Apoptotic cell clearance by macrophages and dendritic cells : immunoregulation in the context of innate immunity

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A pivotal role for innate immunity in the clearance of apoptotic cells

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Summary

Apoptotic cells can be recognized and taken up by both macrophages and dendritic cells. Phagocytosis of apoptotic cells generally leads to active suppression of cytokine production by professional phagocytes. This is different from the response towards cells died by necrosis, which induce a pro-inflammatory cytokine profile. Uptake of apoptotic cells involves a large number of receptors and opsonins, which bind to cellular ligands exposed during the various stages of apoptotic cell death. Among the opsonins of apoptotic cells, complement factors, including C1q, and complement-activating members of the pentraxin family play an important role. This is indicated by *in vitro* phagocytosis studies and supported by the susceptibility to systemic autoimmunity in carriers of genetic deficiencies for early complement proteins. The present review summarizes the role of molecules of innate immunity in the handling of apoptotic cells by macrophages and dendritic cells. It is proposed that C1q and other opsonins prevent autoimmunity and maintain *self*-tolerance by supporting the efficient clearance of apoptotic material, as well as by actively modulating phagocyte function.

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Introduction

The innate immune system is of key importance in primary recognition of invading pathogens. In this respect, a large number of so-called pattern recognition molecules have been identified, including soluble molecules, such as complement factors, members of the collectin family and the pentraxin family, as well as membrane-bound receptors, such as members of the family of Toll-like receptors (TLR) and the family of C-type lectins. Pattern recognition molecules are not only involved in pathogen elimination but also in the clearance of apoptotic cells. The rapid and early removal of apoptotic cells by phagocytosis is directed by early changes of the apoptotic cell surface that precede the release of its intracellular contents. Apoptotic cells are phagocytosed by professional phagocytes, such as macrophages and immature dendritic cells, but also non-professional phagocytic neighbor cells may participate. Identification of molecules and mechanisms involved has revealed important parallels between innate host defense against pathogenic intruders and clearance of *self* debris, thereby shedding a new light on mechanisms of tolerance and immunity.

The present review summarizes recent data on the role of pattern recognition molecules in the recognition and clearance of apoptotic cells, and will elaborate on the way these molecules might direct differential cellular responses upon apoptotic cell phagocytosis.

Molecules involved in recognition and clearance of apoptotic cells

A well-recognized and early event in the apoptotic process is the loss of phospholipid asymmetry of the cell membrane. This leads to exposure of phospholipids such as phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) on the outside of the cell membrane. Exposure of phospholipids is a major factor in the recognition of apoptotic cells by phagocytes, involving membrane-bound receptors, such as the PS-receptor [2] and several scavenger receptors [3,4], as well as a number of soluble opsonins that can bridge apoptotic cells with phagocytes (Table 1 and references therein). Mice deficient for the membrane receptor for PS show a profound defect in the clearance of apoptotic cells, causing abnormal development and neonatal death [5].

Important insight into mechanisms involved in removal of apoptotic cells has been generated by studies in *Caenorhabditis elegans* (reviewed in [6,7]). In this organism, two genetic pathways have been defined, involving the molecules CED-1, CED-6 and CED-7 in the first pathway, and CED-2, CED-5, CED-10 and CED-12 in the second pathway, which are involved in the engulfment of dying cells. A worm homolog of the PS receptor, *psr1*, appeared to be involved in the recognition of cell corpses, and was shown to mediate its function via interaction with the signaling

proteins CED-5 and CED-12 [8]. However, in contrast to observations in humans and mice, disruption of cell engulfment pathways in worms did not lead to overt disease.

Table 1. Opsonins involved in bridging apoptotic cells with phagocytes

Cell surface receptors involved	Opsonin	Ligand on apoptotic cell	Ref.
CD91/calreticulin	C1q	?	1,9-12
Scavenger receptor-A/calreticulin ?	MBL	?	12,13
	SP-A	?	14,15
Other collectin receptors	SP-D	?	15
Via C3b receptors?[a]	C4b	Covalent binding upon activation[b]	10,16
Complement receptor-3 Complement receptor-4	(i)C3b	Covalent binding upon activation[b]	16,17
Pentraxin receptor(s)?	CRP	PC	18,19
Via collectin receptors?[a]	SAP component	PE, chromatin, DNA	20
Via C3b receptors?[a]	Pentraxin 3	?	1,21,22
Via collectin receptors?[a] Via C3b receptors?[a]	IgM	PC	23,24
c-Mer	growth arrest-specific gene 6[c]	PS	25,26
Tyro3? [d]	Protein S	PS	27
$\alpha_v\beta_3$	milk fat globule-EGF-factor 8	PS, PE	28
$\alpha_v\beta_3$ / CD36	thrombospondin-1	?	29,30
?	β2 glycoprotein	PS	31,32

a. Collectin receptors and C3b receptors can mediate phagocytosis following C1q binding and activation of the classical complement pathway, respectively
b. C3b and C4b can bind to complement-activating surfaces via a covalent thiol-ester linkage
c. GAS6 can also bind to other receptors related to c-Mer (Tyro-3, Axl)
d. It is not sure whether human protein S may bind to human Tyro-3 [33,34]

