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Review

The membrane attack complex as an inflammatory trigger

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ABSTRACT

The final common pathway of all routes of complement activation involves the non-enzymatic assembly of a complex comprising newly formed C5b with the plasma proteins C6, C7, C8 and C9. When assembly occurs on a target cell membrane the forming complex inserts into and through the bilayer to create a pore, the membrane attack complex (MAC). On some targets, pore formation causes rapid lytic destruction; however, most nucleated cell targets resist lysis through a combination of ion pumps, membrane regulators and active recovery processes. Cells survive but not without consequence. The MAC pore causes ion fluxes and directly or indirectly impacts several important signalling pathways that in turn activate a diverse series of events in the cell, many of which are highly pro-inflammatory. Although this non-lytic, pro-inflammatory role of MAC has been recognised for thirty years, no consensus signalling pathway has emerged. Recent work, summarised here, has implicated specific signalling routes and, in some cells, inflammasome involvement, opening the door to novel approaches to therapy in complement-driven pathologies.

Contents

The membrane attack complex

The membrane attack complex (MAC), the cytolytic coup de grace of complement activation, is a membrane-traversing pore formed from the five terminal pathway component proteins. It is a member of a large and heterogeneous group of pore-forming proteins that play roles in attack and defence in organisms from bacteria to man (Rosado et al., 2008; Iacovache et al., 2008; Gilbert et al., 2014). All in this diverse group share the capacity to create physical or functional pores that breach biological membranes, but the pore structures vary considerably. Two pore-forming protein complexes play important roles in mammalian immunity; perforin, a protein present in granules of cytolytic T cells and NK cells that binds target membranes and oligomerises to form pores, and the MAC (Kondos et al., 2010). There are numerous structural and functional similarities between these immune effectors that are beyond the scope of this brief review.

MAC assembly begins with cleavage of C5 by a C5 convertase enzyme of the classical/lectin (C4b2a3b) or alternative (C3bBbC3b) pathways (Pangburn and Rawal, 2002). This cleavage is the final enzymatic event in the complement pathway and the first step in the terminal pathway (Müller-Eberhard, 1985; Esser, 1994). The biologically potent pro-inflammatory peptide C5a is released, leaving the large C5b fragment still attached to its parent enzyme. Nascent C5b binds the plasma proteins C6 and C7, triggering a conformational change that releases the tri-molecular C5b67 complex to the fluid phase and creates a labile hydrophobic membrane binding site in the complex. The large majority of C5b67 complexes formed decay in the fluid phase through hydrolysis of the

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membrane binding site and/or binding fluid-phase inhibitors that include clusterin, vitronectin and, notably, C8 – the next component in the sequence. Those fortunate few that encounter membrane before the binding site decays or is blocked by protein inhibitors, lock tightly onto the membrane. Bound C5b67 sequentially recruits C8 and multiple copies of C9 from plasma, and induces major conformational change in these molecules, unfolding and aggregating as the complex embeds more deeply into the membrane, finally creating a transmembrane pore containing one molecule each of C5b, C6, C7 and C8 with as many as twelve C9 molecules, these forming the walls of the MAC pore (Podack and Tschopp, 1984).

MAC assembly in the membrane is regulated by CD59, a 20 kDa glycolipid-anchored protein that binds tightly into the forming MAC at the C5b-8 stage and prevents further recruitment of C9 into the complex – thereby preventing pore formation (Davies and Lachmann, 1993). CD59 is broadly expressed on human cells and in all mammalian species studied.

Sublytic MAC as an activator of target cells

The MAC has evolved to deliver lytic killing of pathogens; indeed, deficiencies of MAC component proteins predispose to infection, albeit only for *Neisseria* species infections (Ram et al., 2010). When complement is activated on self-cells, multiple defence mechanisms and regulators limit MAC assembly and accelerate MAC removal from the membrane (Morgan, 1989). Self-cells are thus protected from lytic killing in all but the most extreme circumstances. CD59 on the cell surface restricts pore formation but, because it is a suicide inhibitor consumed in the act of inhibition, may become depleted. Pores that do form in the membrane cause inward leakage of water and ions – events that in metabolically inert targets, such as aged erythrocytes, progress to lysis but in nucleated cells are countered by ion pumps. Pumps consume energy and can be overwhelmed if inward leakage continues; however, nucleated cells also actively remove MAC lesions from the membrane, either by budding off (ectocytosis) or engulfment (endocytosis). MAC lesions are thus transient, allowing cells to survive.

Assembly of pores at sublytic levels in nucleated cell membranes is not without consequence; many different effects have been described in different cell types, including on cell cycle and proliferation (either enhancing or inhibiting), apoptosis (accelerating or delaying), protein synthesis, membrane lipid composition, granule release *etc.* (Morgan, 1992; Cole and Morgan, 2003; Elimam et al., 2013; Takano et al., 2013). Proinflammatory consequences of sublytic MAC have been reported in many cell types. Neutrophils (and macrophages) were induced to synthesise and secrete inflammatory cytokines and triggered to degranulate, releasing their arsenal of inflammatory mediators (Morgan, 1992). Sublytic MAC triggered mesangial cells and microglia to release inflammatory cytokines (Zhang et al., 2014; Yang et al., 2014). Retinal epithelial cells exposed to sublytic MAC were stimulated to release IL-6, IL-8, MCP-1, and VEGF (Lueck et al., 2011). Platelet activation by MAC in the absence of lysis is described in many reports; effects include release of microparticles and surface changes causing increased stickiness (Martel et al., 2011).

