REVIEW

Complement Membrane Attack Complex

New Roles, Mechanisms of Action, and Therapeutic Targets

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The complement membrane attack complex (MAC) is classically known as a cytolytic effector of innate and adaptive immunity that forms pores in the plasma membrane of pathogens or targeted cells, leading to osmolysis. Nucleated cells resist MAC-mediated cytotoxicity by expression of inhibitors that block MAC assembly or by rapid removal of MAC through endocytosis or shedding. In the absence of lysis, MAC may induce intracellular signaling and cell activation, responses implicated in a variety of autoimmune, inflammatory, and transplant disease settings. New discoveries into the structure and biophysical properties of MAC revealed heterogeneous MAC precursors and conformations that provide insights into MAC function. In addition, new mechanisms of MAC-mediated signaling and its contribution to disease pathogenesis have recently come to light. MAC-activated cells have been found to express proinflammatory proteins—often through NF- κ B—dependent transcription, assemble inflammasomes, enabling processing, and facilitate secretion of IL-1 β and IL-18, as well as other signaling pathways. These recent insights into the mechanisms of action of MAC provide an updated framework to therapeutic approaches that can target MAC assembly, signaling, and proinflammatory effects in various complement-mediated diseases. (*Am J Pathol* 2020, 190: 1138–1150; <https://doi.org/10.1016/j.ajpath.2020.02.006>)

Complement membrane attack complex (MAC) was first identified as an effector of the immune system in which certain classes of antibodies trigger assembly of a pore-forming complex that inserts into the lipid bilayer of the plasma membrane (the classic pathway), complementing the ability of antibodies to kill cells.¹ It was subsequently discovered that MAC could also be formed in response to cell-bound mannose-binding lectin (the lectin pathway) or C3 tickover (the alternative pathway). All three pathways converge on the C3b-mediated proteolytic cleavage of C5 to form C5a and C5b and the C5b-mediated sequential assembly of C5b-7, C5b-8, and MAC (C5b-9), referred to as the terminal complement cascade.² Early studies using aged erythrocytes and antibodies against red blood cell membranes to trigger the complement cascade demonstrated the dramatic effects of complement-induced osmotic cell lysis (osmolysis).³ The lytic function of MAC serves as an important defense against Gram-negative bacteria, enveloped viruses, and parasites; and genetic deficiencies in

terminal complement proteins C5 through C9 predispose to recurrent infections, particularly the *Neisseria* species.¹

In contrast to erythrocytes and microbes, nucleated mammalian cells are typically resistant to osmolysis by MAC, although this resistance can be overcome experimentally by activation of (heterologous) complement proteins from a different species. Nucleated cells have multiple defense mechanisms to resist MAC lytic killing. Complement regulatory factors on human cells, including CD46, CD55, and CD59, inhibit early complement activation and amplification, preventing MAC pore assembly. Often, the function of these proteins is species restricted, a phenomenon called homologous restriction, explaining the lytic effects of heterologous complement.⁴ Other defense

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mechanisms include removal from the plasma membrane by shedding of membrane fragments (ectocytosis) or by internalization (endocytosis).^{5,6} In the absence of lysis, MAC may initiate signaling pathways that induce a variety of biological responses consistent with cell activation, including up-regulation of adhesion molecules,⁷ release of proinflammatory cytokines,^{8,9} and increased cell proliferation.^{10,11} MAC-activated cells have enhanced immunogenicity and increased ability to activate adaptive immune responses, which can propagate disease pathogenesis.^{9,12,13}

The precise mechanisms of action of MAC on different cell types and its proinflammatory consequences are an area of active investigation. This review discusses how recent advances in MAC structure and formation inform our understanding of how it can modulate cell activation. Recent discoveries elucidating novel proinflammatory intracellular signaling events initiated by MAC that lead to cell activation and altered cell function will also be discussed, and the role of MAC signaling in human diseases will be reviewed. Finally, new potential therapeutic strategies to inhibit the effects of MAC through targeting its assembly or signaling will also be reviewed.

Recent Advances in MAC Assembly and Structure and Implications for Mechanisms of Action

New understanding of MAC-mediated osmolysis or signaling has come from insights into MAC structure, assembly, and interactions with plasma membrane components. MAC is generated through sequential assembly from the soluble complement proteins C5b, C6, C7, C8, and C9. Experimentally, MAC can be assembled using heterologous sera⁴; zymosan-activated sera, which activates complement in the fluid phase by the alternative pathway and in the absence of cell membranes to generate soluble MAC¹⁴; sequential addition of recombinant C5b6, C7, C8, and C9; and high-titer panel reactive antibodies, which react primarily with nonself class I and class II major histocompatibility complex molecules on endothelial cells and assemble MAC by the classic pathway.^{9,13,15} MAC sequential assembly on nucleated cells using minimally diluted concentrations of complement components is largely noncytolytic,¹⁶ and forms of MAC deposit on but do not fully insert into cell membranes.⁷ The various methods of complement activation lead to MAC structures with differential interactions with the plasma membrane and complement regulatory proteins, leading to differential cytolytic and proinflammatory activities.

