Review

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Soluble innate immune pattern-recognition proteins for clearing dying cells and cellular components: implications on exacerbating or resolving inflammation

Michael L. Litvack[1,2], Nades Palaniyar[1,2,3]

[1]*Lung Innate Immunity Research Laboratory, Program in Physiology and Experimental Medicine, Hospital for Sick Children, Toronto, Ontario, Canada*
[2]*Institute of Medical Sciences and*
[3]*Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada*

Soluble innate immune pattern-recognition proteins (sPRPs) identify non-self or altered-self molecular patterns. Dying cells often display altered-self arrays of molecules on their surfaces. Hence, sPRPs are ideal for recognizing these cells and their components. Dying cell surfaces often contain, or allow the access to different lipids, intracellular glycoproteins and nucleic acids such as DNA at different stages of cell death. These are considered as 'eat me' signals that replace the native 'don't eat me' signals such as CD31, CD47 present on the live cells. A programmed cell death process such as apoptosis also generates cell surface blebs that contain intracellular components. These blebs are easily released for effective clearance or signalling. During late stages of cell death, soluble components are also released that act as 'find me' signal (*e.g.* LysoPC, nucleotides). The sPRPs such as collectins, ficolins, pentraxins, sCD14, MFG-E8, natural IgM and C1q can effectively identify some of these specific molecular patterns. The biological end-point is different depending on sPRP, tissue, stage of apoptosis and the type of cell death. The sPRPs that reside in the immune-privileged surfaces such as lungs often act as opsonins and enhance a silent clearance of dying cells and cellular material by macrophages and other phagocytic cells. Although the recognition of these materials by complement-activating proteins could amplify the opsonic signal, this pathway may aggravate inflammation. Clear understanding of the involvement of specific sPRPs in cell death and subsequent clearance of dying cell and their components is essential for devising appropriate treatment strategies for diseases involving infection, inflammation and auto-antibody generation.

Keywords: innate immune proteins, apoptotic cell clearance, efferocytosis, programmed cell death, apoptotic cell receptors, apoptotic cellular ligands, DNA, DNA-binding proteins

Abbreviations: SLE, systemic lupus erythematosus; PS, phosphatidylserine; LysoPC, lysophosphatidylcholine; PA, phosphatidic acid; PC, phosphatidylcholine; $iPLA_2$, Ca^{2+}-independent inducible phospholipase A_2; NETs, neutrophil extracellular traps; EETs, eosinophil extracellular traps; sPRPs, soluble innate immune pattern-recognition proteins; CRD, collectin carbohydrate recognition domain; SP-A, surfactant protein A; SP-D, surfactant protein D; MBL, mannose-binding lectin; MASPs, MBL-associated serine proteases; CRP, C-reactive protein (CRP); SAP, serum amyloid-P; DAMP, danger-associated molecular pattern

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Correspondence to: Nades Palaniyar, The Hospital for Sick Children, 555 University Ave, Toronto, ON M5G 1X8, Canada. Tel: +1 416 813 7654 ext 2328; Fax: +1 416 813 5771; E-mail: nades.palaniyar@sickkids.ca

Introduction

Recent studies have identified many forms of programmed cell death including ETosis, autophagy, pyroptosis, oncosis and necroptosis.[1–6] These death processes are significantly different from the well-studied apoptosis.[7] Depending on the type of death process, different surface ligands or patterns are exposed on the surface of the dying cells. Apoptotic programmed cell death is essential for many biological processes including elimination of unwanted immune cells. This type of cell death is associated with reduction or resolution in tissue inflammation.[8,9] In contrast, necrosis represents uncontrolled cell death caused by injury, infection or unknown agents, and is likely to worsen or intensify inflammation of the surrounding tissue. Late apoptotic cells that become permeable to various agents are referred to as secondary necrotic cells. Tissues with inflammation often secrete cytokines to recruit additional immune cells, such as neutrophils, T-cells and eosinophils. When these new recruits have completed their responsibilities, they die by various types of programmed cell death and need to be cleared.[1–4,9–11]

Excess inflammation, cell death, accumulation of dying cells, tissue injury and remodelling are hallmarks of many acute and chronic infectious lung diseases (*e.g.* influenza, pneumonia), respiratory distress syndrome, cystic fibrosis and asthma.[11–13] In some circumstances, poor dead-cell clearance causes an active immune response that generates auto-antibodies to material derived from dying cells, and this complicates attempts to resolve tissue inflammation and may aggravate chronic inflammation.[14,15] A well-studied example for this condition is systemic lupus erythematosus (SLE).[14,16] Under normal circumstances, macrophages play an active role in engulfing and clearing apoptotic cells (efferocytosis) from local tissues to prevent dead cell accumulation.[11,17] A wide spectrum of soluble innate immune pattern-recognition proteins (sPRPs), that opsonize apoptotic cells, play an important role in efferocytosis or the clearance of dead cells by macrophages and other phagocytic cells.