In mice and humans, impaired clearance of apoptotic cells and material derived from these cells is associated with the development of a systemic SLE-like autoimmune disease. Systemic autoimmunity was observed in mice deficient for serum amyloid P-component (SAP), c-Mer, C1q, C4, and IgM, all molecules potentially involved in such clearance processes (Table 2 and references therein).

Furthermore, there is a striking association between genetic deficiencies of the components of the classical complement pathway (C1q, C1r, C1s, C4, C2) and SLE in humans [35,35]. This association is hierarchical, and the association of C1q deficiency with SLE is almost 100 %. Several groups have established that complement components (C1q, mannose-binding lectin (MBL), C4, C3) directly and indirectly interact with apoptotic cells (Table 1), as is further discussed below.

Involvement of the complement system in the opsonization and clearance of apoptotic cells

The complement system can be activated via three different pathways, namely the classical pathway (CP), the alternative pathway (AP), and the lectin pathway (LP), which converge at the level of C3 activation (Figure 1). Whereas the LP and the AP primarily use a direct target recognition mechanism, the CP is mainly activated via binding of the initiating factor C1q to target-bound IgG or IgM antibodies. C1q is able to directly bind to apoptotic cells via its globular head domain [1,11], which may induce complement activation with subsequent deposition of C4b and C3b [1]. Moreover, C1q can also indirectly bind to apoptotic cells via several intermediates. Binding of IgM autoantibodies to apoptotic cells may be responsible for the major part of C1q binding and complement activation by apoptotic cells exposed to normal human serum [23,24]. The pentraxin family members C-reactive protein (CRP), pentraxin-3 (PTX3), and SAP bind to apoptotic cells [18,20,21], which can lead to a direct interaction with phagocyte receptors [4], as well as to secondary C1q binding and complement activation [18,22].

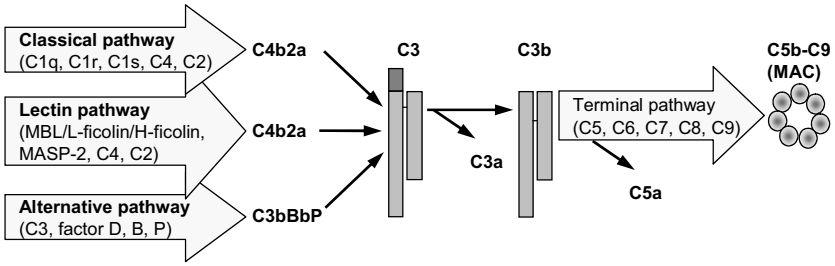

Figure 1. The three pathways of complement activation. Each complement activation pathway generates a C3 convertase (C4b2a / C3bBbP), which mediate cleavage of C3, followed by activation of the terminal complement pathway and formation of the membrane attack complex (MAC).

C1q, being the recognition molecule of the CP, is functionally and structurally related to MBL, a major recognition molecule of the LP [36]. MBL belongs to the family of collectins, soluble multimeric pattern recognition molecules, characterized by C-type lectin domains that serve for ligand recognition and collagenous domains

that interact with receptors. MBL can bind to apoptotic cells via its lectin domains [12,13]. However, this interaction does not lead to complement activation [13]. Not only MBL but also L-ficolin, a member of the ficolin family that is able to activate the LP [37], is able to bind to apoptotic cells (M. Lund Jensen and P. Garred, unpublished observation). Other members of the collectin family include the lung surfactant proteins A and D (SP-A, SP-D). These molecules mediate phagocytosis of apoptotic cells by human and rat macrophages [14,15]. A direct interaction with apoptotic cells has been demonstrated for SP-A [14], which was reported to be lectin domain-independent [14], suggesting that the mechanism involved is different from that used by MBL.