Recent studies have visualized the heterogeneity of MAC structures and their varied degrees of membrane insertion and distortion using cryogenic electron microscopy. As C5 is cleaved by C3b to yield C5a and C5b, C5b rapidly binds C6. On recruitment of C7, C5bC6C7 is stabilized by

binding to the outer lamella of the plasma membrane. Recruitment of C8 leads to formation of the C5bC6C7C8 (C5b-8) initiator complex that allows for the initial insertion into the membrane. This partially inserted initiation complex acts as a nucleation site and recruits soluble C9 to propagate pore growth. The incorporation of the first C9 is a rate-limiting step, which lowers the energy for bending the lipid bilayer, and additional soluble C9 monomers may be recruited after membrane insertion.¹⁷ Pore formation is rapidly completed in a unidirectional, clockwise oligomerization.¹⁸ Although multiple plasma-soluble regulatory factors inhibit C3 and C5 convertase formation, CD59 is the only membrane-associated inhibitor in human cells and acts by inhibition of terminal complement activation. CD59 acts by either binding to the C5b-8 precursor or after adding C9 to prevent further oligomerization.¹⁸ Thus, MAC precursors and terminal complexes have distinct interactions with and do not all transverse the lipid bilayer.

MAC was classically believed to be a rigid trans-membrane pore, allowing water and ions to passively traverse and cause osmolysis, as observed in experiments on aged erythrocytes. Recent experiments revealed MAC to be a flexible immune pore with open and closed conformations (Figure 1). Moreover, MAC is not assembled into a symmetric and completely closed ring. Instead, MAC assumes an asymmetrical split-washer configuration composed of three regions: the asymmetric region (C5b, C6, C7, and C8), a hinge region (C7, C8, and two C9), and a C9 oligomer.¹⁹ In the open conformation, the asymmetric region juts into the lumen to exaggerate the split-washer conformation and MAC has a 30-Å wide chasm traversing the length of the pore (Figure 1A). The asymmetric region can rotate with respect to the C9 oligomer to vary the curvature of C9, and the chasm is sealed in the closed conformation (Figure 1B). It is not clear if these different conformations affect the patency of the pore that is formed, but alternative conformations of MAC may differ in their effects on membrane curvature, elimination from the plasma membrane (discussed below), or pore-independent pathways of signal induction.²⁰

The plasticity of the MAC pore structure and how MAC interacts with the lipid bilayer have implications for its subsequent mechanisms of action that have yet to be fully characterized.^{19,21} For instance, the membrane-interacting β -hairpin structures composing the MAC pore differ in length and charge properties. Moreover, the MAC β -pore barrel and scaffold composition were observed to be highly glycosylated, and glycan removal altered the structural integrity of the β -barrel and led to irregular pore structures.¹⁹ Mutational variants of C9 discovered in patients with the complement-associated disease, age-related macular degeneration, revealed that changes affecting the negatively charged patch on C9 altered recruitment of C9 by its positively charged face and the rate of C9 self-polymerization.¹⁹ These structural variants, thereby, influence MAC assembly and interactions with target cells, and may also

Figure 1 Conformational flexibility of membrane attack complex (MAC) pore structure. **A:** Open conformation of MAC pore based on cryogenic electron microscopy reconstruction,¹⁹ demonstrating a split-washer configuration with a 30-Å wide chasm running the length of the pore. The asymmetric region (C5b, C6, C7, and C8) juts into the lumen of the β-barrel pore by the hinge region (C7, C8, and two C9s), as shown in cross-section. **B:** The chasm is sealed in the closed conformation of MAC pore by the asymmetric region rotating out to meet the C9 oligomer (18 C9 monomers) to vary the curvature of C9 and generating an asymmetric β-barrel pore. The transmembrane regions are indicated, and the cross-sectional views shown are indicated by the **dashed lines** in the side views.

alter the efficacy of CD59 inhibition, which associates directly with MAC components to inhibit C9 oligomerization. Although MAC does not have any known surface receptors or lipid dependency for its binding, MAC is observed to be aggregated in specific lipid microdomains and glycosylphosphatidylinositol (GPI)-anchored proteins.²² This clustering may be due to the biophysical properties of the β-barrel pore, asymmetry, or conformations that confer a selectivity. Clustering of MAC on the cell membrane may be a process regulated by the targeted cell to control membrane distortion or destabilization and aid more efficient host cell recovery by MAC endocytosis or shedding.