Recent research of innate immunity has revealed that sPRPs recognize many surface ligands present on apoptotic cells, whose exposure is mostly dependent on the stages of cell death or apoptosis. These opsonins often share structural similarities, but interact with different specific molecular patterns. These sPRPs have been isolated from the lungs, serum, liver, and kidneys.[10,18] Immune response (*e.g.* inflammation, phagocytosis, auto-antibody generation) depends on the types of sPRPs and coating procedures (*e.g.* opsonization versus complement activation). Moreover, on-going work on innate immunity has revealed some of the membrane-associated receptors for the sPRPs, or other novel pattern-recognition receptors on the surface of phagocytic cells. In this review, we critically evaluate the functions of different sPRPs in the context of clearing dying immune cells and their intracellular components.

Apoptosis

The concept of apoptotic cell death in contrast with necrotic cell death was first clarified and described in the early 1970s. The most notable difference was that cells undergoing this newly identified form of cell death did not cause inflammation to local tissues.[7] Apoptosis can be identified by its morphological and membrane-associated changes including chromatin condensation, cytoplasmic condensation, nuclear fragmentation, membrane phospholipid flipping and the formation of cell-surface protrusions or blebs, which separate from the dying cells. These blebs vary in size and often contain nucleic acids, various cytoplasmic components and membranes. Resident phagocytes can internalize the entire apoptotic cells or blebs. Throughout the apoptotic programmed death sequence, lipids, proteins and nucleic acids are re-arranged in such a fashion that their release to the extracellular spaces or to nearby phagocytes remains silent to the inflammation-driven branch of the immune system.[19] Phospholipids such as phosphatidylserine (PS) flip to the outer leaflet of the cellular membrane as a result of the cessation of function of aminophospholipid translocase, the enzyme responsible for restricting PS to the inner leaflet.[20,21] It is well documented that this exposure of PS to the outside of the apoptotic cell enhances its capacity to be phagocytosed.[20,21] In addition to the exposure of PS on apoptotic cells, lysophospholipids (lysophosphatidylcholine; LysoPC), phosphatidic acid (PA), cardiolipin, aminosugars, mannose, adhesion molecules are also revealed or released.[8,11,20,22]

During the later stages of apoptosis when the plasma membrane is permeable, phosphatidylcholine (PC) is hydrolysed by the Ca^{2+}-independent inducible phospholipase A_2 ($iPLA_2$) and the resultant LysoPC is exposed on the surface of apoptotic cells.[22] Phosphatidic acid of the late apoptotic cells is known to inhibit the uptake of these cells by phagocytes. Other studies suggest that chromatin and the cellular DNA are also accessible during later stages of apoptosis.[23,24] LysoPC and nucleotides are soluble molecules, and they may act as chemo-attractant or 'find me' signals. Thus, phagocytes can easily identify the late apoptotic cells releasing LysoPC and nucleotides for clearance.[25–27] The ligands that are exposed on apoptotic cells act as 'eat me' signals and help to enhance their engulfment by resident phagocytes.[21]

In contrast to these 'eat me' signals exposed on apoptotic cells, vital cells expose 'don't eat me' signals. For example, constitutive surface expression of CD31 or CD47 discourages activated phagocytes from attempting to engulf those cells.[20,21] Non-activated phagocytes appear to have the ability internalize CD47-containing cells.[28]

ETosis or extracellular trap formation

ETosis is a recently described novel form of cell death in neutrophils and eosinophils.[1–3] Neutrophil extracellular traps (NETs) contain genomic DNA and mitochondrial DNA whereas eosinophil extracellular traps (EETs) contain mitochondrial DNA.[2,3] Both NETs and EETs contain intracellular cytotoxic granular proteins of the cells.[1] Upon exerting microbial killing function, these NETs and EETs should be cleared by phagocytes. These altered self or the intracellular materials such as DNA are likely targets for sPRPs including collectins.[29,30] All of these examples of exposed apoptotic cell molecular patterns illustrate the importance of targets for 'pattern recognition' by a wide variety of innate immune proteins, which opsonize apoptotic cells to bridge and prepare them for phagocytic clearance and reduce the potential for accumulation-associated inflammation.

