Beyond lysis: how complement influences cell fate

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ABSTRACT

Complement is a central component of the innate immune system involved in protection against pathogens. For many years, complement has been known to cause death of targets, either indirectly by attracting and activating phagocytes or directly by formation of a membrane pore, the membrane attack complex. More recently, it has been recognized that complement may cause other ‘non-classical’ effects that may not directly be aimed at killing of pathogens. Products of complement activation collaborate with the adaptive immune system to enhance responses to antigens. The membrane attack complex of complement, apart from lysing cells, can also trigger diverse events in target cells that include cell activation, proliferation, resistance to subsequent complement attack and either resistance to, or induction of, apoptosis. Various complement products play important roles in signalling for clearance by phagocytes of apoptotic self cells. Here we review some of these non-classical activities of complement and stress the roles that they may play in maintaining the integrity of the organism.

INTRODUCTION

Complement is a central part of the innate immune system, on the front line in defence of the body from invading pathogens and in clearance of potentially damaging debris. From its very earliest description over a century ago, complement has been inextricably linked with cell death [1]. The complement system was first identified as a heat-sensitive fraction of serum that acted in concert with antibody to kill bacteria [2]. A century on, research in the field is flourishing, and the fruits of the work are influencing clinical practice, with a number of therapeutics designed to modulate complement activity currently undergoing clinical trials [3].

Of course, complement does more than just kill cells. It has varied and wide-ranging functions that include a crucial role in the efficient phagocytosis of pathogens and cellular debris by opsonizing them with molecules such as C3b [4]. By doing so, it aids the solubilization and clearance of immune complexes that would otherwise lodge in capillary beds and cause damage. Another of its important functions is to act as a stimulus to inflammation, and here the small anaphylatoxic fragments C5a and C3a are involved by directly activating cells bearing the appropriate receptors. More recently, an important role for complement in linking innate and adaptive immunity has been revealed, for example by contributing a second signal to B lymphocytes that have recognized a complement-opsonized antigen [5].

The complement system itself is remarkable in its simplicity. Unfortunately, this simplicity is lost in a quagmire of difficult terminology, a relic of history that has never been adequately addressed. Once over the terminology hurdle, the system reveals its true nature (Figure 1A). It consists of three recognition systems that permit the identification of appropriate targets, several...
Figure 1  For legend, see facing page
enzyme complexes that greatly amplify a stimulus and produce inflammatory mediators and opsonins, and a single terminal pathway that results in the formation of the membrane attack complex (MAC) [4,6]. The MAC is the main perpetrator in the classical story of complement-mediated cell death, known for blasting holes in the cell membrane.

Although complement is designed to protect us, it sometimes does just the opposite. Bacteria and other organisms are targeted efficiently by complement, but, with such a powerful pro-inflammatory system being activated in the body, there is the potential for considerable damage from ‘friendly fire’ [1]. The body therefore has many complement regulators, both fluid phase and membrane bound, to minimize the chances of this occurring [7]. Despite these defences, complement still causes damage to self and has been implicated in the pathogenesis of a large number of inflammatory and immunological diseases, including rheumatoid arthritis [8], glomerulonephritis [9] and multiple sclerosis (MS) [10,11]. Although not necessarily an initiating factor in these conditions, it is often responsible for promoting and perpetuating inflammation. Cell death is often seen in these conditions, but it is not at all clear that lysis is a dominant feature in the in vivo activities of complement.

This review will describe some of the ‘non-classical’ activities of complement, focusing attention on the roles of the MAC as a trigger to cell activation, and the roles of the MAC and other complement products as modulators of apoptotic cell death.

**COMPLEMENT-MEDIATED LYSIS OF CELLS**

As noted above, complement was first recognized because of its capacity, in conjunction with antibody, to lyse cells, in particular bacteria [2,12]. Since then, much attention has focused on how the complement system achieves this, notably the dissection of the molecular mechanisms leading to assembly of the MAC and subsequent cell death. Lysis is an important part of the response to infectious organisms, utilized as a defence not only in the early stages of infection, but also once adaptive immunity has developed [1,5]. Despite this, individuals deficient in components of the membrane

attack pathway are not markedly immunocompromised, a tribute to the remarkable efficiency of complement opsonization and phagocyte clearance mechanisms. However, the MAC has been blamed for much of the damage done when complement is activated in the wrong place or at the wrong time.