Signalling of sublytic effects of MAC

Among the ions entering the cell, Ca^{2+} is particularly relevant to downstream effects because of the large concentration gradient and the importance of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) as a first signal for cell activation. Ca^{2+} influx through the pore increases $[Ca^{2+}]i$ that in turn triggers Ca^{2+}-activated Ca^{2+} release from endoplasmic reticulum stores; as a result, $[Ca^{2+}]i$ increases from low nanomolar resting levels into the micromolar range within seconds (Morgan, 1989). Increased $[Ca^{2+}]i$ engages intracellular signalling pathways by binding multiple cytoplasmic Ca^{2+} binding proteins, notably calmodulin. When $[Ca^{2+}]i$ exceeds a critical threshold, Ca^{2+} ions bind calmodulin, triggering major conformational changes that enable activation of downstream calmodulin-dependent kinases to drive events in the cell (Fig. 1).

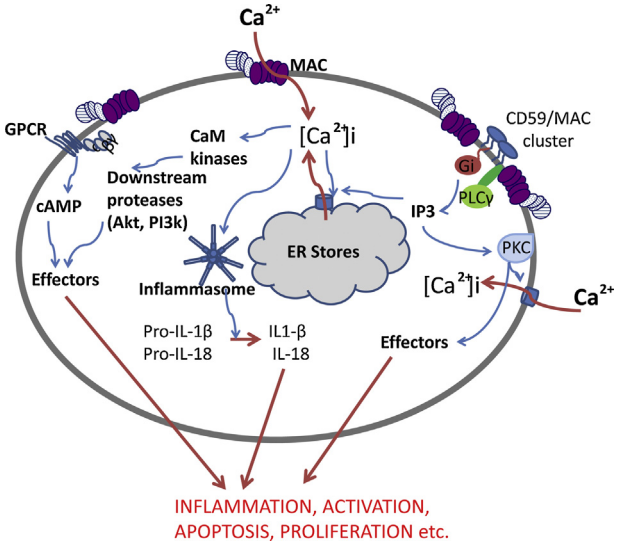

Fig. 1. *MAC pathways to cell activation and inflammation.* The MAC pore permits Ca^{2+} influx thereby increasing intracellular Ca^{2+} concentration ($[Ca^{2+}]i$). Elevated cytosolic $[Ca^{2+}]i$ triggers the opening of Ca^{2+} channels in the endoplasmic reticulum (ER) and release of Ca^{2+} from stores, further elevating $[Ca^{2+}]i$. Calmodulin (CaM) is activated by increased $[Ca^{2+}]i$ and switches on multiple downstream proteases, including Akt and PI3k (phosphatidylinositol-3-kinase), that in turn trigger multiple downstream effectors of inflammation and other activation events. Increased $[Ca^{2+}]i$ also triggers assembly and activation of the NLRP3 inflammasome with resultant cleavage of the pro-forms of the inflammatory cytokines IL-8 and IL-1β to generate the active, secreted cytokines. MAC may also cause cell activation through interactions with other signalling molecules in the membrane. Association with G-protein-coupled receptors may enable MAC to engage cAMP (cyclic adenosine monophosphate)-mediated activation pathways. Clustering with the GPI-anchored MAC regulator CD59 may trigger GPI-associated G-proteins and phospholipases that generate IP3 (inositol-3-phosphate) with resultant opening of Ca^{2+} channels in the plasma membrane and ER and activation of downstream effectors.

Although the prevailing evidence supports the concept that $[Ca^{2+}]i$, through engagement of Ca^{2+}-dependent signalling pathways is the principle mediator of sublytic MAC effects, MAC induces activation in some cell types even when Ca^{2+} influx is prevented by removal of extracellular Ca^{2+} and/or intracellular Ca^{2+} chelation. A direct interaction of the MAC with the Giα-subunit, classically linked to G-protein-coupled receptor family members, has been described (Niculescu et al., 1997), providing a possible Ca^{2+}-independent route to regulation of cyclic AMP (cAMP) production. Precisely how MAC, formed from five plasma proteins with no obvious G-protein binding motifs, intercalates with the Giα-subunit and other signal transducers in the membrane is unresolved. The recent emergence of evidence showing that MAC interacts functionally and physically with toll receptors, GPCRs and other signalling receptors in the membrane provides a possible explanation (Liu et al., 2011; Mastellos et al., 2013). No structure/function explanation has yet emerged for these MAC interactions, although it has been suggested that MAC localises to the same membrane microdomains as these receptors (Morgan et al., 1987; Hänsch 1992; Dunstone and Tweten, 2012).

The membrane regulator of MAC assembly, CD59, has been implicated in MAC signalling. CD59 is a glycosyl phosphoinositol lipid (GPI)-anchored protein and, in common with other GPI-anchored proteins, has the capacity to signal when cross-linked

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