Opsonins of apoptotic cells

The soluble proteins that recognize the patterns exposed on apoptotic cells and aid in their subsequent clearance come in a variety of classes. The major focus here will be on the collectins, ficolins, pentraxins and the well characterized innate-adaptive immunity linking complement proteins (Figs 1 and 2).

Collectins

Innate immune collectins (**col**lagenous **lectins**) are part of a larger group of soluble proteins called C-type lectins and are large multimeric proteins that consist of a collagen and a Ca^{2+}-dependent carbohydrate-binding domain.[10,18,31,32] The collagen domain is characterized by a Gly–X–Y repeat amino acid sequence and is N-terminal proximal whereas the C-terminal proximal lectin domain is globular in structure and contains the characteristic collectin carbohydrate recognition domain (CRD). This CRD is the 'pattern-recognition' region of collectins. Two well-characterized collectins were originally isolated from lung surfactant, subsequently characterized and named surfactant protein (SP-)A and D. Their structures are similar in that the basic collectin structures of SP-A and SP-D consist of mutually unique

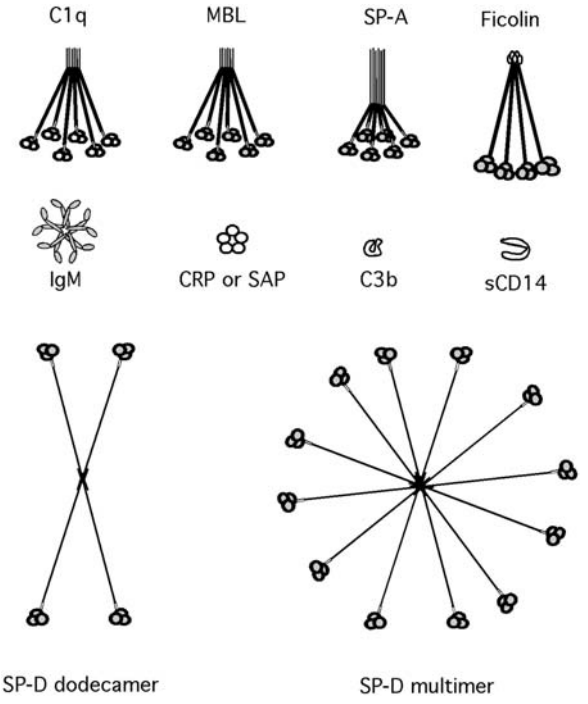

Fig. 1. Major innate immune sPRPs involved in apoptotic cell clearance. Proteins are drawn approximate to the scale. Surfactant protein D is a ~100 nm protein.

homotrimers of 35-kDa and 43-kDa peptides, respectively. The oligomeric structure of SP-A consists of six units of the homotrimer, generating an octodecamer where all the globular CRDs are unidirectionally positioned so that the oligomeric structure resembles a bouquet of flowers. The oligomeric structure of SP-D is much larger than SP-A and forms when the SP-D homotrimers assemble into four units mutually joined at their N-termini with their CRDs directed in opposite directions, to create an X- or asterisk-shaped oligomer. These structures are thought to provide multivalent interactions with the repeating molecular pattern. Both surfactant collectins have been implicated in the clearance of apoptotic cells and other biological materials.[10,18,32,33]

Initially, SP-A was noted to serve a structural purpose for the maintenance of surfactant, which helps to reduce surface tension in the lungs.[34] Recently, however, a wide variety of novel studies have highlighted SP-A as a major soluble innate immune protein of the lung.[10,32,35] Surfactant protein A can bind to and enhance the clearance of a variety of bacteria, viruses and apoptotic cells by lung alveolar macrophages.[36,37]

It has been shown that SP-D binds to genomic DNA using its CRD and collagen-like regions and can enhance the clearance of DNA.[29,30] The ligand to which SP-D binds apoptotic cells is not specifically known; thus, the data illustrating that SP-D binds to genomic DNA raise a