The terminal pathway, resulting in the formation of the MAC and subsequent cell lysis, is illustrated in Figure 1(B). The key molecule in the MAC is C9, an amphipathic molecule that inserts through the cell membrane and is then able to polymerize to form the ‘pore’ of the MAC [6]. There may be as many as 16–18 C9 molecules per MAC, which yield the well-known tubular channel structure visible in electron micrographs (Figure 1C) [6,13]. However, only one or two molecules of C9 are required to form functional pores, and these smaller complexes do not form tubular structures. This has caused some controversy with regard to how the MAC interacts with the membrane to cause lysis. Some investigators have favoured a ‘leaky patch’ hypothesis, whereby the MAC disrupts the order of the target membrane, but does not form true, discrete pores [14]. Others have argued that the MAC does form pores, but when C9 is present in low copy numbers these are functional rather than rigid, hollow protein-lined channels [13]. It is this latter view that is now widely held, and the weight of evidence is certainly in its favour.

Erythrocytes were among the first cells to be investigated with regard to the lytic mechanism of complement action, and they are still used extensively today in the complement haemolytic assays for the two pathways [i.e. measurement of haemolytic activity at 50% lysis in the classical (CH50) and alternative (AH50) pathways] [15]. It has been shown that a single functional MAC in the membrane of a metabolically inert, aged erythrocyte is sufficient to lyse the cell by colloid osmosis [16]. The breached membrane permits entry of water into the cell, driven by the osmotic gradient, causing the cell to swell and burst. A similar picture occurs when liposomes are attacked [17]. Not surprisingly, the efficiency with which lysis occurs depends on the number of MACs in the cell membrane, and on the composition of the extracellular fluid.

This simple picture of osmotic cell lysis does not extend to analyses of MAC-mediated killing of nucleated cells. Metabolically active nucleated cells are, in general,
much more resistant to the lytic effects of complement when compared with erythrocytes [18]. Complement-mediated lysis of nucleated cells displays ‘multi-hit’ kinetics, implying a requirement for many MACs on the cell surface, and factors other than colloid osmotic dysregulation, notably the presence of calcium in the extracellular fluid, influence the efficiency of killing [16,19]. In general, nucleated cells differ from aged erythrocytes in that they possess a variety of protective mechanisms that restrict complement-mediated lysis. These include ion pumps that can compensate for membrane pores and the capacity to remove MACs from the cell surface [18,20].

Calcium plays a crucial role in deciding the fate of a cell attacked by complement. The earliest detectable event following MAC attack is a large influx of calcium into the cell [21,22], and increasing levels of extracellular calcium speed the progression to cell death [23]. The ensuing crisis is centred on the mitochondrion. Excess calcium causes loss of the mitochondrial transmembrane potential, resulting in an energy crisis in the cell, as energy-consuming ion pumps frantically try to redress the balance [24]. This precarious situation is exacerbated further by the loss of ATP and its precursors via the MAC pore into the extracellular environment [25]. The decrease in ATP through consumption and leakage renders the cell incapable of sustaining its essential metabolic processes, leading to necrosis.

As indicated above, the MAC is not essential for the killing of most bacteria. Gram-positive bacteria possess an efficient MAC avoidance strategy. The thick cell wall characteristic of these organisms prevents the MAC from reaching and breaching the inner plasma membrane of the bacterium [1]. MAC deposition on the cell wall is without consequence. Gram-negative organisms, however, lack this thick protective coat. Complement activation first permeabilizes the outer membrane, then aids degradation of the thin cell wall and finally exposes the inner membrane to MAC formation and lysis. MAC may also target the zones of apposition of the inner and outer membranes and breach both at the same time [26,27]. Lysis by MAC is a major route for killing of Gram-negative organisms of the genus Neisseria; as a consequence, individuals deficient in the components of the MAC, such as C6 and C7, have markedly increased susceptibility to infections by Neisseria species that frequently cause meningitis [28].

