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Chapter 34

Should I Stay or Should I Go? Trafficking of Sub-Lytic MAC in the Retinal Pigment Epithelium

Aparna Lakkaraju, Kimberly A. Toops and Jin Xu

Abstract Assembly of sub-lytic C5b-9 membrane attack complexes (MAC) on the plasma membrane of retinal pigment epithelial cells contributes to the pathogenesis of age-related macular degeneration. C5b-9 pores induce calcium influx, which activates signaling pathways that compromise cell function. Mechanisms that limit sub-lytic MAC activity include: cell surface complement regulatory proteins CD46, CD55, and CD59 that inhibit specific steps of MAC formation; elimination of assembled MAC by exocytosis of membrane vesicles or by endocytosis and subsequent lysosomal degradation; and rapid resealing of pores by the exocytosis of lysosomes. Aging in the post-mitotic retinal pigment epithelium is characterized by the accumulation of cellular debris called lipofuscin, which has also been associated with retinal diseases such as age-related macular degeneration. Lipofuscin has been shown to activate complement components both in vitro and in vivo, suggesting that it could contribute complement-mediated dysfunction in the retinal pigment epithelium. Here, we discuss emerging evidence that vesicular trafficking in the retinal pigment epithelium is critical for efficient removal of MAC from the cell surface and for limiting inflammation in the outer retina.

Keywords Retinal pigment epithelium • Age-related macular degeneration • Inflammation • Lysosome exocytosis • Complement-regulatory proteins • Exosomes • Membrane integrity • Membrane attack complex

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Abbreviations

AMD	Age-related macular degeneration
ESCRT	Endosomal sorting complexes required for transport
GPI	Glycosylphosphatidylinositol
MAC	Membrane attack complex
RPE	Retinal pigment epithelium
SNARE	Soluble N-ethylmaleimide sensitive factor Attachment protein Receptor

34.1 Introduction

Age-related macular degeneration (AMD) is a multifactorial disease with many genetic and environmental factors that contribute to disease susceptibility and progression [1]. Development of effective therapies for the chronic version of the disease, called geographic atrophy or dry AMD, has been hampered by a lack of clear insight into disease mechanisms [2]. However, it is now accepted that the initial damage that eventually results in vision loss occurs in cells of the retinal pigment epithelium (RPE) [3, 4]. The alternative pathway of the complement system has been implicated in AMD pathogenesis. Complement factor H polymorphisms that reduce complement-inhibitory activity are associated with $\geq 50\%$ of AMD cases [3]. Many complement components, including activated proteins and complement-regulatory molecules, have been detected in drusen, the extracellular lipid-protein aggregates that accumulate beneath the RPE and are a risk factor for AMD [5, 6].

The final step in the complement cascade is the formation of the C5b-9 membrane attack complexes (MAC, also called “terminal complement complexes”) by a sequential assembly of C5b, C6, C7, C8, and C9 proteins on the cell membrane to form a lytic pore. The C9 protein is an amphipathic molecule that, when inserted in the cell membrane, polymerizes to form rigid channel. Deposition of a large number of MAC blasts holes in membranes, leading to cell lysis and death [7]. The earliest detectable event after MAC pore formation is a large influx of calcium into the cell. At high concentrations, calcium causes loss of mitochondrial membrane potential and eventually cell death. It is now appreciated that at low numbers or at “sub-lytic” doses, MAC pores do not cause cell lysis, but can nevertheless lead to compromised cell function. Since calcium is an important second messenger, a sustained influx of calcium into the cell can cause aberrant signaling and secretion of pro-inflammatory mediators [7, 8]. In the RPE in vitro, assembly of sub-lytic MAC either alone [9] or in combination with oxidative stress [10] has been shown to increase the secretion of interleukins IL-6, IL-8 and other pro-inflammatory proteins and pro-angiogenic proteins such as vascular endothelial growth factor. Further, lipofuscin and its major component, A2E, have been shown to activate complement both in vitro in the RPE and in vivo in the *abca4*^{-/-} mouse model of recessive Stargardt’s macular dystrophy [11, 12]. These data suggest that lipofuscin-mediated activation of complement and subsequent chronic inflammation could be one significant mechanism that contributes to AMD.