The cellular receptors for C1q have been a matter of debate for a long time. Via peptide sequencing, the endothelial receptor for C1q, called cC1qR, was shown to be identical to calreticulin (CRT). CRT can not only bind to C1q, but also to MBL, SP-A, and SP-D [38]. CRT, as well as several host-derived heat shock proteins (gp96, HSP70, HSP90), can bind to the α2-macroglobulin receptor CD91 [39]. CD91 is a transmembrane receptor that can mediate phagocytosis, and is homologous to the *C. elegans* protein CED-1. Indeed, a complex of CD91 and CRT serves as a collectin receptor on the surface of macrophages and can mediate the phagocytosis of apoptotic cells opsonized with C1q, MBL, SP-A or SP-D [12,15]. Furthermore, scavenger receptor A has now been reported as a second receptor involved in binding and internalization of CRT and gp96 [40]. Scavenger receptor A is prominently expressed not only on macrophages but also on dendritic cells and therefore might also be involved in the uptake of apoptotic material opsonized with C1q and collectins.

Opsonization in relation to the stage of cell death

Depending on the ligand on the apoptotic cell, opsonins may support clearance of apoptotic cells in early or later stages of the apoptotic process. Molecules that bind to exposed phospholipids, such as the PS receptor, several scavenger receptors, as well as a number of opsonins for apoptotic cells (Table 1) presumably interact with early apoptotic cells. For other opsonins, such as C1q, MBL, SP-A, and PTX3, the ligand on the apoptotic cell surface is currently unknown, but, in case of C1q and MBL [1,13], probably distinct from PS. Accordingly, C1q and MBL binding to apoptotic cells are rather late events in the cell death process: C1q binding to early apoptotic cells is much weaker than to late apoptotic cells and binding of MBL and PTX3 was exclusively demonstrated to late apoptotic cells [1,13,21]. Furthermore, SAP, an opsonin binding to PE, is able to bind to early apoptotic cells, but again the binding to late apoptotic cells is of much higher affinity [20]. Also deposition of C4 and C3 on apoptotic cells, as a consequence of activation of the complement cascade, is a late event during apoptosis [24,41].

C1q, MBL and PTX3 do not only bind to intact apoptotic cells, but also to microparticles that are released from the cell during apoptosis [1,13]. Noteworthy, whereas we found that C1q, MBL and PTX3 always bind to a subpopulation of these microparticles, SAP showed strong binding to the complete population of microparticles collected from the supernatant of apoptotic Jurkat cells (Fig. 2), suggesting that this molecule, which is constitutively present in human serum in a high concentration, may play a prominent role in the clearance of such apoptotic debris.

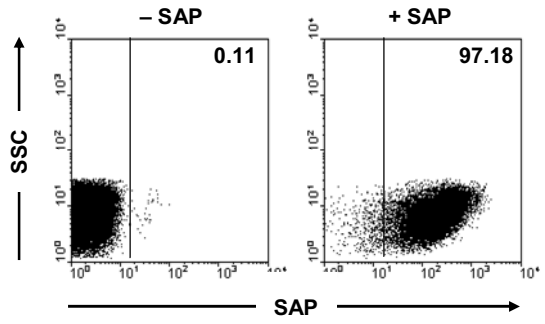

Figure 2. Binding of SAP to cell-derived microparticles generated during induction of apoptosis. Jurkat cells were induced to apoptosis by treatment with 40 µM etoposide during 48 hours in the presence or absence of purified human SAP (20 µg/ml). Microparticles were isolated from the culture supernatant as described [1] and stained for the binding of SAP using a monoclonal antibody directed against SAP, followed by PE-conjugated goat anti-mouse antibodies for detection. SAP and mAb anti-SAP were a kind gift of Dr. Hack and Dr. Familian.

Together, these data suggest that C1q, MBL and the pentraxins are primarily important in the clearance of apoptotic cells in later stages of the cell death process, after they have become leaky and/or have fallen apart in membrane-enclosed apoptotic bodies. Therefore, in the normal clearance of early apoptotic cells, other clearance mechanisms may prevail, involving molecules such as the PS receptor. It is difficult to estimate how often late apoptotic cells do occur *in vivo*. Furthermore, *in vitro* and *in vivo* phagocytosis experiments use a mixture of early and late apoptotic cells, since these cells progress into late stages during handling. Therefore, the role of opsonins in the uptake of early apoptotic cells remains to be established. However, since C1q-deficient mice show impaired clearance of apoptotic cells [42], and since both C1q-deficient mice and humans are strongly susceptible to autoimmunity, the contribution of C1q to the clearance system is apparently important. Complement-dependent clearance mechanisms could be especially relevant during e.g. trauma or inflammation, resulting into an increased load of apoptotic material.

Noteworthy, MBL [13] and C1q (W. Xu et al. unpublished) do not only directly bind to late apoptotic cells but also to cells dying via necrosis. Necrotic cells activate the classical complement pathway, largely via an IgM-dependent mechanism [Ciurana et al. submitted for publication, [41]]. Also necrotic cells expose phospholipids that may serve as ligands for receptors and opsonins. Therefore, the clearance mechanisms for apoptotic cells are presumably also involved in the clearance of

necrotic material and therefore these molecules may function as general scavengers of cellular debris.