This section has focused on the ‘classical’ capacity of MAC to kill cells, and has highlighted the fact that nucleated cells are often very difficult to kill. The recognition of this fact raised the possibility that MAC deposition on nucleated cells might cause other effects relevant to health and disease. These ‘non-lethal’ consequences of MAC assembly on nucleated cells are increasingly recognized as key events in life-or-death decisions in cells.

**CELL SURVIVAL IN THE FACE OF COMPLEMENT MEMBRANE ATTACK**

We have already noted that metabolically active nucleated cells are more resistant to complement attack than erythrocytes because of the presence of ion pumps and mechanisms for removal of the MAC [16,18]. Additional protection is provided by the presence of membrane-bound complement regulators [7]. Cells are protected from MAC formation by a membrane-bound molecule, CD59, that blocks assembly of the lytic pore [29]. In addition, nucleated cells may express ecto-proteases on their surface that can cleave complement components, or ecto-kinases that can inactivate them by phosphorylation [30]. Finally, when complement is activated on cells, surviving cells can become protected against subsequent attack [30,31]. The mechanisms of this ‘induced protection’ phenomenon are uncertain, although it has been shown to depend upon RNA and protein synthesis [31].

A protein complex known as the large complement-induced protein (L-CIP), related to heat-shock proteins, has been shown to be induced, which translocates to the cell membrane, although a protective function of L-CIP has not been shown [32,33]. ‘Induced protection’ is not limited to MAC attack. Non-lethal amounts of the MAC also protect against other pore formers such as perforin, melittin and streptolysin O which, in turn, can induce protection from MAC attack [34]. All of these protective mechanisms are important in limiting damage to host cells in areas of inflammation, but may also be put to more sinister purpose, for example when they are utilized by tumour cells in order to evade complement-mediated killing.

**Non-lytic consequences of complement membrane attack**

Removal of MACs represents an important mechanism of cell resistance to, and recovery from, complement attack, and is also one of the best defined of the non-lethal effects of MAC assembly (Figure 2). MACs are removed either by shedding on membrane vesicles (ectocytosis) or by internalization and degradation, depending on the cell type [20,35,36]. The efficiency with which this occurs is temperature dependent [18]. Signalling of MAC removal has been studied and, again, calcium is implicated, acting in its well-known capacity as a second messenger [37]. Calcium influx occurs via the pore, but even in the absence of extracellular calcium the MAC still induces an increase in intracellular calcium by triggering calcium release from stores [38]. Protein kinase C activation occurs, both triggered directly by MAC and signalled by calcium. Events further downstream are poorly defined and the precise mechanism of MAC shedding is completely unknown. Calcium therefore plays a double role, being important in protecting cells when the MAC is
Non-classical effects of complement

Figure 2  Range of cellular effects of the MAC

The combination of effects observed depends on the cell type and other environmental factors. PGE$_2$, prostaglandin E$_2$.

Present in non-lytic doses, but contributing to cell death when damage is more extensive [21].

The MAC has been implicated in a variety of other non-lytic effects that differ according to the nature of the target cell and the system interrogated. On phagocytes (neutrophils and macrophages), cell types that are intrinsically resistant to complement-mediated lysis, the MAC induces profound activation, with the production and release of inflammatory mediators such as prostaglandins, thromboxanes, leukotrienes and reactive oxygen species [39]. These processes contribute to homeostasis by stimulating the inflammatory response and arming the host immune system to deal with invading organisms. On the other hand, these same events may be the cause of much damage. For example, glomerular epithelial cells respond to sublytic MAC levels by the induction of cyclo-oxygenase 2 and the production of prostaglandins that contribute substantially to pathology in membranous glomerulonephritis [40].