Fig. 34.1 Mechanisms that help cells remove or recover from damage induced by sub-lytic membrane attack complexes (MAC) deposition. 1 Complement-regulatory proteins on the cell membrane such as the GPI-anchored *CD55* and *CD59* and the transmembrane *CD46* inhibit specific steps of MAC assembly. 2 MAC can also be removed by shedding from the cell surface or by secretion of exosomes; complement-regulatory proteins can also be secreted via exosomes, presumably to protect neighboring cells from MAC attack. 3 Fusion of lysosomes with the plasma membrane in response to calcium influx (lysosome exocytosis) is a crucial survival mechanism that helps maintain membrane integrity. 4 MAC can also be removed from the cell membrane by endocytosis and subsequent lysosomal degradation. These mechanisms are discussed in detail in the text in relation to the RPE

Nucleated cells have evolved numerous ways to protect themselves from sub-lytic MAC attack (Fig. 34.1) including (1) cell-surface complement regulatory proteins, (2) secretion of anti-inflammatory proteins via exosomes and removal of MAC by surface shedding of microvesicles, (3) resealing MAC pores by lysosome exocytosis, and (4) endocytosis and subsequent lysosomal degradation of MAC [7, 13]. Here, we will review how these mechanisms operate in the RPE and how endo-lysosome trafficking is central to the efficient functioning of these protective mechanisms.

34.2 Membrane-Bound Complement-Regulatory Proteins

CD46, CD55, and CD59 are cell-surface complement-regulatory proteins that inhibit specific steps of MAC formation [14]. CD59 in particular blocks the assembly of the C9 pore and prevents MAC deposition [15]. CD46 is a trans-membrane protein whereas CD55 and CD59 are attached to the membrane by a glycosylphosphatidylinositol (GPI) anchor. GPI-anchored proteins preferentially associate with

cholesterol-enriched domains of the cell membrane and upon internalization and undergo slow recycling back to the cell surface [16]. Studies have shown that recycling of GPI-anchored proteins depends on the cholesterol content of the cell, and recycling kinetics can be accelerated by depleting the cell of cholesterol [17]; conversely, excess cholesterol diverts GPI-anchored proteins away from the recycling pathway towards the lysosomal degradative pathway. In both early and late AMD, studies have documented decreased expression of CD59 apically and CD46 basolaterally on the RPE [18, 19]. These data suggest that a decrease in the amount of cell-surface complement-regulatory proteins would facilitate the deposition of sub-lytic MAC on the RPE membrane and predispose towards inflammation.

34.3 Exosomes and Microvesicles

MAC can be removed from the plasma membrane either by the secretion of exosomes or by shedding of microvesicles from the cell surface [13]. Exosomes are internal vesicles of late endosomes that are released upon fusion of the late endosomal-limiting membrane with the plasma membrane [20]. Exosomes have emerged as an important mode of intercellular communication and have been implicated in various diseases including cancer, Alzheimer's disease, and Parkinson's disease [21, 22]. Exosomes released by antigen-presenting cells have CD55 and CD59, which presumably protect them from complement-mediated lysis [23]. Alternatively, complement-regulatory proteins carried by RPE cell exosomes could protect neighboring cells from MAC attack. Cells also shed MAC-enriched microvesicles, called "ectosomes," from the surface as a rapid mechanism to limit the residence time of MAC on the plasma membrane. In many cell types, including oligodendrocytes, platelets, erythrocytes, and neutrophils, multiple membrane protrusions enriched in C9 appear on the cell surface following MAC deposition [24, 25]. These protrusions are then shed from the cell by pinching off. In addition to MAC and complement-regulatory proteins, both exosomes and ectosomes are enriched in cell-specific repertoire of proteins and lipids, indicative of active sorting processes that contribute to vesicle biogenesis [13].