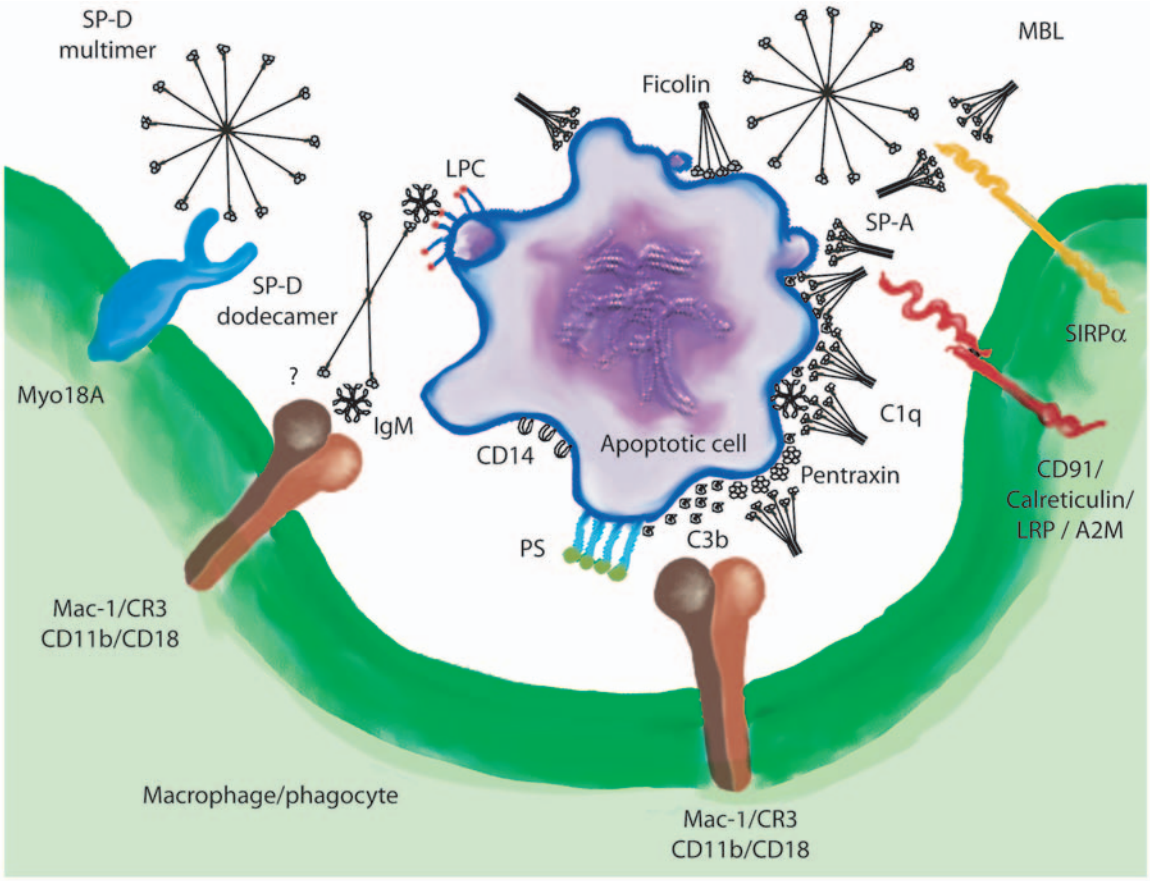

Fig. 2. Interaction among the major innate immune sPRPs, apoptotic cell surface ligands and phagocytic cell surface receptors. Collectins such as SP-A and SP-D are present primarily in the mucosal surfaces including the airways. Apoptotic cell surface ligands for these proteins are not clearly established. Myo18A (or SPR-210), SIRPα, calreticulin/CR91 (or LRP) and integrin α2β1 are putative receptors for these collectins. Collectins can bind CD91 ligand alpha-2 macroglobulin (A2M); hence, A2M may act as an adaptor molecule. Natural IgM binds late apoptotic cells via LysoPC and directly, or indirectly via complement activation, enhances the clearance of these cells by macrophages. C1q binds apoptotic cells either directly via PS and indirectly via IgM and pentraxins. These pentraxins CRP and SAP are considered to bind preferentially to phosphorylcholine and nucleosomes, respectively. Other complement activating proteins, ficolins and collectin MBL can also bind apoptotic cells. Ficolin preferentially binds late apoptotic or necrotic cells. Activation of complement cascade deposits C3b on the surface of apoptotic cells and enhances opsonization. CR3 (Mac1 or CD11b/CD18) recognizes C3b and internalizes the opsonized particles. However, complement activation by-products such as anaphylatoxins C3a increase tissue inflammation. CR3 is also considered as a putative receptor for IgM. The ratio SP-D:IgM may also modulate apoptotic cell clearance. MFG-E8 (or lactadherin) interacts with PS and enhances apoptotic cell clearance via the integrin α5β3. PG is a putative ligand for CD14, which acts as tethering receptor. Soluble CD14 dimer could interfere with apoptotic cell clearance. In general, the mucosal collectins SP-A and SP-D are considered to opsonize apoptotic cells directly and their fragments to enhance their clearance by macrophages without causing inflammation. Complement activating proteins generally increase C3b opsonic signal together with increased inflammatory signal (C3a). DNA present in/on the late apoptotic cells or necrotic cells and neutrophil extracellular trap and eosinophil extracellular traps may be accessible to most of the sPRPs (*e.g*. SP-A, SP-D, MBL, C1q, ficolins, CRP, SAP, C4b binding protein). Soluble LysoPC and nucleotides could act as chemo-attractant or 'find me' signals. On the apoptotic cells, pattern-recognition proteins may bind to DNA (purple), their blebs (small and medium sized