The heterogeneity of MAC precursors and terminal structures, conformations, and membrane interactions on various cell types are indicative that a single mechanism of action downstream of MAC is unlikely. Further study on what factors mediate conformational changes of the MAC pore and the interplay between the host cell, the various MAC intermediaries and conformations, and the environmental milieu that may influence MAC assembly, elimination, and downstream MAC-initiated intracellular signaling remain to be more precisely elucidated. Human disease-associated variants and mutations will be informative in

studying abnormalities in MAC assembly, structure, and signaling.

MAC Initiates Intracellular Signaling that Can Induce Cell Activation and Alter Cell Function

MAC Signaling Initiated by Ion Flux Across the Plasma Membrane

As a pore-forming complex, MAC insertion in the plasma membrane of a cell can lead to collapse of physiological ion gradients across this barrier. For example, K^+ ion efflux is a signal for ATP entry and nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3) inflammasome assembly (Figure 2A).²³ At the same time, large increases in Ca^{2+} ion concentrations in the cytosol can activate prolytic factors, such as calpain, Bcl-2 homology 3 domain (BH3) interacting domain death agonist, receptor-interacting serine/threonine-protein kinase 1, receptor-interacting serine/threonine-protein kinase 3, and mixed lineage kinase domain-like protein.^{24,25} Elevated cytosolic Ca^{2+} ion concentrations also trigger a large number of cell survival promoting events mediated by activation of calcium-binding proteins, such as calmodulin,

Figure 2 Membrane attack complex (MAC)—initiated intracellular signaling pathways lead to altered cell functions and cell activation. **A:** On complement activation, MAC deposition, and insertion into the plasma membrane, MAC can induce downstream prolytic and prosurvival, proliferative, and proinflammatory pathways that are dependent on MAC functioning as a pore and allowing small-molecule or ion flux, including potassium (K^+) efflux, ATP entry, or calcium (Ca^{2+}) influx. **B:** MAC can induce signaling independent of membrane pore formation. MAC precursor complements C5b-7 and C5b-8 and MAC (C5b-9) can interact with signaling molecules and enzymes that induce the production of secondary messengers, such as cAMP, diacylglycerol (DAG), and ceramide. Resulting proliferative and proinflammatory signaling events induced include activation of phosphoinositide 3-kinase (PI3K)/Akt/forkhead box protein O1 (FOXO1), extracellular signal-regulated kinase (ERK), and NF- κ B signaling. Bax, Bcl-2–associated X protein; Bad, Bcl-2–associated death promoter; Bid, Bcl-2 homology 3 domain (BH3) interacting domain death agonist; CaM, calmodulin; CaMK, CaM kinase; COX, cyclooxygenase; MLKL, mixed lineage kinase domain-like protein; JNK, Jun N-terminal kinase; PLA₂, phospholipase A₂; PKA, protein kinase A; PKC, protein kinase C; RIPK, receptor-interacting serine/threonine-protein kinase.

and activation of protein kinase C, which, in turn, mediates activation of members of the mitogen-activated protein kinase cascade, such as extracellular signal-regulated kinase (ERK).²⁶ In K562 human erythroleukemic cells, Ca^{2+} -dependent activation of protein kinase C and protein kinase C-mediated ERK activation increases the ability of the cells to resist MAC osmolysis by increasing membrane vesiculation and MAC internalization by endocytosis.²⁶ In addition, MAC triggers Ca^{2+} intracellular influx in lipopolysaccharide-primed lung epithelial cells, leading to mitochondrial dysfunction and activating NLRP3 inflammasomes and proinflammatory IL-1 β secretion.²⁷

MAC Signaling Initiated Independently of Ion Flux

There is emerging evidence that MAC activates signaling in certain cell types independently of Ca^{2+} influx (ie, when an increase in cytosolic Ca^{2+} ion is prevented by removal of extracellular Ca^{2+} or by chelation of intracellular Ca^{2+}). Similarly, signaling may occur when MAC is assembled from non-transmembrane MAC precursors that cannot form pores. For instance, C5b-7 deposition can induce production of cAMP and lipid-derived signal messengers diacylglyceride and ceramide. Although these responses may be further increased with the assembly of C5b-8 and C5b-9, the response in the absence of C8 and C9 indicates that the activation of certain phospholipases is independent of ion flux^{28,29} (Figure 2B). In glomerular epithelial cells (GECs) and tubular epithelial cells, MAC (C5b-9) assembly and deposition induces cytosolic phospholipase A2 to hydrolyze phospholipids at the plasma membrane, endoplasmic reticulum membrane, and nuclear envelope, leading to arachidonic acid production and release that is independent of Ca^{2+} flux.³⁰ MAC significantly increases GEC expression of cyclooxygenase 2 and increased metabolism of arachidonic acid to prostanoids, exacerbating proteinuria in rat models of membranous nephropathy. MAC-induced cytosolic phospholipase A2 activation and phospholipid hydrolysis damages the endoplasmic reticulum membrane, inducing endoplasmic reticulum stress proteins bip and grp94 expression.³¹ MAC-induced stimulation of arachidonate metabolism also results in release of prostaglandin E2 from macrophages, leukotriene B4 from neutrophils, thromboxane B2 from human platelets, and prostanoids, IL-1, and reactive oxygen species from human monocytes.^{32,33}

