Complement: Terminal Pathway

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Terminal pathway of complement refers to the sequence of interactions between components C5b, C6, C7, C8 and C9 which lead to the production of a 'membrane attack complex' that has the ability to disrupt cell membranes.

Secondary article

Article Contents

Introduction

The complement system comprises approximately 35 proteins, enzymes, receptors and regulatory components. Activation occurs by three distinct pathways and is initiated by the interaction of complement with immune complexes (classical pathway) or with the surface of pathogenic organisms (lectin and alternative pathways). All pathways lead to cleavage of component C3 and formation of C5 convertase, an enzyme complex that activates the 'terminal pathway'. This pathway is referred to as terminal because it leads to the end-product of complement activation. This end-product is the membrane attack complex or 'MAC', which is composed of components C5b, C6, C7, C8 and C9. MAC facilitates the killing of bacteria and other pathogens by altering the permeability of their membranes.

Activation

Central to activation of the terminal pathway is cleavage of a single Arg–Leu bond at amino acid position 74–75 in the α chain of C5. Cleavage is catalysed by the serine protease C2a in the C5 convertase ([C4b,2a,3b]) of the classical and lectin pathways or Bb in the C5 convertase ([$(C3b)_2$,Bb,P]) of the alternative pathway. Both are surface-bound enzyme complexes that cleave C5 in the fluid phase to produce C5a (M_r = 11 200) and C5b (M_r = 179 000). C5a expresses a variety of biological activities during an inflammatory response. These include smooth muscle contraction, histamine release from mast cells, vasodilation, and increased vascular permeability. It is also a potent chemoattractant for neutrophils and other leucocytes. C5b is a labile product and is rapidly inactivated unless it binds to C6, in which case it forms a stable C5b-6 dimer. Subsequent binding of C7, C8 and C9 leads to formation of the MAC (Müller-Eberhard, 1986).

Components

Human C5 (M_r = 190 000) is composed of an α chain (M_r = 115 000) and a β chain (M_r = 75 000) linked by a disulfide bond. C5 is a structural homologue of components C3 and C4. All three proteins are similar in size, amino acid sequence and genomic organization and all are members of the α_2-macroglobulin superfamily. All are synthesized as single-chain precursors but are present in the circulation as multisubunit proteins (α-β in C3 and α-β-γ in C4). Cleavage of each during complement activation produces small anaphylatoxic peptides (C3a, C4a or C5a) and larger products (C3b, C4b or C5b) that are involved in a variety of protein–protein interactions important for complement function.

Human C6 (M_r = 105 000) and C7 (M_r = 92 000) are single-chain proteins that are similar in size and structural organization. C8 is an oligomeric protein (M_r = 151 000) consisting of an α (M_r = 64 000), β (M_r = 64 000) and γ (M_r = 22 000) subunit, each of which is encoded in a separate gene. In C8 purified from serum, the subunits are arranged as a disulfide-linked α-γ dimer and a noncovalently associated β chain. C8α-γ and C8β can be dissociated, purified in stable form and recombined to form C8. Human C9 (M_r = 72 000) is a single-chain protein that has the distinction of being able to self-polymerize. Polymerization occurs during formation of MAC and by exposure to divalent cations such as Zn^{2+}, elevated temperature or upon partial digestion with proteases. Poly C9 nominally contains 12–18 C9 monomers arranged as a hollow cylindrical structure rimmed with a torus at one end (Podack and Tschopp, 1982; DiScipio and Hugli, 1985). It is estimated to have an inner diameter of ~10 nm, outer diameters of ~21 nm (torus) and ~18 nm (cylinder), and an overall length of ~15 nm. The ultrastructure of poly C9 resembles that of the pore-like structure formed by MAC on membranes.

Human C6, C7, C8α, C8β and C9 are structurally and genetically related proteins collectively referred to as the 'MAC protein family' (Plumb and Sodetz, 1998). All have

