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Chapter 34
Should I Stay or Should I Go? Trafficking of Sub-Lytic MAC in the Retinal Pigment Epithelium

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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 ≥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.

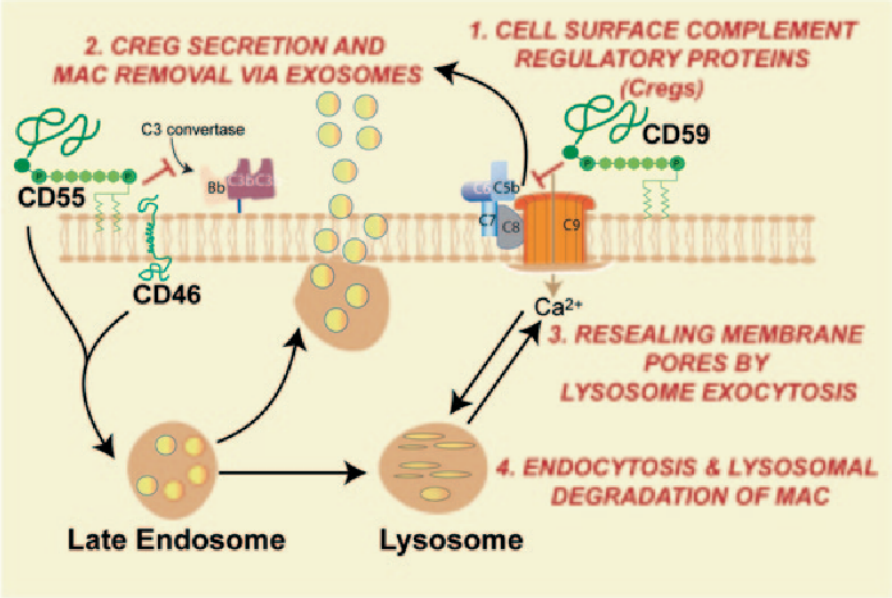

Fig. 34.1 Mechanisms that help cells remove or recover from damage induced by sub-lytic membrane attack complexes (MAC) deposition. *1* Complement-regulatory proteins on the cell membrane such as the GPI-anchored *CD55* and *CD59* and the transmembrane *CD46* inhibit specific steps of MAC assembly. *2* 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 syntaptotagmin 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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