MAC can interact directly with G_i protein, activating phosphatidylinositol 3-kinase/Akt/forkhead box protein O1 and ERK1 pathways that induce cell cycle activation³⁴ (Figure 2B). In human aortic smooth muscle cells, MAC induces cell cycle entry and proliferation by activating mitogen-activated protein kinase pathways, phosphatidylinositol 3-kinase, and p70 S6 kinase.¹⁰ In cultured GECs, MAC-induced ERK-dependent activation of guanine nucleotide exchange factor H1 (GEF-H1), a guanine nucleotide exchange factor, and subsequent RhoA activation, providing a possible mechanism for MAC-induced

actin cytoskeleton condensation and podocyte foot effacement in membranous nephropathy.³⁵ Furthermore, MAC can cause lysosomal membrane permeabilization, blocking fusion of autophagosomes with the lysosome and impairing lysosomal degradation of autophagosomes in podocytes and tubular epithelial cells.³⁶

MAC also triggers intracellular signaling independent of K^+ efflux, leading to inflammasome assembly and subsequent processing and secretion of IL-1 β and IL-18. Although bacterial pore-forming proteins can elicit inflammasome assembly, this treatment activates both NOD-like receptor family CARD domain-containing protein 4 (NLRC4) and NLRP3 inflammasomes,³⁷ whereas antibody-mediated deposition of MAC on human endothelial cells (ECs) only activates NLRP3 inflammasomes.¹³ In this case, MAC signaling is initiated on Rab^+ MAC^+ endosomes and not on the plasma membrane so that NLRP3 inflammasome assembly in human endothelium can be abrogated by inhibiting clathrin-mediated endocytosis^{13,15} (Figure 3). Age-dependent increases in amyloid β induce MAC deposition on rodent retinal pigment epithelium (RPE) and trigger inflammasome assembly, caspase-1 activation, and secretion of IL-1 β and IL-18.³⁸ In systemic lupus erythematosus (SLE), engagement of the CD3/T-cell receptor (TCR) complex on T cells involves rewiring to spleen tyrosine kinase (Syk) rather than the tyrosine kinase homolog, zeta-chain-associated protein kinase 70 (ZAP70), and induces enhanced TCR-induced signaling T-cell responses; a study found that binding of immune complexes purified from SLE patients and MAC deposition on human naïve CD4^+ T cells triggered activation of Syk and signaling by preferential recruitment of the fragment crystallizable region receptor gamma ($\text{Fc}\gamma$) chain.³⁹ Because Syk has been described to be required for pro-IL-1 β processing by and activation of the NLRP3 inflammasome,⁴⁰ the implications of these MAC-mediated effects on enhanced pathogenic autoimmune T-cell responses remain to be more fully studied.

MAC Signaling Initiated by Internalization

MAC has been shown to be removed from the cell surface of nucleated cells of multiple types,^{41,42} by endocytosis (internalization) and/or budding off of plasma membrane, a process termed ectocytosis. These processes allow for active and rapid removal of MAC lesions from the plasma membrane and cell recovery. Neutrophils use both endocytosis and ectocytosis of MAC, shedding MAC-enriched membrane vesicles with increased levels of cholesterol and diacylglycerol, suggestive of active lipid sorting.^{5,43,44} In K562 erythroleukemia cells, MAC endocytosis and loading into early endosomes is dependent on caveolin-1, implicating uptake through caveolae.⁴⁵ MAC vesicular shedding from K562 cells is dependent on mortalin/glucose-regulated protein 75 (GRP75), a mitochondrial chaperone and member of the heat shock