From studies in vitro on diverse cell types and in vivo focusing on inflammatory diseases, much has been learnt about the signal transduction pathways utilized by the MAC. However, the very diversity of cells and systems studied has created a considerable complexity. Numerous signalling pathways for MAC effects, initiated by the activation of calcium flux [37,41], receptor tyrosine kinases [42] and G-proteins [43], have been described. These in turn can activate other signalling cascades in the target cell, including mitogen-activated protein kinase (MAP kinase) and c-Jun N-terminal kinase pathways [44]. Downstream activation of nuclear factor κB (NFκB) and activator protein-1 may result in gene transcription [40]. Current knowledge, although considerable, represents a patchwork of tenuously connected datasets obtained from different targets. No unifying concept of how the MAC triggers activation events has emerged. As a consequence, it is now necessary to step back and critically evaluate the literature in an attempt to identify common pathways of MAC signalling.

Non-lytic effects of the complement MAC are implicated in several diseases. A role in glomerulonephritis was noted above, and recently, dilated cardiomyopathy has come under the spotlight. MAC deposits are found on apparently viable cardiac myocytes in biopsies from patients with this disease, a finding that was correlated with the expression of tumour necrosis factor-α (TNF-α), a powerful pro-inflammatory cytokine and negative inotropic factor [45]. In relevant cell types in vitro, TNF-α is induced by the MAC via NFκB, providing a possible explanation for the findings in vivo. Many other pathogenic processes in heart disease are being traced to similar non-lytic activating effects of the MAC, including a role in vascular smooth muscle proliferation and remodelling in atherosclerosis [46]. The role of the MAC in the pathological proliferation of cells is now receiving considerable attention.

Complement and cell proliferation

The MAC has been implicated as a stimulus to cell proliferation in a number of scenarios over the past 10 years. In vivo models of mesangioproliferative glomerulonephritis, for example, have shown that mesangial
cell proliferation is markedly reduced in C6-deficient animals, which cannot form the MAC, compared with controls [47]. In other pathological states, such as atherosclerosis, MAC deposition occurs in areas where active proliferation is taking place [46]. From these studies it is not clear whether the MAC induces proliferation directly, or indirectly by stimulating the release of growth factors from activated cells, which then stimulate cell division.

Work on endothelial cells has supported the latter explanation by demonstrating that platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) are produced when the MAC is present on the cell membrane [48]. This process is crucially regulated by CD59: when CD59 is inactivated by glycation, as may occur in diabetes, endothelial cells increase their production of PDGF and bFGF [49]. It has been suggested that this effect contributes to the proliferative disorders seen in diabetes, such as retinopathy and nephropathy. These data also indicate that levels of functional CD59 on a cell surface may be critical not only for protecting the cell, but also in dictating its response to sublytic complement attack.

In studies using aortic smooth muscle cells [50] and Schwann cells [51], non-lethal MAC attack did indeed directly stimulate cell proliferation. Other studies have shown an increase in DNA synthesis in response to the MAC, which in replication-competent fibroblasts probably reflects increased proliferation [52], but in end-cells such as oligodendrocytes [53] and mesangial cells [54] does not. Thus the MAC induces entry to the cell cycle in many cell types, but only takes cells right through to division if they are intrinsically capable of doing so. The MAC can also amplify mitogenic signals from other growth factors, such as PDGF, adding a further level of complexity.

Although the MAC has been clearly implicated in pathological cell proliferation, such as occurs in atherosclerosis, physiologically this response may be important in repairing tissues following inflammation. Thus, while the proliferative effects of the MAC may be damaging in the acute phase of inflammation, during resolution it may assume an important role in repair.

The data summarized above show that the MAC of complement must now be seen in a new light: while the MAC certainly can lyse cells, its non-lytic effects may be of much greater physiological and pathological relevance (summarized in Figure 2). MAC can promote inflammation, increase the resistance of cells to further lytic attack by all sorts of pore formers, and even drive cells to divide. Studies in disease are not only yielding interesting information on how the MAC signals to the cell, but also promising to strongly influence the direction of future therapeutic approaches. Already, it is clear that lytic cell death and the MAC are not related in the simple and straightforward manner that was the accepted dogma.