similar amino acid sequences and a highly conserved structural organization (**Figure 1**). The genes of each are similar with respect to length of exons and the location and phases of exon boundaries (Hobart *et al.*, 1995). A distinctive feature of the MAC proteins is the presence of several cysteine-rich modules that are ~40–80 amino acids in length. These are structurally similar to modules found in several functionally unrelated proteins. In many cases the modules are involved in mediating protein–protein interaction, which is also thought to be their role within the MAC. Included is the thrombospondin type I (TSP1) module repeated three times in thrombospondin, the low-density lipoprotein receptor class A (LDLRA) module that is repeated seven times in the LDL receptor and an EGF module that exhibits sequence similarity to epidermal growth factor. Both C6 and C7 contain additional, tandemly repeated modules in their C-terminal regions. The complement control protein (CCP; also known as the short consensus repeat or SCR) module is repeated multiple times in several complement regulatory proteins while the factor I module (FIM) occurs once in factor I. Although not a module, the internal region of each protein is designated MACPF to emphasize sequence similarity between this segment of the MAC proteins and perforin. Perforin (M_r = 70 000) is a protein released from the secretory granules of cytotoxic T lymphocytes. During lymphocyte-mediated cytolysis, perforin self-polymerizes in the presence of Ca^{2+} to form transmembrane channels on target cells. Polymers of perforin have physical and functional characteristics similar to polymers of C9.

Human C8γ is structurally unrelated to any complement protein (Kaufman and Sodetz, 1994). Based on similarities in amino acid sequence and genomic organization, it has been assigned to the 'lipocalin' family of proteins, which is part of a larger superfamily known as the 'calycins' because of the cup-shaped structure of its members. The lipocalins are widely distributed in both vertebrates and invertebrates. They are characterized by similarities in amino acid sequence, molecular weight (M_r ~ 20 000) and a common ability to bind and transport small, hydrophobic ligands. Examples include retinol-binding proteins such as serum retinol-binding protein, β-lactoglobulin from milk and tear prealbumin. Others include serum apolipoprotein D which binds cholesteryl esters, the odorant-binding proteins of the nasal mucosa and pheromone-binding proteins such as mouse urinary protein and rat α-2u-globulin. In insect haemolymph, the lipocalin insecticyanin binds biliverdin for coloration and camouflage, while coloration in lobster is provided by α-crustacyanin complexed with the chromophore astaxanthin. Crystallographic analyses of several lipocalins revealed a conserved folding pattern that consists of a characteristic β barrel structure with a cup-shaped ligand-binding site. This site is lined with nonpolar residues that confer specificity towards hydrophobic ligands. Molecular modelling of C8γ suggests it has a similar folding pattern; however a ligand has yet to be identified.

Description of Pathway

Aside from proteolytic cleavage of C5, all steps in the MAC assembly process are nonenzymatic. Individually, the components have properties of hydrophilic proteins but when combined they form a complex capable of binding to and disrupting the structural integrity of membranes.

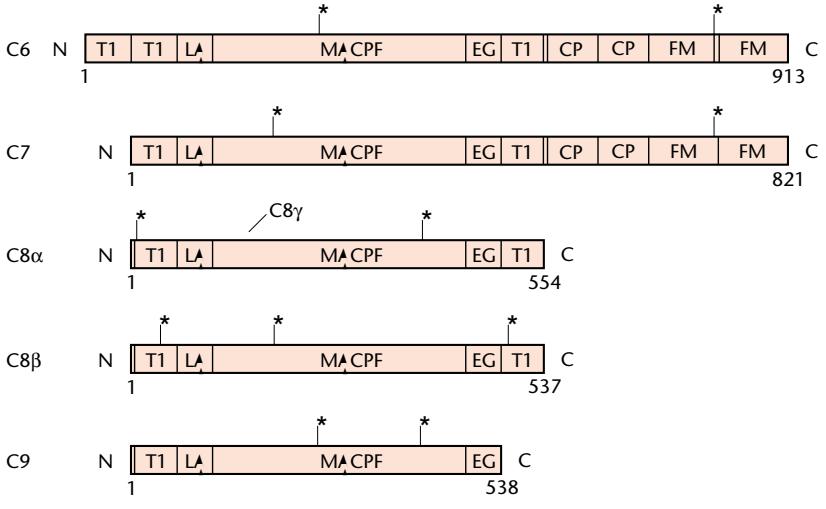


Figure 1 Modular structure of the membrane attack complex (MAC) protein family. Modules are labelled as follows: T1, thrombospondin type I; LA, low-density lipoprotein receptor class A; EG, epidermal growth factor; CP, complement control protein; FM, factor I module. MACPF is an extended region of sequence similarity found within all family members and perforin. Numbers correspond to amino acid residues in the mature proteins. Possible *N*-glycosylation sites are identified by (*). C8γ is linked to Cys164 in C8α.