Figure 3 Internalized membrane attack complexes (MACs) activate interferon- γ (IFN- γ)–primed human endothelial cells (ECs) by a MAC/endosomal NF- κ B–inducing kinase (NIK)/nucleotide-binding oligomerization domain-like receptor (NLR) family pyrin domain containing three (NLRP3) inflammasome/IL-1 signaling pathways and increase their ability to recruit and activate alloreactive T cells. Antibody-mediated complement activation leads to MAC deposition on the surface of IFN- γ –primed human ECs. MAC is internalized by a clathrin-mediated endocytosis and delivered to Ras-related protein Rab5⁺ endosomes. The Rab5/zinc finger FYVE-type containing 21 (ZFYE21)/SMAD–specific E3 ubiquitin protein ligase 2 (SMURF2)/phosphorylated Akt signaling pathway modifies the phosphoinositide composition of Rab5⁺MAC⁺ endosomes, which recruit and stabilize mitogen-activated protein kinase kinase 14 [alias NF- κ B–inducing kinase (NIK)], preventing its tumor necrosis factor receptor–associated factor 3–mediated polyubiquitylation. Endosomal NIK activates noncanonical NF- κ B signaling to synthesize pro-IL-1 β and the assembly of an NLRP3 inflammasome that processes and secretes mature IL-1 β and IL-18. IL-1 β feeds back on the ECs to induce proinflammatory genes through a canonical NF- κ B pathway that increases the capacity of the ECs to activate alloreactive CD4⁺ T effect or memory cells. IL-18 selectively expands alloreactive peripheral helper T cells, an IL-21–producing T-cell subset. ASC, apoptosis-associated Speck-like protein containing CARD; CCL5, chemokine (C-C motif) ligand 5; CCL20, chemokine (C-C motif) ligand 20; GSDMD, gasdermin D; ICAM-1, intercellular adhesion molecule 1; MHC, major histocompatibility complex; VCAM-1, vascular cell adhesion molecule 1.

protein 70 family, that binds directly to C9⁴⁶ in potential cooperation with heat shock protein 90.⁴⁷ Vesicular trafficking in RPE is critical for MAC removal and limiting inflammation.⁴⁸ MAC deposition on rat kidney GECs in experimental membranous nephropathy is found in clathrin-coated pits, and MAC is also exocytosed into urine.⁴⁹ As noted above, alloantibody-mediated MAC deposited on the surface of human ECs that is internalized in a clathrin-mediated process and rapidly transferred to Rab5⁺ endosomes within minutes⁹ (Figure 3). MAC⁺ Rab5⁺ endosomes act as a signaling platform by

recruiting the Rab5 effector zinc finger FYVE-type containing 21 (ZFYE21), leading to SMAD specific E3 ubiquitin protein ligase 2 (SMURF2)-dependent ubiquitylation of phosphatase and tensin homolog (PTEN), followed by its proteasome-dependent degradation.¹² This alters phosphoinositide composition of the Rab5⁺ MAC⁺ endosomes to allow for recruitment of signalosome components, phosphorylated Akt and NF- κ B–inducing kinase (NIK). Binding to the endosome stabilizes NIK by preventing its tumor necrosis factor receptor–associated factor 3–mediated

polyubiquitinylation and rapid proteosomal degradation.¹⁵ Endosomal NIK activates two responses: initiation of noncanonical NF- κ B signaling, leading to transcription; and translation of pro-IL-1 β and NLRP3 recruitment from the endoplasmic reticulum and initiation of inflammasome assembly and caspase-1 activation. Activated caspase-1 cleaves and activates pro-IL-1 β and pro-IL-18 to form mature (bioactive) IL-1 β and IL-18, respectively, and cleaves gasdermin D, the amino terminal fragments of which form a pore that allows IL-1 β and IL-18 to be secreted from the cytosol.¹³ Circulating levels of interferon- γ , which acts on endothelium *in situ* to up-regulate major histocompatibility complex proteins, also prime ECs for MAC-induced NLRP3 inflammasome assembly and function by up-regulating expression of NLRP3, procaspase 1, and gasdermin D expression.¹³ IL-1 β , processed and released by MAC-activated ECs, initiates autocrine/paracrine signaling and induces up-regulation of proinflammatory genes, including chemokines, cytokines, and adhesion molecules, through activation of canonical NF- κ B pathway.¹³ MAC induction of the EC NLRP3 inflammasome also led to IL-18 secretion from ECs, and IL-18 can act to selectively expand alloreactive peripheral helper T cells, an IL-21-producing T-cell subset,⁵⁰ that may act on a variety of other cell types, including B cells and endothelium. The net result of MAC-induced IL-1 β (and possibly IL-18) secretion is to provide endothelium with an enhanced capacity to locally recruit and activate leukocytes, including alloreactive effector memory T cells.^{9,13} Prior studies have also shown that MAC deposition on human ECs induces activation dependent on NF- κ B activation, characterized by increased expression of IL-8, IL-1 α , IL-1 β , E-selectin, intracellular adhesion molecule-1, vascular cell adhesion molecule-1, I κ B α , plasminogen activator inhibitor-1, and monocyte chemoattractant protein-1.^{8,51,52} Previous reports inferring a direct link between MAC deposition and canonical NF- κ B signaling in ECs may need to be reconsidered in light of the role that inflammasomes and IL-1 β have been shown to play in this response.⁸ In addition to ECs, MAC activation of NF- κ B signaling and gene transcription has also been observed in GECs, exacerbating proteinuria and experimental model of membranous nephropathy,⁵³ and human vascular smooth muscle cells,⁵⁴ which may exacerbate the pathogenesis of atherosclerosis.