Opsonization modulates the uptake of apoptotic cells

Opsonization generally increases the efficiency of the interaction between phagocyte and apoptotic cell and induce receptor-mediated phagocytosis, which accelerates the kinetics of the phagocytosis process and thus enhances the capacity of the phagocytic system. Most of the molecules implicated in apoptotic cell clearance have been shown to contribute to the uptake of apoptotic cells by macrophages *in vitro*. *In vivo* support for their role in clearance of self debris is in a number of cases available by studies in knockout mice, as presented in table 2.

Table 2. In vivo support for a role of receptors and opsonins in the clearance of self debris

Molecule	Phenotype of knockout mice	Ref.
C1q	Systemic autoimmunity with autoantibodies and glomerulonephritis Increased numbers of apoptotic cells in glomeruli Delayed clearance of apoptotic cells from the peritoneal cavity	10,42
SP-D	Delayed clearance of apoptotic cells from the lung	15
C4	Systemic autoimmunity with autoantibodies and glomerulonephritis Delayed clearance of apoptotic cells from the peritoneal cavity	10,43
SAP	Systemic autoimmunity with autoantibodies and glomerulonephritis Accelerated chromatin degradation, enhanced immune response against chromatin	44
IgM	Autoimmunity to nuclear antigens, renal IgG and complement deposition	45,46
c-Mer	Autoimmunity to nuclear antigens, renal IgG and complement deposition	25,47
PS receptor	Defect in clearance of apoptotic cells in the thymus and spleen Accumulation of apoptotic cells in brain and lungs Abnormal development and neonatal lethality	5

Opsonins can contribute to the clearance of apoptotic cells in a tissue-specific way. Mice deficient for SP-D show decreased uptake of apoptotic cells that are administered into the lung [15]. Mice deficient for C1q and C4 show a delayed clearance of apoptotic cells that are injected into the peritoneal cavity [10]. However, $C1q^{-/-}$ mice show a normal uptake of apoptotic cells in the lung [15]. Similarly, despite the fact that C1q is able to bind to apoptotic keratinocytes [9], it does not seem to contribute to the uptake of apoptotic cells in the skin [48]. C1q supports the uptake of apoptotic cells by macrophages but not by renal mesangial cells [49], indicating that a defect in clearance by mesangial cells cannot explain the increased numbers of apoptotic cells in glomeruli from C1q-deficient mice [42].

The data available indicate that deficiency of apoptotic cell opsonins such as complement factors delays the *in vivo* uptake of apoptotic material by professional phagocytes [10]. This may result into a prolonged exposure of the immune system to apoptotic cells and to a progression of these apoptotic cells into later stages of cell death, thereby increasing the risk of induction of autoimmunity. Apoptotic cells are a rich source of autoantigens that are known targets of autoantibodies in systemic autoimmune diseases such as SLE. Uptake of these autoantigens by dendritic cells (DC) may lead to professional presentation to naïve T cells and thereby to a loss of tolerance. DCs are professional antigen presenting cells that are responsible for induction of primary immune responses. It is now clear that DCs, depending on their state of maturation, also play a critical role in induction and maintenance of tolerance [50]. Walport and colleagues have introduced the waste disposal hypothesis to explain the role of C1q in the prevention of systemic autoimmunity [35]. In this hypothesis, the main role of C1q is to promote the uptake of apoptotic debris by macrophages. Without C1q, the efficiency of this process is too low, leading to an overload of apoptotic cells which will then be taken up also by immature dendritic cells.

Recent data obtained by our group indicate that C1q and MBL do not only enhance the uptake of apoptotic material by macrophages, but also by immature dendritic cells (A.J. Nauta et al., submitted for publication). Therefore, it seems that these apoptotic cell opsonins do not make a distinction between macrophages and immature DCs, but allow uptake of apoptotic cells in both cell types. A potential role for C1q in antigen uptake by immature DCs is also strongly supported by our recent observation that immature DCs, but not mature DCs, are strong producers of C1q, even stronger than macrophages [51].

Not only C1q and MBL, but also complement activation products promote the uptake of apoptotic cells by DC, involving the complement receptors CR3 and CR4 [17,52]. Data obtained from complement-deficient mice and humans indicate that C1q and complement are involved in maintenance of tolerance. Since C1q and complement components promote apoptotic cell uptake by both macrophages and immature DC, we hypothesize that these apoptotic cell opsonins have important immunomodulatory effects.