Further, complement and the MAC may even contribute to cell death through non-lytic processes, and it is to this issue that we will now turn.

**COMPLEMENT AND APOPTOSIS**

Lytic cell death, the ‘classical’ role of complement and the MAC, is associated with necrosis—a pro-inflammatory form of cell death in tissues whereby the cell contents are released to propagate inflammation. In contrast, apoptotic cell death does not involve the release of cell contents and is considered to be non-inflammatory in nature [55, 56]. Nevertheless, complement and the MAC play important roles in regulating apoptosis in vivo. Apoptosis is important in development and homoeostasis. Apoptotic processes regulate cell numbers and fate in embryogenesis and tissue remodelling, contribute to the removal of virus-infected cells [57], and are involved in many diseases characterized by abnormal cell turnover [58]. Thus diseases as diverse as AIDS, cancer and neurodegeneration may involve altered apoptotic pathways.

Apoptosis can be defined by morphological criteria: classically, cellular shrinkage, membrane blebbing, nuclear condensation and DNA fragmentation are seen. At the molecular level, the execution of apoptosis centres on a group of enzymes known as the caspases that cleave various proteins in the cell to produce the typical morphological changes [59]. The decision to commit suicide by apoptosis is largely governed by the Bcl-2 family of proteins. These integrate signals from pro-apoptotic and anti-apoptotic stimuli from both the death receptor (e.g. Fas–Fas-ligand) and mitochondrial pathways of induction. They are able to homo- and hetero-dimerize, and the relative levels of the pro-apoptotic (e.g. Bax) versus the anti-apoptotic (e.g. Bcl-2) members of the family dictate whether or not apoptosis occurs. These signalling proteins act at the level of the mitochondrial membrane to maintain its integrity and regulate the release of cytochrome c, which is responsible for triggering the execution caspases such as caspase 3 [59, 60].

Complement influences apoptosis at two distinct levels: first, in deciding the fate of the cell, and secondly, in helping phagocytes to dispose of the corpses of apoptosed cells.

**Complement in the initiation or inhibition of apoptosis**

Several different products of complement influence the decision to proceed to apoptosis. C5a, one of the small anaphylatoxic fragments of complement, inhibits the spontaneous apoptosis of neutrophils, extending their lifespan after recruitment to sites of inflammation [61]. Other inflammatory mediators may facilitate this protective function of C5a; for example, perforin, a pore-
Non-classical effects of complement

Figure 3  Proposed signalling events involved in the initiation and inhibition of apoptosis by the MAC and C5a

Formation of the MAC results in the influx of calcium. In pro-apoptotic doses, this results in the loss of the mitochondrial transmembrane potential (ΔΨm), leading to the release of cytochrome c, which forms the apoptosome with APAF-1 (apoptosis protease-activating factor-1) and caspase 9. Apoptosis is executed by caspase 3. In cells where the MAC inhibits this, G-proteins are activated, and phosphoinositide 3-kinase (PI-3-K) phosphorylates Bad, and/or MAP kinase pathways increase the expression of Bcl-2. These events act to inhibit apoptosis at the level of the mitochondrion. MAP kinase pathways also inhibit the cleavage of pro-caspase 3. The effects of C5a are dependent on the density of the C5a receptor: at low density, apoptosis is inhibited by a G-protein-dependent pathway, whereas at high density, we propose that C5a receptors may act in a fashion analogous to death receptors such as TNF receptors.

forming molecule produced by cytotoxic T cells, can up-regulate the expression of the C5a receptor on neutrophils when present in non-lethal doses [62]. C5a can also protect neurons. Glutamate is an excitatory neurotoxin that triggers the apoptotic death of neurons in vitro and is implicated in neuronal death in both Alzheimer’s disease and MS [63]. In a model of excitotoxic neuronal death, C5a inhibited apoptosis via inhibition of caspase 3 activity [64]. Confusingly, in a related system, C5a was found to increase the apoptosis of a neuronal cell line [65]. The anaphylatoxin C3a has also been shown to protect neurons in vitro from apoptosis induced by the excitotoxic agent N-methyl-D-aspartate (NMDA) [66]. Interestingly, in stroke the C3a receptor is up-regulated on neurons, and it is tempting to speculate that this may aid cell survival [67].