MAC Signaling in Human Disease

The effects of MAC have been studied in various cell types and disease settings, revealing intracellular signaling that increases cell activation, alters their functions, and promotes inflammation and disease pathogenesis. Numerous downstream signaling pathways have been implicated with

various human and animal model cell types and different sources of complement and MAC activation. Although a unifying MAC signaling pathway is unlikely, there are common proliferative and proinflammatory signaling events that emerge, including activation of phosphatidylinositol 3-kinase/Akt/forkhead box protein O1 and ERK, inflammasome assembly, secretion of IL-1 β and IL-18, and NF- κ B signaling. Moreover, the context of MAC signaling and integration with the recent biophysical, kinetics, and structural advances will be important in future studies of MAC signaling.

MAC in IRI

MAC has been implicated as a mediator in ischemia/reperfusion injury (IRI).^{55,56} IRI commonly occurs after restoration of blood after a prolonged period of occlusion, leading to widespread tissue damage, such as post-transplantation, stroke, or myocardial infarction. IRI induces EC changes that allow binding of natural IgM, mannose-binding lectin, or collectin-11, which can all activate complement, leading to MAC insertion and activation of the endothelium.^{57–59} IRI can cause alternative pathway complement activation on murine cells but classic pathway complement activation mediated by IgM on human cells.⁶⁰ Allografts with IRI are associated with worse outcomes and are at higher risk for the development of graft rejection. Eculizumab is an anti-C5 monoclonal antibody that inhibits the generation of C5a anaphylatoxin and C5b, which blocks formation of MAC. Several studies have investigated the effect of MAC inhibition on IRI. In experiments with a humanized mouse model of IRI, blocking terminal complement activation on human graft ECs lining a human coronary artery xenograft using anti-mouse C5 monoclonal antibody attenuated the IRI-exacerbated allograft vasculopathy.⁶¹ Similarly, murine complement inhibitors complement receptor 2 (CR2)-CD59a, which inhibits terminal MAC assembly, and CR2-complement receptor-1 related gene/protein Y (Crry), which inhibits C3 activation, were found to be similarly protective against hepatic IRI.⁶² CR1g/FH, a complement receptor of the immunoglobulin superfamily (CR1g)-targeted complement inhibitor that connects the functional domains of CR1g and Factor H (FH), is protective in murine renal IRI by reducing complement activation C3d and MAC deposition in renal IRI, potentially via phosphatidylinositol 3-kinase/Akt activation.⁶³

MAC in Transplant

Transplant rejection, especially after the first postoperative year, is associated with complement-activating (fixing) donor-specific antibody (DSA) formation.⁶⁴ Assessment of allograft biopsies frequently shows evidence of complement deposition, as detected as C4d, but we have observed

C5b-9 as well.^{12,13} Complement fixation is initiated by DSA binding nonself major histocompatibility complex I and II complexes on graft ECs, leading to up-regulation of proinflammatory genes characteristic of EC activation without causing cell death. Because MAC-activated ECs have enhanced ability to recruit and activate allogeneic memory T cells,^{9,12,13,15} this pathway may explain how DSA binding to graft ECs increases T-cell recruitment and secretion of interferon- γ , a cytokine that produces vasculopathic changes in the arterial wall. Allograft vasculopathy reduces organ perfusion, ultimately resulting in late graft failure. In organ transplantation, IRI not only exerts a direct effect on complement activation through the mechanisms described above, but also promotes the development of DSA and leads to sustained (or periodic) MAC activation of graft ECs. Mechanistically, the IgM-dependent MAC activation of human ECs induced by IRI leads to IL-18 secretion and selective expansion of IL-21-secreting peripheral helper T cells that promote B-cell production of DSA.⁵⁰

MAC in Cancer

The interactions of cancer cells with MAC activation on and around cancer cells are complex. MAC has been found to activate pathways in cancer cells that inhibit death signals and induce mechanisms of evasion of complement-dependent cytotoxicity, such as blocking MAC assembly and increasing MAC elimination from the cell surface.⁶⁵ Increased presentation of CD59 on leukemic cancer cells reduces effectiveness of rituximab by inhibiting apoptosis^{66,67} and on tumor cells leads to increased tumor progression in a model of neuroblastoma.⁶⁸ Cancer cells activate protective pathways, including CD46, CD55, and CD59, to inhibit complement activation to reduce number of MAC inserted to counteract death signals.⁶⁵