Opsonization may direct the cellular response to apoptotic cells

Phagocytosis of apoptotic cells by professional phagocytes not only prevents the release of their harmful intracellular contents, but also actively modulates phagocyte function [4,53]. Phagocytosis of apoptotic neutrophils by human macrophages was shown to inhibit production of cytokines and chemokines, whereas production of TGF-β1 was increased [54]. The addition of apoptotic cells to macrophages also modulates cytokine production induced by TLR ligands [54,55],

which partially involves production of TGF-β1. Apoptotic cell-induced release of TGF-β1 *in vivo* was shown to require exposure of PS [56].

With respect to dendritic cells, it has been shown that DC can engulf both apoptotic and necrotic cells [57-59]. Similarly to macrophages, DC show suppressed cytokine production upon their exposure to apoptotic cells [17,59]. Furthermore, antigens delivered to DC via apoptotic cells induced tolerance *in vivo* [60]. However, under certain circumstances, exposure of DC to apoptotic cells can also induce DC maturation and immunity [61,62]. This might be dependent on the amount of apoptotic cells, as well as on the presence of pro-inflammatory signals and opsonizing antibodies.

At this moment, there is only little information available about how the known apoptotic cell opsonins interfere with the modulation of phagocytic responses by apoptotic cells. Apoptotic cells opsonized with CRP and complement induced expression of TGF-β by macrophages [18]. Furthermore, stimulation of the iC3b receptor was shown to inhibit cytokine production by DC [17]. In contrast, the interaction of SP-A and C1q with the CD91/CRT complex on macrophages was recently demonstrated to be a pro-inflammatory interaction [63]. However, this has not been studied in the context of these opsonins present on apoptotic cells, which could make a crucial difference.

Contrary to the phagocytosis of apoptotic cells, which is in most cases described as a non-inflammatory process, phagocytosis of necrotic cells leads to DC maturation and macrophage activation [57,60,64]. The pro-inflammatory effects of necrotic cells have been linked to the release of heat shock proteins [65], the chromosomal protein HMBG1 (high mobility group box chromosomal protein 1) [66], and high concentrations of uric acid [67]. Strikingly, heat shock proteins and HMBG1 were not released from cells dying by apoptosis, also not in a late stage of cell death.

Heat shock proteins are now described as endogenous ligands of several members of the TLR family, including TLR2 and TLR4 [68]. Interestingly, SP-A was recently also shown to bind to TLR4 [69]. Furthermore, heat shock proteins can signal towards phagocytic cells via CD91 and via scavenger receptor A [39,40]. This involves an interaction between heat shock proteins and these receptors in a similar way as described for calreticulin, the molecule proposed to be involved in the binding of C1q and collectins [12,63]. Therefore, it is presently unclear whether and how phagocytic cells may distinguish between signals coming from heat shock proteins, derived from necrotic cells, and signals coming from calreticulin bound to C1q, present on the surface of apoptotic cells. It has been proposed that the PS receptor is an important "molecular switch" in this respect, determining whether or not the phagocytic cell should mount a pro-inflammatory or a tolerogenic response

[70]. This hypothesis includes the premise that necrotic cells are somehow able to prevent signaling via the PS receptor.

Concluding remarks

Opsonization of apoptotic cells is required for their safe elimination. Whereas appropriate opsonization will direct a rapid and anti-inflammatory clearance of apoptotic material by macrophages and DC, inappropriate opsonization, either in a quantitative or qualitative way, may lead to disturbed uptake and phagocyte modulation, finally resulting in a loss of self tolerance (Figure 3).