C5a plays an important role in sepsis, in which a massive systemic inflammatory response occurs that can result in multi-organ failure and death [68]. One of its features is a catastrophic dysregulation of the immune response. Immunosuppression occurs in part as a result of immune cells undergoing apoptosis, and C5a is implicated as a trigger. Thymocytes undergo intense apoptosis in the caecal ligation and puncture model of sepsis [69,70]. Prior to the onset of apoptosis, thymocytes up-regulate C5a receptor expression in response to lipopolysaccharide and interleukin-6 [69]. When these cells are then exposed to C5a, they undergo apoptosis in a caspase-dependent manner (Figure 3). This does not happen to thymocytes with a normal C5a receptor density, and therefore is only seen in the acute septic situation. Importantly, this effect may be blocked with anti-C5a antibodies, a treatment that is still effective in the model even after the onset of sepsis, when most other therapies are without effect [68,71].

The MAC, among its many non-lytic effects, also plays a role in modulating apoptosis; the proposed signalling pathways are presented in Figure 3. Again, diseases of the
nervous system provide good examples. The primary target cell in MS is the oligodendrocyte, and demyelination is the hallmark of the disease [43]. Complement has been implicated as a pathogenic factor in MS, and MAC-mediated killing of oligodendrocytes has been demonstrated in vitro. However, far from being destructive, at non-lytic doses the MAC has been found to promote the survival of oligodendrocytes [72]. The MAC inhibits apoptosis by increasing Bcl-2 transcription and suppressing the activation of caspase 3. More recently, in vitro studies using DNA microarrays have shown that the MAC up-regulates anti-apoptotic genes and down-regulates pro-apoptotic genes in the central nervous system in the rodent MS model, experimental allergic encephalomyelitis [73], providing some confirmation of the relevance of this effect. However, as we have already seen, the consequences of non-lytic membrane attack go further than this. The MAC can reverse the differentiated phenotype of oligodendrocytes in vitro, and promote their entry into the cell cycle, suggesting that it may have a role in brain repair as well as in promoting survival [53].

Complement has also been implicated in Guillain–Barre syndrome, a devastating illness characterized by ascending paralysis, areflexia and autonomic dysfunction. Guillain–Barre syndrome is a demyelinating disease of the peripheral nervous system, in which Schwann cells, the peripheral nervous system equivalent of the oligodendrocyte, are targets for the MAC [74]. For Schwann cells too, the MAC is anti-apoptotic when present in non-lytic doses, a finding that accords well with its ability to induce proliferation in this cell type, again indicating a role in repair [51,75].

In other circumstances, the MAC has been implicated as a trigger to apoptosis. Complement is an important player in the pathogenesis of a number of renal inflammatory diseases in which substantial deposits of complement components are found in the kidney. Recent studies in vitro have shown that exposure of renal mesangial cells to non-lytic amounts of MAC caused the cells to undergo apoptosis [76]. MAC-triggered cell death occurred through a caspase-dependent pathway, specifically via caspase 3, and the cells displayed characteristic features of apoptosis. Evidence from animal models of renal disease also implicates the MAC in triggering apoptosis. In antibody-dependent glomerulonephritis models, such as those induced by concanavalin A or anti-Thy1.1, the abundant glomerular cell apoptosis seen in normal rats was much reduced in C6-deficient rats, in which the MAC cannot form [77,78].