34.4 Membrane Repair

Rapid resealing of membrane holes is a crucial survival mechanism that is important for patching MAC pores and preventing further influx of calcium. The additional membrane required to reseal the pore is provided by organelles such as lysosomes, endosomes, or small vesicles. Calcium-induced fusion of lysosomes with the plasma membrane has emerged as an important mechanism of membrane repair in non-polarized cells [26]. This phenomenon, called lysosome exocytosis, involves the fusion of lysosomes docked at the cell periphery with the plasma membrane and

was first identified during cell invasion by the protozoan parasite *Trypanosoma cruzi* [27]. Lysosome exocytosis can also function as an exit route for cellular debris: In rat hepatocytes, secretion of copper-laden lysosomes into bile is one mechanism by which cells deal with excess copper [28]; in a mouse model of the lysosomal storage disease metachromatic leukodystrophy, lysosomes containing sulfatide are exocytosed in response to increased $[Ca^{2+}]_i$ [29]. Machinery required for Ca^{2+} -induced lysosome exocytosis in non-polarized cells includes the calcium sensor synaptotagmin VII, the vesicular soluble N-ethylmaleimide sensitive factor attachment protein receptor (v-SNARE) VAMP7, and the target SNAREs (t-SNAREs) SNAP23 and syntaxin 4 [30]. A localized increase in intracellular calcium induces a conformational change in synaptotagmin VII, which then allows the formation of the four helical SNARE bundles involving the v- and t-SNAREs [26]. We recently investigated the mechanism of lysosome exocytosis in polarized epithelial cells in response to calcium ionophores and pore-forming toxins. Our data implicate the actin cytoskeleton, membrane cholesterol content, and the t-SNARE syntaxin 4 in polarized lysosome exocytosis [31]. It is therefore likely that in the RPE, lysosome exocytosis participates in membrane repair following MAC deposition (Toops, Xu, Lakkaraju, et al., in preparation).

34.5 Endocytosis and Degradation

MAC and other pore-forming toxins can also be removed by rapid endocytosis and subsequent lysosomal degradation [32, 33]. Once internalized, MAC can be transported to late endosomes or multivesicular bodies and secreted out as exosomes. Alternatively, MAC can be trafficked to the lysosome for degradation. In erythroleukemic cells, recent work shows that endocytosis of MAC occurs by a clathrin-independent, caveolin-1-dependent route [32]. Endocytosis of MAC requires membrane cholesterol (which maintains the structure of caveolae) and the large GTPase dynamin-2, which is required for fission of endocytic vesicles. In normal rat kidney cells, the pore-forming toxin streptolysin O first induces lysosome exocytosis to facilitate membrane repair, followed by rapid removal of streptolysin O pores by internalization into endosomes. The toxin is then targeted to lysosomes by ubiquitination/ESCRT-dependent sorting and subsequently degraded [33]. It remains to be seen if the endosomal sorting complexes required for transport (ESCRT) machinery is also required for lysosomal degradation of MAC.

34.6 Conclusions and Perspectives

The RPE, like other nucleated cells, has numerous protective mechanisms that help limit damage caused by sub-lytic MAC deposition. The studies summarized in this review indicate that many, if not all, of these processes depend on efficient endo-

lysosome trafficking in the RPE. We have shown that the lipofuscin component A2E causes cholesterol storage in RPE late endosomes and lysosomes [34], and this excess cholesterol inhibits organelle motility (Toops, Xu, Lakkaraju, et al., in preparation). In agreement with this hypothesis, we have recently shown that excess cholesterol inhibits lysosome exocytosis in polarized epithelia [31]. Ongoing work in our laboratory is focused on how lipofuscin and cholesterol accumulation affect lipid-protein trafficking, secretion of exosomes, and lysosome exocytosis in the RPE. With age, declining lysosome function in the RPE can lead to a reciprocal increase in lipofuscin formation and, possibly, cholesterol storage, which can in turn cause intracellular traffic jams and interfere with endo-lysosome function. A decrease in the efficiency of protective mechanisms that help the cell remove and recover from sub-lytic MAC attack would compromise RPE membrane integrity, lead to aberrant signaling, and likely contribute to a chronic inflammatory environment in the outer retina.