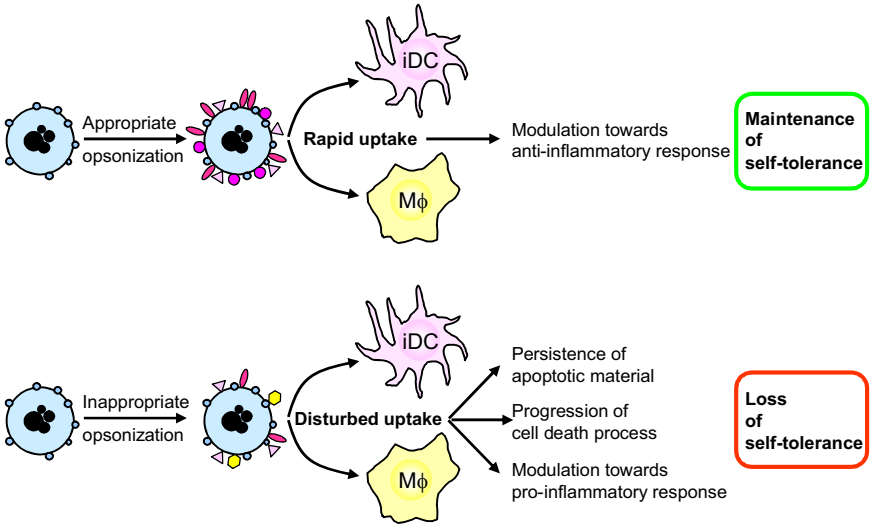

Figure 3. The role of opsonins in the handling of apoptotic cells. Appropriate opsonization of apoptotic cells leads to their rapid uptake by macrophages (Mϕ) or immature dendritic cells (iDC), and to a modulation of these phagocytic cells, leading to an anti-inflammatory response and maintenance of tolerance. In contrast, quantitatively or qualitatively inappropriate opsonization may disturb the apoptotic cell uptake, leading to a loss of tolerance, as illustrated in the lower panel.

Dendritic cells are well recognized as the central mediators of both immunity and tolerance. Recent studies have revealed that also for the induction of tolerance immature DC need to reach a certain stage of maturation [71]. In this respect we hypothesize that a combination of stimuli, such as opsonins, membrane-associated ligands, cytokines and TLR ligands, will determine whether the DC will induce immunity or tolerance upon encountering dying cells. Further definition of these signals and their effect on phagocyte responses towards apoptotic cells are required to understand why deficiencies in molecules involved in the handling of apoptotic cells are associated with systemic autoimmunity.

Acknowledgments

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Reference List

1 Nauta AJ, Trouw LA, Daha MR et al. Direct binding of C1q to apoptotic cells and cell blebs induces complement activation. Eur J Immunol. 2002;32:1726-1736.

2 Fadok VA, Bratton DL, Rose DM et al. A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature. 2000;405:85-90.

3 Giles KM, Hart SP, Haslett C, Rossi AG, Dransfield I. An appetite for apoptotic cells? Controversies and challenges. Br J Haematol. 2000;109:1-12.

4 Nauta AJ, Daha MR, Kooten C, Roos A. Recognition and clearance of apoptotic cells: a role for complement and pentraxins. Trends Immunol. 2003;24:148-154.

5 Li MO, Sarkisian MR, Mehal WZ, Rakic P, Flavell RA. Phosphatidylserine receptor is required for clearance of apoptotic cells. Science. 2003;302:1560-1563.

6 Grimsley C, Ravichandran KS. Cues for apoptotic cell engulfment: eat-me, don't eat-me and come-get-me signals. Trends Cell Biol. 2003;13:648-656.

7 Conradt B. With a little help from your friends: cells don't die alone. Nature Cell Biol. 2002;4:E139-E143.

8 Wang X, Wu YC, Fadok VA et al. Cell corpse engulfment mediated by C elegans phosphatidylserine receptor through CED-5 and CED-12. Science. 2004;302:1563-1566.

9 Korb LC, Ahearn JM. C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited. J Immunol. 1997;158:4525-4528.

10 Taylor PR, Carugati A, Fadok VA et al. A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells in vivo. J Exp Med. 2000;192:359-366.

11 Navratil JS, Watkins SC, Wisnieski JJ, Ahearn JM. The globular head of C1q specifically recognizes surface blebs of apoptotic vascular endothelial cells. J Immunol. 2001;166:3231-3239.

12 Ogden CA, deCathelineau A, Hoffmann PR et al. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. J Exp Med. 2001;194:781-795.

13 Nauta AJ, Raaschou-Jensen N, Roos A et al. Mannose-binding lectin engagement with late apoptotic and necrotic cells. Eur J Immunol. 2003;33:2853-2863.

14 Schagat TL, Wofford JA, Wright JR. Surfactant protein A enhances alveolar macrophage phagocytosis of apoptotic neutrophils. J Immunol. 2001;166:2727-2733.

15 Vandivier RW, Ogden CA, Fadok VA et al. Role of surfactant proteins A, D, and C1q in the clearance of apoptotic cells in vivo and in vitro: calreticulin and CD91 as a common collectin receptor complex. J Immunol. 2002;169:3978-3986.

16 Mevorach D, Mascarenhas JO, Gershov D, Elkon KB. Complement-dependent clearance of apoptotic cells by human macrophages. J Exp Med. 1998;188:2313-2320.

17 Morelli AE, Larregina AT, Shufesky WJ et al. Internalization of circulating apoptotic cells by splenic marginal zone dendritic cells: dependence on complement receptors and effect on cytokine production. Blood. 2003;101:611-620.

18 Gershov D, Kim SJ, Brot N, Elkon KB. C-reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: implications for systemic autoimmunity. J Exp Med. 2000;192:1353-1363.