MAC in Autoimmunity

In SLE, autoantibodies forming immune complexes trigger complement activation, leading to cell membrane and soluble MAC. MAC deposition is associated with disease intensity and is a marker for poor treatment response.⁶⁹ Altered CD55 and CD59 expression was found on peripheral blood cells from SLE patients with increased CD55 and CD59 lymphocytes, which may explain the cytopenia commonly found in SLE patients.⁷⁰ A study also found evidence of complement activation and MAC deposits in brain lesions of patients with SLE.⁷¹ Although the exact initiation mechanism of rheumatoid arthritis is not known, it is thought that serum collagen autoantibodies can bind to cartilage components of antigen presented on the surface of articular cartilage, leading to complement activation.⁷² Evidence of complement activation and MAC have been found in the synovial fluid of rheumatoid arthritis

patients⁷³ and on synovial ECs with significant MAC-mediated activation of the Rab5-ZFYVE21-SMURF2-phosphorylated Akt axis, leading to enhanced immune cell infiltration.¹² Soluble MAC was found to up-regulate osteoprotegerin expression and release by ECs.⁷⁴ Although not generally considered a form of autoimmunity, osteoarthritis is also associated with complement activation with MAC deposition and signaling on synovial microvessels.^{12,75}

MAC in Membranous Nephropathy

Idiopathic membranous nephropathy, one of the most common forms of nephrotic syndrome in adults, is characterized by immune complex and MAC deposition in the subepithelial space, causing thickening of the glomerular basement membrane and functional impairment of the glomerular capillary wall, manifested as proteinuria. An experimental model of membranous nephropathy is Heymann nephritis, and recent studies have found that M-type phospholipase A₂ receptor as a major target antigen and IgG4 anti-phospholipase A₂ receptor autoantibodies can activate complement by the lectin pathway.⁷⁶ The deposition of MAC on podocytes initiates cellular dysfunction and is thought to be a critical step in membranous nephropathy development.³⁶

MAC in Age-Related Macular Degeneration

Age-related macular degeneration is characterized by lesions at the RPE/choroid interface of the macular regions. MAC assembly and deposition by the alternative pathway may contribute to the pathogenesis of age-related macular degeneration. Oxidative stress was found to decrease levels of membrane-bound complement inhibitor CD59.⁷⁷ MAC in RPE cells was found to increase vascular endothelial growth factor release and induce loss of barrier function, propagating choroidal neovascularization.⁷⁷ MAC activation by lipofuscin or in combination with oxidative stress on RPE has also been observed to induce increased secretion of IL-6, IL-8, and vascular endothelial growth factor.^{78,79} ECs of the choriocapillaries are the main sites on MAC deposition in the macula and can induce proinflammatory signaling, resulting in secretion of IL-6, IL-8, and vascular endothelial growth factor.^{80,81} Age-related macular degeneration—associated mutations, discovered in patients, have been shown to alter the charged interface by which C9 polymerizes and drive both increased and decreased C9 self-polymerization, depending on the variant.¹⁹

MAC in Other Diseases

Additional diseases in which MAC signaling may be implicated include sleep apnea,⁸² osteoarthritis,¹² Alzheimer disease, and atherosclerosis. Apolipoprotein E, which has been implicated to Alzheimer disease, was found to attenuate classic complement activation by high-affinity binding

to the initiating C1q protein.⁸³ In apolipoprotein E-deficient mice, complement activation led to leukocyte infiltration of the choroid plexus and reduction of C5 by siRNA decreased the complement-induced choroid plexus inflammation, atherosclerosis, and amyloid β -associated microglia. MAC, along with S-protein, C3d, and apolipoprotein B, has been detected in human arterial tissues with atherosclerosis.⁸⁴ In CD59 and apolipoprotein E knockout mice, CD59 was found to protect against atherosclerosis by restricting MAC-mediated EC dysfunction and injury and foam cell formation.⁸⁵

New Insights into Therapeutic Strategies to Inhibit MAC Assembly, Signaling, and Proinflammatory Effects

Inhibiting MAC Assembly

CD59-Promoting Therapy

Inhibiting MAC assembly with CD59 had been shown to attenuate complement-mediated pathologies in several disease models. In traumatic brain injury patients, MAC is implicated in secondary injury and neuronal loss in acute setting. Inhibition of MAC assembly by CD59a prevented microglial accumulation, mitochondrial stress, and axonal damage in a murine model of traumatic brain injury.⁸⁶ In a mouse model of age-related macular degeneration, increased MAC deposition was observed on retinal pigment epithelium and increased with age-related macular degeneration. Inhibition of MAC using the murine complement inhibitor CR2-CD59 to inhibit MAC assembly reduced ocular injury severity.⁸⁷