Lipids in the target membrane are not degraded but instead undergo a disruptive rearrangement as a result of MAC insertion. This increases membrane permeability which results in osmotic lysis of simple cells such as erythrocytes or initiation of a variety of intracellular signalling events in the case of nucleated cells. In bacteria, MAC disrupts the outer membrane, thereby increasing permeability and inducing lethal changes in the inner membrane.

All five MAC components circulate independently in the bloodstream but interact in a highly specific and sequential manner once C5b is formed. As each intermediate complex in the pathway is assembled, binding specificity changes so as to recognize the next component in the pathway. Once associated, the affinity between components is high despite the noncovalent nature of their interaction. Dissociation can only be accomplished by solubilization of the membrane or denaturation of the MAC.

Once C5 is cleaved by surface-bound C5 convertase, C5b binds C6 to produce a stable, soluble C5b-6 dimer (**Figure 2**). This dimer then interacts with C7 to produce C5b-7, a trimer composed of one molecule each of C5b, C6 and C7. Within this complex, C6 and C7 are in close association with the cleaved α chain of C5b (α′); thus this chain probably contains binding sites for both C6 and C7. During C5b-7 formation, the constituent proteins undergo a hydrophilic to amphiphilic transition to produce a complex that has a high affinity for lipid. This enables C5b-7 to bind tightly to the surface of the same cell that initiated complement activation. Binding is noncovalent and relatively nonspecific in that C5b-7 can also bind to phospholipid or detergent micelles, synthetic lipid vesicles and lipoproteins. In the absence of a membrane or inhibitor of MAC formation, C5b-7 self-aggregates through its hydrophobic membrane-binding site to form inactive protein micelles. Although relatively nonspecific in its affinity for lipid, C5b-7 deposition may nonetheless be localized to specific structures on the cell surface. For simple cells such as erythrocytes, evidence suggests C5b-6 binds to sialic acid on surface molecules such as gangliosides or glycophorin. Subsequent binding of C7 disrupts this ionic interaction, resulting in exposure of hydrophobic domains and direct binding of C5b-7 to membrane lipid.

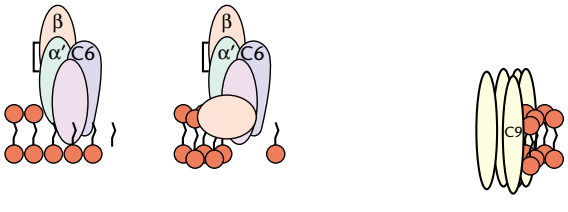
In membrane-bound C5b-7, C5b is largely exposed to the aqueous phase while C6 and C7 form a stalk-like structure that penetrates into the bilayer; however, penetration is not sufficient for membrane leakage or lysis. Studies suggest that C5b-7 associates with the outer lipid surface and only minimally penetrates into the hydrocarbon phase. Subsequent binding of C8 and C9 induces structural changes suggestive of deeper membrane bilayer penetration, which is required for MAC lytic activity.

Once it is formed, C5b-7 binds C8 to produce a tetrameric complex referred to as C5b-8. Binding involves mutually recognized sites on the C8β subunit and on C5b within the C5b-7 complex. Although relatively inefficient when compared to fully formed MAC, C5b-8 produces some membrane leakage. When deposited at a high density, it causes leakage from synthetic lipid vesicles, promotes slow osmotic lysis of erythrocytes and induces signalling responses in nucleated cells. Evidence suggests that C8 contributes directly to membrane perturbation by C5b-8. The C8α subunit in particular is a major component inserted into the hydrocarbon phase of the lipid bilayer. C5b, C6, C7 and C8β are also inserted but to a lesser extent. C8γ resides on the MAC surface and is not inserted.

The primary function of membrane-bound C5b-8 is to serve as a receptor for C9 in the final step of MAC formation. Initially, C9 interacts with a specific binding site on the C8α chain. This is a reversible interaction at 0°C but becomes irreversible at 37°C, when C9 unfolds and inserts into the membrane. Binding of the first C9 mediates incorporation of additional C9 molecules, presumably through C9–C9 interactions. Conformational changes in C9 lead to exposure of a membrane-binding domain believed to be centred on a segment defined by amino acids 287–345. Under certain conditions, multiple C9 molecules can be incorporated, thereby producing a MAC whose ultrastructure resembles that of Zn^{2+}-induced poly C9.