19 Chang MK, Binder CJ, Torzewski M, Witztum JL. C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: Phosphorylcholine of oxidized phospholipids. Proc Natl Acad Sci U S A. 2002;99:13043-13048.

20 Familian A, Zwart B, Huisman HG et al. Chromatin-independent binding of serum amyloid P component to apoptotic cells. J Immunol. 2001;167:647-654.

21 Rovere P, Peri G, Fazzini F et al. The long pentraxin PTX3 binds to apoptotic cells and regulates their clearance by antigen-presenting dendritic cells. Blood. 2000;96:4300-4306.

22 Nauta AJ, Bottazzi B, Mantovani A et al. Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. Eur J Immunol. 2003;33:465-473.

23 Kim SJ, Gershov D, Ma X, Brot N, Elkon KB. I-PLA_2 activation during apoptosis promotes the exposure of membrane lysophosphatidylcholine leading to binding by natural immunoglobulin M antibodies and complement activation. J Exp Med. 2002;196:655-665.

24 Zwart B, Ciurana C, Rensink I et al. Complement activation by apoptotic cells occurs predominantly via IgM and is limited to late apoptotic (secondary necrotic) cells. Autoimmunity. In press.

25 Scott RS, McMahon EJ, Pop SM et al. Phagocytosis and clearance of apoptotic cells is mediated by MER. Nature. 2001;411:207-211.

26 Nakano T, Ishimoto Y, Kishino J et al. Cell adhesion to phosphatidylserine mediated by a product of growth arrest-specific gene 6. J Biol Chem. 1997;272:29411-29414.

27 Anderson HA, Maylock CA, Williams JA et al. Serum-derived protein S binds to phosphatidylserine and stimulates the phagocytosis of apoptotic cells. Nat Immunol. 2003;4:87-91.

28 Hanayama R, Tanaka M, Miwa K et al. Identification of a factor that links apoptotic cells to phagocytes. Nature. 2002;417:182-187.

29 Ren Y, Silverstein RL, Allen J, Savill J. CD36 gene transfer confers capacity for phagocytosis of cells undergoing apoptosis. J Exp Med. 1995;181:1857-1862.

30 Savill J, Hogg N, Ren Y, Haslett C. Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. J Clin Invest. 1992;90:1513-1522.

31 Balasubramanian K, Chandra J, Schroit AJ. Immune clearance of phosphatidylserine-expressing cells by phagocytes. The role of b2-glycoprotein I in macrophage recognition. J Biol Chem. 1997;272:31113-31117.

32 Balasubramanian K, Schroit AJ. Characterization of phosphatidylserine-dependent b_{2}-glycoprotein I macrophage interactions. Implications for apoptotic cell clearance by phagocytes. J Biol Chem. 1998;273:29272-29277.

33 Stitt TN, Conn G, Gore M et al. The anticoagulation factor protein S and its relative, Gas6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases. Cell. 1995;80:661-670.

34 Godowski PJ, Mark MR, Chen J et al. Reevaluation of the roles of protein S and Gas6 as ligands for the receptor tyrosine kinase Rse/Tyro 3. Cell. 1995;82:355-358.

35 Walport MJ. Complement. Second of two parts. N Engl J Med. 2001;344:1140-1144.

36 Jack DL, Klein NJ, Turner MW. Mannose-binding lectin: targeting the microbial world for complement attack and opsonophagocytosis. Immunol Rev. 2003;180:86-99.

37 Matsushita M, Endo Y, Fujita T. Complement-activating complex of ficolin and mannose-binding lectin-associated serine protease. J Immunol. 2000;164:2281-2284.

38 Sim RB, Moestrup SK, Stuart GR et al. Interaction of C1q and the collectins with the potential receptors calreticulin (cC1qR/collectin receptor) and megalin. Immunobiol. 1999;199:208-224.

39 Basu S, Binder RJ, Ramalingam T, Srivastava PK. CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. Immunity. 2001;14:303-313.

40 Berwin B, Hart JP, Rice S et al. Scavenger receptor-A mediates gp96/GRP94 and calreticulin internalization by antigen-presenting cells. EMBO J. 2003;22:6127-6136.

41 Gaipl US, Kuenkele S, Voll RE et al. Complement binding is an early feature of necrotic and a rather late event during apoptotic cell death. Cell Death Differ. 2001;8:327-334.

42 Botto M, Dell'Agnola C, Bygrave AE et al. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. Nat Genet. 1998;19:56-59.

43 Chen Z, Koralov SB, Kelsoe G. Complement C4 inhibits systemic autoimmunity through a mechanism independent of complement receptors CR1 and CR2. J Exp Med. 2000;192:1339-1352.