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References

1. Swaroop A, Chew EY, Rickman CB, Abecasis GR (2009) Unraveling a multifactorial late-onset disease: from genetic susceptibility to disease mechanisms for age-related macular degeneration. *Annu Rev Genomics Hum Genet* 10:19–43
2. Bird AC (2010) Therapeutic targets in age-related macular disease. *J Clin Invest* 120(9):3033–3041
3. Bok D (2005) Evidence for an inflammatory process in age-related macular degeneration gains new support. *Proc Natl Acad Sci U S A* 102(20):7053–7054
4. Ambati J, Fowler BJ (2012) Mechanisms of age-related macular degeneration. *Neuron* 75(1):26–39
5. Anderson DH, Mullins RF, Hageman GS, Johnson LV (2002) A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol* 134(3):411–431
6. Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, Hageman JL, Stockman HA, Borchardt JD, Gehrs KM, Smith RJ, Silvestri G, Russell SR, Klaver CC, Barbazetto I, Chang S, Yannuzzi LA, Barile GR, Merriam JC, Smith RT, Olsh AK, Bergeron J, Zernant J, Merriam JE, Gold B, Dean M, Allikmets R (2005) A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A* 102(20):7227–7232
7. Cole DS, Morgan BP (2003) Beyond lysis: how complement influences cell fate. *Clin Sci* 104(5):455–466
8. Bohana-Kashtan O, Ziporen L, Donin N, Kraus S, Fishelson Z (2004) Cell signals transduced by complement. *Mol Immunol* 41(6-7):583–597
9. Lueck K, Wasmuth S, Williams J, Hughes TR, Morgan BP, Lommatzsch A, Greenwood J, Moss SE, Pauleikhoff D (2011) Sub-lytic C5b-9 induces functional changes in retinal pigment epithelial cells consistent with age-related macular degeneration. *Eye* 25(8):1074–1082
10. Kunchithapautham K, Rohrer B (2011) Sublytic membrane-attack-complex (MAC) activation alters regulated rather than constitutive vascular endothelial growth factor (VEGF) secretion in retinal pigment epithelium monolayers. *J Biol Chem* 286(27):23717–23724