Complement has been implicated in cell death in many models of ischaemia/reperfusion injury, e.g. myocardial infarction and stroke [79,80]. Cell death occurs via apoptosis as well as necrosis in these injuries [81], and the MAC contributes to both. In a myocardial ischaemia/reperfusion model, anti-C5 antibodies inhibited damage following reperfusion and markedly reduced the number of apoptotic cells in the myocardium [81]. Thus blocking the MAC and C5a by this method attenuated both lysis and apoptosis, providing an exciting avenue for improving the efficacy of thrombolytic therapy. Importantly, apoptosis has been found to occur prior to the onset of necrosis in a renal model of ischaemia/reperfusion injury [82]. When apoptosis was inhibited by caspase inhibitors or by survival factors such as insulin-like growth factor-1, inflammation was prevented and renal damage diminished markedly. These results are, at first sight, counter-intuitive, in that apoptotic cell death is considered to be non-inflammatory. However, there is evidence that apoptosis can in some circumstances exacerbate inflammation, and here too complement is implicated. For example, in an in vitro model of apoptosis in human umbilical vein endothelial cells, apoptotic cells activated the classical pathway, resulting in C3 deposition and the release of pro-inflammatory molecules [83].

A note of caution must be injected at this point. Studies of complement-triggered apoptosis usually rely heavily on observation of the typical morphological changes of apoptosis. However, cells attacked by complement may undergo a type of death that has the nuclear features of apoptosis, such as nucleosomal fragmentation, but the cytoplasmic features of necrosis, such as swelling and disruption of organelles [84]. It has been suggested that this represents another form of cell death, ‘apoptotic necrosis’. Such features have also been noted and investigated in vitro, and these studies have shown that such death is primarily necrotic, but with secondary features that resemble nuclear apoptosis, most likely due to an extracellular DNase entering through the disrupted membrane [85]. Studies that identify apoptosis by DNA fragmentation and TUNEL (terminal deoxynucleotidyl UDP nick-end labelling) assay may therefore be misleading.

**Complement in the recognition and clearance of apoptotic cells**

Complement is also involved in the clearance of apoptotic cells. The safe clearance of cells dying by apoptosis is essential in order to prevent an inflammatory response [55,86]. If apoptotic cells are not cleared efficiently, they undergo secondary necrosis, releasing pro-inflammatory mediators into the environment. Most apoptotic cells are cleared by professional phagocytes such as macrophages, although they can be cleared by other cells, albeit less efficiently. Numerous cell surface features of apoptotic cells have been implicated in their recognition by phagocytes [55,87,88]. The contribution of complement had received surprisingly little attention until quite recently [89]. The surface blebs that are characteristic of cells undergoing apoptosis were shown to bind C1q through the globular head domains, a conformation that permits activation of the classical pathway and deposition of other complement fragments [90]. C1q binds specific
receptors on the phagocyte surface, including CD91 and calreticulin, to initiate phagocytosis [87]. Mannose-binding lectin, the lectin pathway analogue of C1q, also binds apoptotic cells and recruits phagocytes through these same receptors. Apoptotic cells may also activate the alternative pathway directly [83], and all three pathways may result in C3 deposition and activation of the terminal pathway. Down-regulation of membrane complement regulators on cells undergoing apoptosis may also contribute to increased ‘opsonization’ of the apoptotic cell [91]. Fragments of C4 and C3 deposited on the apoptotic cells will bind the phagocyte-expressed receptors CR1 (complement receptor 1), CR3 and CR4 to further aid recognition and clearance [92]. Binding through CR3 also signals the phagocyte to down-regulate the secretion of interleukin-12 and interferon-γ. This response has an additional anti-inflammatory effect by dampening cell-mediated immunity [93]. From such studies, it has become increasingly clear that phagocytes respond to complement and other apoptotic cell surface signals in the context of their environment, thus defining the ‘meaning’ of cell death. Phagocytes may respond by altering the susceptibility of neighbouring cells to death, or by modulating the inflammatory response.