Anti-C5 Therapy

Anti-C5 antibody binds to C5, preventing cleavage into C5a, the anaphylatoxin, and C5b, a terminal complement component, and thus, inhibiting MAC formation. Anti-C5 monoclonal antibody (eculizumab) was approved for treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. Anti-C5a receptor antibodies block the binding of C5a to its receptor, C5aR, but do not inhibit MAC formation. Treatment with anti-C5 therapy, which blocks C5a and MAC formation and targeted at ischemic ECs, attenuated IRI injury. Anti-C5 inhibited complement activation and tissue damage in *ex vivo* rat model of renal IRI.⁸⁸ In a humanized mouse model of allograft vasculopathy and IRI, anti-C5 antibody was effective in attenuating T-cell-mediated injury but not anti-C5a, implicating C5b and terminal complement.⁶¹ In mouse disease models of atherosclerosis and Alzheimer disease, C5 was only made by the liver, allowing siRNA therapy, which naturally targets hepatocytes, to abrogate late complement signaling and reduction in disease severity.⁸³ Complement receptor 1 is a complement regulatory protein that binds to C3b, C4b, C1q, and mannose-binding lectin to inhibit complement activation but is present in circulation at low

concentrations; soluble complement receptor 1 treatment in patients undergoing cardiac surgery has been shown to prevent terminal complement activation and limit ischemic damage.⁸⁹

Inhibition of MAC Signaling and Pro-Inflammatory Effects

NIK Inhibition

MAC signaling leads to NIK stabilization on the surface of Rab5⁺ endosomes, which induces NLRP3 inflammasome formation and noncanonical NF- κ B signaling. The NLRP3 inflammasome leads to the secretion of active IL-1 β and IL-18, and knockdown of NIK inhibited inflammasome assembly.¹³ Noncanonical NF- κ B signaling contributes to IL-1 β secretion by inducing sustained synthesis of pro-IL-1 β . Inhibiting NIK may prevent the subsequent MAC signaling events, such as NLRP3 inflammasome activation and IL-1 β secretion and signaling. Small-molecule NIK inhibitors have been screened for and tested in different disease contexts.^{90–92}

Inflammasome Inhibition

MAC signaling activates the NLRP3 inflammasome, which leads to the activation of caspase-1 that processes the pro-proteins into active IL-1 β and IL-18.¹³ Inhibition of the inflammasome by the NLRP3 inhibitor MCC950⁹³ inhibited the formation of the MAC-induced NLRP3 inflammasome in human ECs lining a human coronary artery xenograft *in vivo*. Pharmacologic inhibitors of the inflammasome may be potential therapy to block downstream MAC-induced inflammasome activation and IL-1 secretion. Moreover, an additional advantage of inflammasome inhibition would be also blocking IL-18 secretion. MCC950 is being tested in early-phase clinical trials and evaluated for its anti-IL-1 effects.⁹⁴ In addition, promoting endogenous regulatory proteins of the inflammasome, including caspase activation and recruitment domain [CARD16; constitutive photomorphogenesis protein 1 homolog (COP1)], CARD17 [inhibitory caspase recruitment domain CARD protein (INCA)], and CARD18 (ICEBERG), which are expressed only in humans, may be another strategy to inhibit the inflammasome.⁹⁵

Anti-IL-1 Therapy

IL-1 receptor antagonist (or anakinra) is a competitive inhibitor and binds to IL-1 receptor 1 with similar affinity as IL-1 α and IL-1 β without agonist behavior. Anti-IL-1 β antibody (canakinumab) targets IL-1 β and was shown in the Canakinumab Anti-Inflammatory Thrombosis Outcome Study (CANTOS) to decrease incidents of cardiovascular events in patients with atherosclerosis who had a myocardial infarction and incidentally also reduced cancer mortality.⁹⁶ IL-1 β is a major product of the NLRP3 inflammasome, which has been shown to be activated by MAC signaling.^{13,27,38} IL-1 β released downstream of the MAC-

induced NLRP3 inflammasome in ECs has been shown to mediate autocrine/paracrine proinflammatory EC activation that can be inhibited by IL-1 receptor antagonist. Inhibiting IL-1 signaling in the ECs diminished the MAC and IL-1 augmented ability of ECs to recruit and activate allogeneic memory CD4⁺ T cells.¹³ In the humanized mouse chimeric model of artery allograft rejection, it was demonstrated that vascular cell-expressed IL-1 promotes allogeneic T-cell intimal infiltration and IL-1 receptor blockade partially reduces basal T-cell-mediated graft injury.⁹⁷ Administration of anakinra to a small number of renal transplant patients appears to be safe and efficacious.⁹⁸ Besides limited study in kidney transplant recipients with familial Mediterranean fever,⁹⁹ canakinumab has yet to be assessed as a disease-modifying therapy in transplant patients.

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