11. Radu RA, Hu J, Yuan Q, Welch DL, Makshanoff J, Lloyd M, McMullen S, Travis GH, Bok D (2011) Complement system dysregulation and inflammation in the retinal pigment epithelium of a mouse model for Stargardt macular degeneration. *J Biol Chem* 286(21):18593–18601
12. Sparrow JR, Ueda K, Zhou J (2012) Complement dysregulation in AMD: RPE-Bruch's membrane-choroid. *Mol Aspects Med* 33(4):436–445
13. Pilzer D, Gasser O, Moskovich O, Schifferli JA, Fishelson Z (2005) Emission of membrane vesicles: roles in complement resistance, immunity and cancer. *Springer Semin Immunopathol* 27(3):375–387
14. Longhi MP, Harris CL, Morgan BP, Gallimore A (2006) Holding T cells in check—a new role for complement regulators? *Trends Immunol* 27(2):102–108
15. Meri S, Morgan BP, Davies A, Daniels RH, Olavesen MG, Waldmann H, Lachmann PJ (1990) Human protectin (CD59), an 18,000–20,000 MW complement lysis restricting factor, inhibits C5b-8 catalysed insertion of C9 into lipid bilayers. *Immunology* 71(1):1–9
16. Mayor S, Riezman H (2004) Sorting GPI-anchored proteins. *Nat Rev Mol Cell Biol* 5(2):110–120
17. Mayor S, Sabharanjak S, Maxfield FR (1998) Cholesterol-dependent retention of GPI-anchored proteins in endosomes. *EMBO J* 17(16):4626–4638
18. Ebrahimi KB, Fijalkowski N, Cano M, Handa JT (2012) Decreased membrane complement regulators in the retinal pigmented epithelium contributes to age-related macular degeneration. *J Pathol* 229(5):729–742
19. Vogt SD, Curcio CA, Wang L, Li CM, McGwin G Jr, Medeiros NE, Philp NJ, Kimble JA, Read RW (2011) Retinal pigment epithelial expression of complement regulator CD46 is altered early in the course of geographic atrophy. *Exp Eye Res* 93(4):413–423
20. Lakkaraju A, Rodriguez-Boulan E (2008) Itinerant exosomes: Emerging roles in cell and tissue polarity. *Trends Cell Biol* 18:199–209
21. Thery C, Ostrowski M, Segura E (2009) Membrane vesicles as conveyors of immune responses. *Nature reviews. Immunology* 9(8):581–593
22. Bellingham SA, Guo BB, Coleman BM, Hill AF (2012) Exosomes: vehicles for the transfer of toxic proteins associated with neurodegenerative diseases? *Frontiers Physiol* 3:124
23. Clayton A, Harris CL, Court J, Mason MD, Morgan BP (2003) Antigen-presenting cell exosomes are protected from complement-mediated lysis by expression of CD55 and CD59. *Eur J Immunol* 33(2):522–531
24. Scolding NJ, Morgan BP, Houston WA, Linington C, Campbell AK, Compston DA (1989) Vesicular removal by oligodendrocytes of membrane attack complexes formed by activated complement. *Nature* 339(6226):620–622
25. Moskovich O, Fishelson Z (2007) Live cell imaging of outward and inward vesiculation induced by the complement c5b-9 complex. *J Biol Chem* 282(41):29977–29986
26. Idone V, Tam C, Andrews NW (2008) Two-way traffic on the road to plasma membrane repair. *Trends Cell Biol* 18(11):552–559
27. Andrews NW (2002) Lysosomes and the plasma membrane: trypanosomes reveal a secret relationship. *J Cell Biol* 158(3):389–394
28. Gross JB Jr, Myers BM, Kost LJ, Kuntz SM, LaRusso NF (1989) Biliary copper excretion by hepatocyte lysosomes in the rat. Major excretory pathway in experimental copper overload. *J Clin Invest* 83(1):30–39
29. Klein D, Bussow H, Fewou SN, Gieselmann V (2005) Exocytosis of storage material in a lysosomal disorder. *Biochem Biophys Res Commun* 327(3):663–667
30. Rao SK, Huynh C, Proux-Gillardeaux V, Galli T, Andrews NW (2004) Identification of SNAREs involved in synaptotagmin VII-regulated lysosomal exocytosis. *J Biol Chem* 279(19):20471–20479
31. Xu J, Diaz F, Carvajal-Gonzalez JM, Mazzoni F, Schreiner R, Rodriguez-Boulan E, Lakkaraju A (2013) Mechanism of polarized lysosome exocytosis in epithelial cells. *J Cell Sci* 2013:In-Press
32. Moskovich O, Herzog LO, Ehrlich M, Fishelson Z (2012) Caveolin-1 and dynamin-2 are essential for removal of the complement C5b-9 complex via endocytosis. *J Biol Chem* 287(24):19904–19915

33. Corrotte M, Fernandes MC, Tam C, Andrews NW (2012) Toxin pores endocytosed during plasma membrane repair traffic into the lumen of MVBs for degradation. *Traffic* 13(3):483–494
34. Lakkaraju A, Finnemann SC, Rodriguez-Boulan E (2007) The lipofuscin fluorophore A2E perturbs cholesterol metabolism in retinal pigment epithelial cells. *Proc Natl Acad Sci U S A* 104(26):11026–11031