44 Bickerstaff MC, Botto M, Hutchinson WL et al. Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. Nat Med. 1999;5:694-697.

45 Ehrenstein MR, Cook HT, Neuberger MS. Deficiency in serum immunoglobulin (Ig)M predisposes to development of IgG autoantibodies. J Exp Med. 2000;191:1253-1258.

46 Boes M, Schmidt T, Linkemann K et al. Accelerated development of IgG autoantibodies and autoimmune disease in the absence of secreted IgM. Proc Natl Acad Sci U S A. 2000;97:1184-1189.

47 Cohen PL, Caricchio R, Abraham V et al. Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase. J Exp Med. 2002;196:135-140.

48 Pickering MC, Fischer S, Lewis MR et al. Ultraviolet-radiation-induced keratinocyte apoptosis in C1q-deficient mice. J Invest Dermatol. 2001;117:52-58.

49 Cortes-Hernandez J, Fossati-Jimack L, Carugati A et al. Murine glomerular mesangial cell uptake of apoptotic cells is inefficient and involves serum-mediated but complement-independent mechanisms. Clin Exp Immunol. 2002;130:459-466.

50 Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. Annu Rev Immunol. 2003;21:685-711.

51 Castellano G, Woltman AM, Nauta AJ et al. Maturation of dendritic cells abrogates C1q production in vivo and in vitro. Blood. 2004;103:3813-3820.

52 Verbovetski I, Bychkov H, Trahtemberg U et al. Opsonization of apoptotic cells by autologous iC3b facilitates clearance by immature dendritic cells, down-regulates DR and CD86, and up-regulates CC chemokine receptor 7. J Exp Med. 2002;196:1553-1561.

53 Savill J, Dransfield I, Gregory C, Haslett C. A blast from the past: clearance of apoptotic cells regulates immune responses. Nat Rev Immunol. 2002;2:965-975.

54 Fadok VA, Bratton DL, Konowal A et al. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-b, PGE2, and PAF. J Clin Invest. 1998;101:890-898.

55 Lucas M, Stuart LM, Savill J, Lacy-Hulbert A. Apoptotic cells and innate immune stimuli combine to regulate macrophage cytokine secretion. J Immunol. 2003;171:2610-2615.

56 Huysmans ACLM, Fadok VA, Henson PM. Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-beta1 secretion and the resolution of inflammation. J Clin Invest. 2002;109:41-50.

57 Gallucci S, Lolkema M, Matzinger P. Natural adjuvants: endogenous activators of dendritic cells. Nat Med. 1999;5:1249-1255.

58 Sauter B, Albert ML, Francisco L et al. Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. J Exp Med. 2000;191:423-434.

59 Stuart LM, Lucas M, Simpson C et al. Inhibitory effects of apoptotic cell ingestion upon endotoxin-driven myeloid dendritic cell maturation. J Immunol. 2002;168:1627-1635.

60 Liu K, Iyoda T, Saternus M et al. Immune tolerance after delivery of dying cells to dendritic cells in situ. J Exp Med. 2002;196:1091-1097.

61 Rovere P, Vallinoto C, Bondanza A et al. Bystander apoptosis triggers dendritic cell maturation and antigen-presenting function. J Immunol. 1998;161:4467-4471.

62 Ronchetti A, Rovere P, Iezzi G et al. Immunogenicity of apoptotic cells in vivo: role of antigen load, antigen-presenting cells, and cytokines. J Immunol. 1999;163:130-136.

63 Gardai SJ, Xiao YQ, Dickinson M et al. By binding SIRPa or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. Cell. 2003;115:13-23.

64 Li M, Carpio DF, Zheng Y et al. An essential role of the NF-k B/Toll-like receptor pathway in induction of inflammatory and tissue-repair gene expression by necrotic cells. J Immunol. 2001;166:7128-7135.

65 Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-k B pathway. Int Immunol. 2000;12:1539-1546.

66 Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature. 2002;418:191-195.

67 Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. Nature. 2003;425:516-521.

68 Vabulas RM, Braedel S, Hilf N et al. The endoplasmic reticulum-resident heat shock protein Gp96 activates dendritic cells via the Toll-like receptor 2/4 pathway. J Biol Chem. 2002;277:20847-20853.

69 Guillot L, Balloy V, McCormack FX et al. The immunostimulatory activity of the lung surfactant protein-A involves Toll-like receptor 4. J Immunol. 2002;168:5989-5992.

70 Henson PM, Bratton DL, Fadok VA. The phosphatidylserine receptor: a crucial molecular switch? Nat Rev Mol Cell Biol. 2001;2:627-633.

71 Lutz MB, Schuler G. Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? Trends Immunol. 2002;23:445-449.