Regulation of Pathway

The formation and lytic activity of the MAC is regulated primarily by the serum proteins vitronectin (S protein) and



clusterin (SP40-40), and the membrane-associated protein CD59. The first two are soluble inhibitors that function to block membrane binding of MAC complexes (e.g. C5b-7, C5b-8, C5b-9) which may diffuse away from the immediate site of complement activation. Thus, their primary role is to protect 'bystander' cells from MAC attack. As a membrane-associated inhibitor, CD59 functions primarily to protect host cells from lysis should MAC form on their surface. Decay-accelerating factor (DAF; CD55) and membrane cofactor protein (MCP; CD46) are two other membrane-associated components that inhibit early reactions in the complement activation cascade and contribute to protection by downregulating formation of C5 convertases.

Vitronectin (M_r = 75 000) is a multifunctional adhesion protein found in the circulation and in different tissues. It mediates cell–matrix and cell–cell adhesion through interaction with integrin receptors. Vitronectin exerts its MAC inhibitory activity primarily by binding to C5b-7 in the fluid phase and blocking its interaction with cell membranes. The result is formation of a soluble form of C5b-7 (SC5b-7) containing an estimated 3–4 molecules of vitronectin. SC5b-7 can bind one C8 and three C9 molecules to form soluble SC5b-8 and SC5b-9, respectively. All of these complexes are unable to bind to membranes, therefore they are lytically inactive and are cleared from the circulation.

Clusterin is a multifunctional heterodimer composed of disulfide-linked α and β subunits each with an M_r of 35 000–40 000. It is a hydrophobic protein that circulates in a complex with lipid and apolipoprotein A-1. Clusterin is normally found along with vitronectin in soluble MAC complexes. It inhibits MAC formation by binding to fluid-phase C5b-7, C5b-8 and C5b-9, and it also can inhibit Zn^{2+}-induced polymerization of C9. Binding studies suggest it interacts directly with C7, C8β and C9 within the MAC.

Protection of host or 'self' cells from MAC-mediated lysis is provided primarily by the inhibitory protein CD59, also known as MAC inhibitory factor (MACIF), membrane inhibitor of reactive lysis (MIRL), protectin, homologous restriction factor 20 (HRF20) or p18. Human CD59 (M_r = 20 000) is expressed as a glycosylphosphatidylinositol (GPI)-linked protein on the membrane surface of many cell types including blood cells (e.g. neutrophils, erythrocytes, lymphocytes, platelets), epithelial cells, endothelial cells and spermatozoa. It inhibits MAC by interacting with C8α and C9 during assembly of the complex on the same cell to which it is attached. This interaction limits the number of C9 molecules bound by C5b-8 and restricts formation of a fully functional MAC. The inhibitory activity of CD59 is species-selective and is most effective towards C8 and C9 from the same species, hence the term 'homologous restriction of lysis' is sometimes used to describe CD59 function. Binding sites for human CD59 have been localized to a segment within amino acids 320–415 of human C8α and 359–411 of human C9.

Nucleated cells exposed to sublytic amounts of MAC undergo responses that promote removal of MAC and recovery of the cells from attack (Morgan, 1992). These responses are triggered by an increase in intracellular calcium that results from an influx of Ca^{2+} through channels formed by the MAC. This can promote K^+ efflux, activation of protein kinases and increased production of cAMP and lipid-derived signal messengers. Depending on the cell type, removal of MAC generally occurs by endocytosis or more commonly by ectocytosis, which is the shedding of MAC-enriched vesicles from the cell surface.

The Membrane Attack Complex

MAC performs its lytic function by restructuring lipid organization within its immediate environment and thereby altering membrane permeability. The composition, ultrastructure and size of the lesion is dependent on the nature of the target membrane (i.e. natural membranes, vesicles, liposomes, etc.) and the amount of C9 available when MAC is formed. The amounts of C5b, C6, C7 and C8 in a single MAC are typically one molecule each whereas the amount of C9 varies considerably. When C9 input is high, MAC formed on simple membranes such as erythrocytes or synthetic vesicles may contain 12–18 C9 molecules per complex and have an ultrastructure resembling that of Zn^{2+}-induced poly C9. This form of MAC consists of a tubular-shaped, pore-like structure of polymerized C9 with C5b-8 attached as an appendage. The pore itself is a transmembrane channel lined with amphipathic helices contributed largely by C9. The pore diameter of this MAC is ~10 nm, which agrees with that for poly C9. Thus, C5b-8 functions much like Zn^{2+} by promoting C9 unfolding and formation of a well-organized structure composed of polymerized C9.