blue and purple circles), phospholipids (*e.g*. PS) or altered phospholipids (LysoPC). Other proteins such as Gas-6, PAI-1, HMGB-1 are either directly or indirectly involved in the modulation of apoptotic cell clearance. Pattern-recognition proteins are depicted in relative size proportion to one another. Cells diagrams are not to scale with each other or pattern-recognition proteins.

plausible binding target for SP-D. This should particularly be relevant to the functions and subsequent clearance of NETs and EETs that contain nucleic acids as their integral components. Other studies have highlighted the ability for SP-D to bind to fatty acids and phospholipids, including phosphatidylinositol in a Ca^{2+}-dependent manner.[38,39] Moreover, SP-D was shown to bind to IgG, IgE, secretory IgA and IgM, of which the latter is considered a candidate for binding to late-stage apoptotic cells.[22,40] These data illustrate a novel linkage between the innate and adaptive immune proteins.

Animal models using SP-A and SP-D knockout mice show profound pathology in the lungs. Surfactant protein D knockout mice in particular are characterized by chronic lung inflammation and accumulation of dying alveolar macrophages.[41–43] Recent studies show that

SP-D$^{-/-}$ mice have elevated levels of auto-antibodies whereas SP-D levels are lowered in SLE patients.[30,44] Conversely, SP-A knockout mice do not display a build-up of apoptotic cells and their lung phenotype is otherwise near normal.[35] Additionally, it has been suggested that SP-A and SP-D can interact directly with resident alveolar macrophages and can potentially suppress apoptotic cell uptake. However, they enhance apoptotic cell uptake by recruited alveolar macrophages.[45,46] It has been proposed that both lung collectins can direct either an anti-inflammatory or pro-inflammatory immune response by binding alternatively to the cell surface receptors SIRPα or CD91/calreticulin/LRP, respectively.[45] Although the receptors for collectins are not clearly established, some studies suggest that $\alpha 2\beta 1$ integrin and Myo18A or SPR-210 could recognize collectins and C1q.[47,48] Collectins also bind to other proteins such as decorin, gp-340 and MFAP-4, but the physiological functions of these interactions are not fully established.[49–51] Together, these findings indicate that soluble mucosal tissue residing pattern-recognition collectins are important for immunity and a silent apoptotic cell clearance.