The relevance of complement for apoptosis is clearly evident in systemic lupus erythematosus (SLE), a systemic autoimmune disorder characterized by autoantibodies to nuclear antigens [94]. The most obvious role that complement plays in this disease is in causing tissue damage when activated by immune complexes deposited in organs such as the kidneys [94]. This results in inflammation in the kidney and other affected organs. However, complement deficiencies, particularly those of the classical pathway, also predispose to pathology resembling SLE, a finding that appears at first to weaken the case for complement involvement [95,96]. The association with SLE is seen most strikingly in C1q deficiency, the strongest single gene association with SLE, and to a lesser extent with C4 and C2 deficiency [95], and can be explained by the roles noted above for C1q and other complement components in clearing apoptotic cells. This is evident in C1q-deficient mice, where multiple apoptotic bodies were seen in the kidney in association with a glomerulonephritis similar to that seen in SLE [97]. Even in the absence of complement deficiency, SLE is associated with complement consumption and with antibodies against C1q, both of which predict severe disease [98]. This has led to the hypothesis that SLE in humans and mice is caused by defects in the removal of apoptotic cell debris, the ‘waste disposal’ hypothesis [94]. Apoptotic cells that are not cleared generate an autoimmune response because cytoplasmic and nuclear antigens that are normally sequestered become exposed at the cell surface or outside the lysed cell [89,99,100]. Apoptotic cells undergoing secondary necrosis may also provide ‘danger’ signals to the antigen-presenting cells and T cells in the area, converting the response to these antigens from tolerogenic into immunogenic [55]. Activated T cells can then stimulate autoreactive B cells to differentiate into plasma cells and start producing autoantibodies [94].

The ‘waste disposal’ hypothesis is further supported by the observations that mice deficient in serum amyloid P (SAP) or DNase 1, both of which are involved in the clearance of apoptotic debris, also develop an SLE-like disease [101,102]. The pentraxin SAP solubilizes DNA and chromatin in the extracellular fluid and transports it to the liver, where it is catabolized, while DNase1 breaks down DNA into non-antigenic fragments. Indeed, SLE patients have low levels of DNase 1, adding support to the involvement of this enzyme, and to the importance of reducing the antigenicity of DNA by cleaving it [102]. C-reactive protein, also a member of the pentraxin family and closely related to SAP, binds small nuclear ribonucleoproteins. These are also autoantigens in SLE, and it is widely recognized that disease flares are associated with a deficient C-reactive protein response, which may be associated with poor clearance of such antigenic material [103].

These studies emphasize the multi-factorial process of efficient waste disposal, and highlight the fact that defects in any one of the key handling processes can cause failed clearance and pathology. They also suggest that treatment strategies targeting the clearance mechanisms for apoptotic cells might be effective in many diseases.

CONCLUSIONS AND FUTURE DIRECTIONS

We are now at a fascinating point in our understanding of the roles of complement in homoeostasis and pathology. The ‘classical’ role of complement as a lytic system remains important, but it is now clear that complement and the MAC have a range of non-lethal effects on cells, acting as a drive to inflammation, but also involved in the induction of proliferation and resistance to killing by both lysis and apoptosis [18,72]. In other circumstances, complement can kill by inducing apoptosis [76], and there is abundant evidence for a role in the efficient clearance of apoptotic cell debris [87]. There are several situations where complement appears to be playing opposing roles in the same system: both promoting cell survival and inducing apoptosis; both causing cell lysis and inducing protection against lysis. These apparent contradictions probably arise due to the artificial systems in which most have been demonstrated, but do illustrate the protean effects of this superficially simple system in complex tissues.

Perhaps the first step towards clarification is to recognize that complement has these diverse activities. Current complement therapies aim to inhibit complement activation in order to control inflammation [3].
What is clear from the above discussion is that it may not always be to the benefit of the patient for complement to be inhibited. The risks relating to infection are well rehearsed, but a loss of the ‘non-classical’ complement activities described above might be of more relevance to the patient. To overcome these problems, complement inhibitors may have to be carefully tailored and targeted to act only in those areas where complement activation needs to be controlled. Ultimately, we need to understand not only how complement influences cell fate, but also how we can alter this when it goes awry in disease.

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