Formation of tubular-shaped poly C9 is not absolutely required for MAC-mediated lysis (Esser, 1994). MAC formed on erythrocytes at typical serum concentrations of C9 contains an average of 3–6 C9 molecules. Some complexes contain more or less than the average number and accordingly may or may not appear as tubular structures. Other studies indicate that MAC complexes containing as little as one C9 are haemolytically active even though they lack the ultrastructural characteristics of poly C9. These nontubular complexes form smaller transmembrane channels or pores because of the limited amount of C9 available. Pore diameters are only ~1–3 nm but sufficient to promote osmotic lysis. Thus, the pore size of the MAC is variable and depends on the number of C9 molecules incorporated.

Relevance in Protection and Pathology

Complement has a role in protection against pathogenic strains of bacteria, viruses, mycobacteria, yeasts, parasites and protozoa. MAC itself is especially important in defence against Gram-negative bacteria such as *Neisseria*. Humans with hereditary deficiencies of C5, C6, C7, C8 or C9 have an impaired ability to form MAC and are particularly susceptible to *N. menin itidis* and *N. onorrhoeae*. These individuals may have recurrent meningococcal and systemic gonococcal infections, respectively. Gram-negative bacteria have an outer lipid bilayer containing embedded lipopolysaccharide (LPS), a central periplasmic space containing peptidoglycan and an inner cytoplasmic membrane. Many strains activate the classical, lectin or alternative pathways and in some cases all three and produce MAC. Channels formed by MAC on the outer membrane of sensitive strains enables the release of periplasmic enzymes and influx of serum lysozyme, which degrades peptidoglycans. However, this alone does not produce lysis and the exact mechanism by which MAC mediates bacterial killing is unknown. Dissipation of the inner membrane potential and loss of cellular energy are required for killing, however this is not caused by MAC directly since the inner membrane is inaccessible to MAC and MAC does not translocate from the outer membrane to form channels on the inner membrane. Experimental evidence suggests a direct role for C9 and a possible mechanism in which C9 or cytotoxic fragments of C9 are released by MAC into the periplasm where they can translocate and interact with the inner membrane (Esser, 1994).

Not all strains of Gram-negative bacteria are susceptible to MAC. Those with shorter LPS chains (less carbohydrate or 'rough phenotype') on the cell surface tend to be more susceptible whereas those with longer LPS chains (more carbohydrate or 'smooth phenotype') tend to be resistant. In the latter case, longer LPS chains and the more hydrophilic nature of the membrane surface impairs insertion of MAC into the membrane. Gram-positive bacteria tend to resist lysis because MAC is unable to penetrate the relatively thick peptidoglycan layer in their cell wall. Bacteria resistant to lysis by MAC are generally killed by opsonization and phagocytosis.

MAC also has a role in protection against viruses. Enveloped viruses are particularly susceptible to MAC-mediated lysis because they have a membrane-like lipid bilayer surrounding the protein shell (capsid) that contains viral genetic material. This lipid bilayer is usually acquired from the membrane of the cell of origin. MAC forms pore-like lesions on these viruses and promotes disruption of viral integrity, release of intraviral constituents and loss of viability. Interestingly, some viruses acquire normal complement regulatory molecules such as DAF, MCP or CD59 during the process of maturation and budding from the membrane of the cell of origin. These are then used to evade destruction by complement and MAC. Some examples are human cytomegalovirus, human T-cell leukaemia virus type I and human immunodeficiency virus type 1(HIV-1). Other viruses encode within their own genome proteins that mimic the regulatory components. Herpesvirus saimiri encodes a protein that is $\sim 50\%$ identical in sequence to human CD59 and is anchored by a GPI linkage. Cells expressing this homologue are resistant to lysis by MAC.