Mannose-binding lectin (MBL) is a collectin that is found in the serum, rather than the mucosal tissues, which binds to mannose and to the oligosaccharide mannan. There is a detailed scientific history of MBL which includes its isolation from rabbit liver in 1978 and from human serum in 1983.[52] The structure of MBL resembles that of SP-A, constituting the similar bouquet-of-flowers-like structure. The globular CRD provides similar functions to MBL as it does for the other collectins. Mannose-binding lectin can bind to non-glycosylated proteins, nucleic acids, and phospholipids.[29,52–54]

Mannose-binding lectin is the initiating component of the lectin pathway of complement activation. This collectin binds to mannose or other carbohydrate residues present on the surface of pathogens and a series of MBL-associated serine proteases (MASPs) assemble in the location and thus initiate complement activation. The ability for MBL to activate the complement system is an important difference between it and SP-A and SP-D, as it suggests that MBL can influence the state of inflammation in a different manner than can the lung collectins.[10,18,32,54] Consistent with this, MBL is known to bind to apoptotic cells in conjunction with the complement component C1q, which shares a striking similarity in structure to MBL but lacks lectin activity. Together, these proteins enhance the phagocytosis of apoptotic cells.[55] This has clinical relevance because often the defective clearance and accumulation of apoptotic cells are associated with autoimmune diseases such as case in SLE. Interestingly, there has been noted association of compromising polymorphisms of the MBL gene in some SLE patients.[56] This suggests that the lack of functional MBL can potentially aggravate SLE.[57] Mannose-binding lectin and deficiencies of MBL have been implicated in other diseases including rheumatoid arthritis, cystic fibrosis, and recurrent miscarriage.[56] There are clinical considerations for implementing MBL replacement therapy for the purpose of disease-modification.[58] Therefore, MBL is a versatile and critically important collectin, mostly because of its interaction with complement and its systemic availability in the serum.

A few other collectins have also been identified and studied in some detail. CL-P1, CL-L1 and CL-K1 (also known as CL-11) are highly expressed in placenta, liver and kidney, respectively.[10,18,59] They are also expressed in few other tissues. There are three collectins called conglutinin, CL-43, and CL-46 that are typically found in the bovine serum. CL-46, in particular, has been noted to be highly express in the bovine thymus and liver.[18] A number of these collectins may also act as soluble receptors for the clearance of dying cells or their componts.[10,31]

Ficolins

In addition to the array of collectins discussed above, the innate immune system uses other pattern-recognition molecules, such as ficolins, in mucosal tissues and in the blood. The most notable difference between collectins and ficolins is the globular C-terminal domain, which is homologous to fibrinogen β and γ.[60] Ficolins have been isolated from tissues including the liver, the lungs – specifically ciliated bronchial epithelial cells and alveolar type II epithelial cells – and are expressed on monocytes and neutrophils.[61,62] Multiple forms of ficolins have been identified with varying degrees of differences in their primary structures. Consequently, the different ficolins perform pattern-recognition for similar, but distinct, targets including GlcNAc and GalNAc and, in some circumstance, can act as phagocytic receptors.[62] Recently, it has been shown that some ficolins can direct the lectin pathway of complement activation much like MBL.[63,64] There is also evidence indicating that ficolins may bind to ligands on late apoptotic cells and can specifically bind to immobilized DNA. In addition to these data, it was suggested that ficolins effectively opsonize necrotic cells and enhances their uptake and clearance by human monocytes.[65] Like other soluble mucosal innate immune pattern recognition proteins, when ficolins enhance the clearance of dying cells by macrophages there is a local release of anti-inflammatory cytokines.[66] This research indicates that ficolins are yet another example of sPRPs that can

direct innate immunity and dead cell clearance with reduction in inflammation.

Pentraxins

These are highly conserved acute-phase innate immune proteins. They are induced and expressed during infection, tissue damage and systemic inflammation. The classical short pentraxins include the C-reactive protein (CRP) and the serum amyloid-P (SAP) which are both produced in hepatocytes. A third pentraxin, PTX3, is considered a long pentraxin and is produced by a variety of cell types including macrophages, fibroblasts, and inflammation-activated endothelial and dendritic cells. All three of these pentraxins can bind to the surface of apoptotic cells and some can bind to nuclear debris derived from apoptotic cells.[67,68]

Similar to the collectins, CRP requires calcium to bind to many of its diverse targets, which include phosphatidylcholine, chromatin, other cellular debris and apoptotic cells. However, CRP and collectins recognize targets by different mechanisms. In binding to apoptotic cells, it has been suggested that CRP initiates a sustained production of TGF-β and increases the phagocytosis of apoptotic cells by macrophages, which readily internalize CRP-coated particles. These events occur during states of inflammation with diverse cytokine secretion including the production of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α.[69] C-Reactive protein may also be able to minimize inflammation while enhancing apoptotic cell clearance.[68]

