (Ricklin and Lambris, 2007). These include small molecule inhibitors such as Compstatin and its derivatives and a variety of recombinant complement inhibitors. Given the important role that complement appears to play in the development of uveitis (Jha et al., 2007) it seems that clinical trials using complement inhibitors to control inflammation in uveitis are overdue.

Acknowledgments

This work was supported by NIH grants EY014189 (RWR and SDV) and NS46032 (SRB); and EyeSight Foundation of Alabama grant FY2002-03-16 (RWR and SDV). Additional support from unrestricted departmental grants from The EyeSight Foundation of Alabama and Research to Prevent Blindness. Dr. Read is a Research to Prevent Blindness Physician-Scientist.

References

- Avichezer D, Silver PB, Chan CC, Wiggert B, Caspi RR. Identification of a new epitope of human IRBP that induces autoimmune uveoretinitis in mice of the H-2b haplotype. *Investigative ophthalmology & visual science*. 2000; 41:127–31. [PubMed: 10634611]
- Bardenstein DS, Cheyer CJ, Lee C, Cocuzzi E, Mizuno M, Okada N, et al. Blockage of complement regulators in the conjunctiva and within the eye leads to massive inflammation and iritis. *Immunology*. 2001; 104:423–30. [PubMed: 11899428]
- Caspi RR, Roberge FG, Chan CC, Wiggert B, Chader GJ, Rozenszajn LA, et al. A new model of autoimmune disease. Experimental autoimmune uveoretinitis induced in mice with two different retinal antigens. *J Immunol*. 1988; 140:1490–5. [PubMed: 3346541]
- Chan CC, Caspi RR, Ni M, Leake WC, Wiggert B, Chader GJ, et al. Pathology of experimental autoimmune uveoretinitis in mice. *Journal of autoimmunity*. 1990; 3:247–55. [PubMed: 2397018]
- Copland DA, Hussain K, Baalasubramanian S, Hughes TR, Morgan BP, Xu H, et al. Systemic and local anti-C5 therapy reduces the disease severity in experimental autoimmune uveoretinitis. *Clinical and experimental immunology*. 2010; 159:303–14. [PubMed: 20002447]
- Durrani OM, Meads CA, Murray PI. Uveitis: a potentially blinding disease. *Ophthalmologica Journal international d'ophtalmologie International journal of ophthalmology Zeitschrift fur Augenheilkunde*. 2004; 218:223–36.
- Gritz DC, Wong IG. Incidence and prevalence of uveitis in Northern California; the Northern California Epidemiology of Uveitis Study. *Ophthalmology*. 2004; 111:491–500. discussion. [PubMed: 15019324]
- Hopken UE, Lu B, Gerard NP, Gerard C. The C5a chemoattractant receptor mediates mucosal defence to infection. *Nature*. 1996; 383:86–9. [PubMed: 8779720]
- Horai R, Caspi RR. Cytokines in autoimmune uveitis. *Journal of interferon & cytokine research: the official journal of the International Society for Interferon and Cytokine Research*. 2011; 31:733–44. [PubMed: 21787221]
- Jha P, Bora PS, Bora NS. The role of complement system in ocular diseases including uveitis and macular degeneration. *Molecular immunology*. 2007; 44:3901–8. [PubMed: 17768108]
- Kerr EC, Copland DA, Dick AD, Nicholson LB. The dynamics of leukocyte infiltration in experimental autoimmune uveoretinitis. *Progress in retinal and eye research*. 2008; 27:527–35. [PubMed: 18723108]
- Kildsgaard J, Hollmann TJ, Matthews KW, Bian K, Murad F, Wetsel RA. Cutting edge: targeted disruption of the C3a receptor gene demonstrates a novel protective anti-inflammatory role for C3a in endotoxin-shock. *J Immunol*. 2000; 165:5406–9. [PubMed: 11067891]
- Li R, Coulthard LG, Wu MC, Taylor SM, Woodruff TM. C5L2: a controversial receptor of complement anaphylatoxin, C5a. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*. 2013; 27:855–64. [PubMed: 23239822]
- Marak GE, Wacker WB, Rao NA, Jack R, Ward PA. Effects of complement depletion on experimental allergic uveitis. *Ophthalmic Res*. 1979; 11:97–107.
- Ohno M, Hirata T, Enomoto M, Araki T, Ishimaru H, Takahashi TA. A putative chemoattractant receptor, C5L2, is expressed in granulocyte and immature dendritic cells, but not in mature dendritic cells. *Molecular immunology*. 2000; 37:407–12. [PubMed: 11090875]
- Read RW, Szalai AJ, Vogt SD, McGwin G, Barnum SR. Genetic deficiency of C3 as well as CNS-targeted expression of the complement inhibitor sCrry ameliorates experimental autoimmune uveoretinitis. *Exp Eye Res*. 2005
- Ricklin D, Lambris JD. Complement-targeted therapeutics. *Nat Biotechnol*. 2007; 25:1265–75. [PubMed: 17989689]

- Sarma JV, Ward PA. New developments in C5a receptor signaling. *Cell health and cytoskeleton*. 2012; 4:73–82. [PubMed: 23576881]
- Sohn JH, Kaplan HJ, Suk HJ, Bora PS, Bora NS. Chronic low level complement activation within the eye is controlled by intraocular complement regulatory proteins. *Investigative ophthalmology & visual science*. 2000; 41:3492–502. [PubMed: 11006244]
- Woodruff TM, Nandakumar KS, Tedesco F. Inhibiting the C5-C5a receptor axis. *Molecular immunology*. 2011; 48:1631–42. [PubMed: 21549429]

Figure 1.

EAU severity is similar between $C3aR^{-/-}$, $C5aR^{-/-}$ and $C3aR^{-/-}/C5aR^{-/-}$ mice compared to wild type controls. EAU was induced as described in Materials and Methods in $C3aR^{-/-}$ ($n=10$), $C5aR^{-/-}$ ($n=13$), $C3aR^{-/-}/C5aR^{-/-}$ ($n=28$) and wild type mice ($n=15-27$). Shown are scattergram plots for all groups of mice and represent the sum of three to five independent experiments. Black bars represent mean for each group.

Table 1

Comparison of EAU severity scores and disease incidence between wild type and complement anaphylatoxin receptor-deficient mice.

Mouse Genotype	Severity Score	Disease Incidence
C3aR ^{-/-} (n=10)	0.79 *	80% (8/10)
Wild type (n=21)	0.75	81% (17/21)
C5aR ^{-/-} (n=13)	0.92 *	85% (11/13)
Wild type (n=15)	1.4	87% (13/15)
C3aR ^{-/-} /C5aR ^{-/-} (n=28)	0.39 *	79% (22/28)
Wild type (n=27)	0.25	74% (2/27)

* not significantly different from wild type mice