Nucleated cells subjected to even sublytic amounts of MAC undergo protective responses to resist lysis, however MAC also elicits other important responses (Mold, 1998). These vary with cell type and in some cases can be elicited by C5b-7 or C5b-8 as well as MAC. Some of these lead to the production and release of inflammatory mediators that are important in host response to injury or in the pathogenesis of disease. For example, superoxide anion and its toxic reactive oxygen metabolites (e.g. hydrogen peroxide, hydroxyl radical) are produced by phagocytic cells during the respiratory burst and are used to kill microorganisms. Both neutrophils and macrophages release these metabolites when exposed to MAC and their release has been implicated in tissue damage associated with the inflammatory response. These cells also release eicosanoids (e.g. prostaglandins, thromboxanes, leucotriene B4, etc.), which are synthesized from arachidonic acid by the cyclo-oxygenase and lipoxygenase pathways. Eicosanoids are diverse activators of pain and inflammation and are also mediators of acute and chronic inflammatory conditions. These responses generally require a calcium influx, activation of protein kinases and phospholipases and thus may involve the same mechanisms that protect cells from lysis.

In platelets, calcium influx in response to sublytic MAC initiates secretion of mediators such as ATP from storage granules, phosphorylation of protein kinases and induction of shape changes. It also enhances the procoagulant properties of platelets by promoting membrane phospholipid rearrangement and exposure of binding sites for the tenase (FVIIIa and FIXa) and prothrombinase (FVa and FXa) enzyme complexes of the coagulation system. MAC is removed by vesiculation and released vesicles provide additional sites for generation of the prothrombinase complex. Vesiculation also tends to activate the platelet surface, thus increasing the ease with which the cells aggregate.

Sublytic MAC formed on endothelial cells elicits several responses. In intact endothelium, it mediates disruption of intracellular junctions leading to formation of gaps that can affect permeability. It activates endothelial cells and increases procoagulant activity by altering membrane phospholipid bilayers that support prothrombinase activity and by inducing cytokine synthesis (interleukin 1α, IL-1α). The latter leads to upregulated expression of tissue factor and plasminogen activator inhibitor 1, both of which contribute to widespread coagulation and persis-

tence of fibrin clots. MAC also promotes attachment of leucocytes to endothelial cells by inducing expression of P selectin on the surface and the synthesis of E selectin, which are cell adhesion molecules. MAC affects vascular tone by stimulating release of prostaglandins (PGI_2) that act as vasodilators. It also promotes *de novo* synthesis of thromboxanes (TxA_2) that function as vasoconstrictors.

MAC is believed to have a role in the pathogenesis of several diseases. In rheumatoid arthritis, the severity of joint disease correlates with complement activation in the joint space and evidence indicates that MAC attack and recovery is ongoing within the joint. MAC has also been shown to stimulate production and release of inflammatory eicosanoids and cytokines from rheumatoid synovial cells and to stimulate collagenase synthesis in synovial fibroblasts. In demyelinating diseases of the central nervous system (e.g. multiple sclerosis), evidence suggests MAC forms on myelin membranes undergoing autoimmune attack and renders myelin basic protein more susceptible to proteolytic degradation. It can also elicit pro-inflammatory responses in myelin-producing oligodendrocytes as well as facilitate their lysis. MAC has been identified in glomerulonephritic lesions in kidney, atherosclerotic plaques and ischaemic myocardium.

The hereditary haematological disorder paroxysmal nocturnal haemoglobinuria (PNH) is caused by an inability to synthesize the GPI linkage used to anchor CD59 and other complement regulatory proteins to the cell membrane. The result is increased susceptibility to the effects of complement activation and MAC in particular. Manifestations of this disorder include haemolytic anaemia and haemoglobinuria caused by increased red cell lysis; venous thrombosis due to the procoagulant effects of MAC on platelets and endothelial cells; and a general abnormality in the function of cells of the haematopoietic system, all of which lack normal CD59.

MAC also has a role in hyperacute graft rejection, a severe rejection reaction that can quickly destroy a transplanted organ. Complement activation is initiated by binding of natural antibodies to the endothelium of the transplanted organ. This ultimately leads to thrombosis, interstitial oedema and necrosis. This is a significant problem in the field of xenotransplantation where organs from phylogenetically disparate species are grafted into recipients. Currently, the pig is being considered as a potential donor for human transplantations because of cost, ease of breeding and fewer concerns about transmission of infectious agents in relation to primates. However, complement regulatory proteins such as DAF and CD59 are species-selective in their action and are most effective against complement from the same species, i.e. homologous complement. Thus, the endothelium in transplanted organs from a pig is not adequately protected from damage by human MAC. Efforts are underway to produce transgenic pigs that express human regulatory proteins on their cell surface, thus making their organs resistant to attack by human MAC (Byrne *et al*., 1997).

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