Serum amyloid-P is closely related to CRP. It has been implicated in the binding of apoptotic cells and their surface blebs, which contain DNA and chromatin. It has been suggested that SAP can interact with C1q of the complement system to help clear cellular debris.[69]

PTX3 is induced to detectable levels in serum by inflammatory cytokines such as IL-1 and TNF-α.[70,71] This pentraxin can bind to membrane domains of late-stage apoptotic cells but less efficiently to necrotic cells.[72] PTX3 also increases the accumulation of C1q and C3 on apoptotic cells, which eventually directs their appropriate clearance from local tissues. PTX3 can, therefore, function as an opsonin by coating particles and increasing their clearance by macrophages.[72,73] Thus, this pentraxin is involved in many innate immune activities that control and regulate both dying cell clearance and the inflammatory/non-inflammatory arms of immunity.

Antibodies and complement components

Antibodies such as immunoglobulin M (IgM) can also modulate apoptotic cell clearance. Studies have indicated that IgM can directly interact with CR3 and enhance the phagocytosic capacity of macrophages.[74,75] However, IgM-mediated direct clearance of apoptotic cells has not yet been clearly established. Of great importance is the finding that IgM binds to late apoptotic cells.[22,76] Late apoptotic cells are often referred to as secondary necrotic cells; on particular, accumulation of these cells in tissues is considered to exacerbate inflammation.[7–9] The ligand(s) to which IgM binds on late apoptotic cells is speculated to be an altered form of phospholipids (LysoPC).[22] Binding of IgM to late apoptotic cells can activate complement system.[22,25] This suggests that IgM-mediated apoptotic cell clearance may exacerbate tissue inflammation.[77–80]

C1q is very similar in structure to the collectins SP-A and MBL, but it lacks lectin activity at its globular head region.[81] Interestingly, C1q can also directly bind to surface blebs of apoptotic cells.[82] Additionally, PS has been considered as a potential ligand for C1q.[83] Moreover, it has been noted that mice lacking C1q exhibit a severe accumulation of apoptotic cells in their kidneys, which leads to glomerulonephritis.[16] Little is known about the alternative complement pathway in apoptotic cell clearance. Recent work suggests that late apoptotic or secondary necrotic cells may be cleared by this pathway.[84] Overall, there is sufficient evidence illustrating the role of the complement system in apoptotic cell clearance and linking the innate and adaptive immune systems for effectively clearing immune complexes.

Other soluble proteins

Another innate immune protein CD14 has also been implicated in apoptotic cell recognition.[85,86] $CD14^{-/-}$ mice show defective apoptotic cell clearance. These animals do not generate auto-antibodies suggesting an interesting uncoupling of the accumulation of dying cells and autoimmunity. However, the precise mechanisms involved in CD14-mediated apoptotic cell clearance are not completely understood. A recent report indicates that CD14 could recognize phosphatidylglycerol.[87,88] Hence, phosphatidylglycerol present on the dying cells could be a ligand for CD14. The milk fat globule epidermal growth factor 8 (MFG-E8; lactadherin) is a peripheral membrane glycoprotein.[15] It is secreted by a subset of macrophages and directly binds to PS present on the surface of apoptotic cells. The MFG-E8 can cross-link the dying cells to the macrophages via integrin 5β3.[89] These two proteins appear to work together to regulate apoptotic cell clearance during bacterial infection where the expression of MFG-E8 is down-regulated via LPS–CD14 pathway.[90] C4b-binding protein also recognizes DNA and necrotic cells.[91] This interaction may limit complement activation on dying cells to

prevent tissue damage. Dead-cell clearance is also modulated by growth arrest-specific (Gas)–6 protein, plasminogen activator inhibitor (PAI)–1 and the high-mobility group box (HMGB)–1 protein. These proteins have been implicated in the clearance of dead and dying cells and the regulation of inflammation. With the identification of PS as an 'eat me' signal on apoptotic cells, Gas-6 has been considered as putative PS-receptor.[92] Conversely, PAI-1 has been identified as a 'don't eat me signal', distinguishing viable neutrophils from dead and dying cells.[93] The HMGB-1 protein is considered to be a chemo-attractive protein that is released from necrotic cells and activated cells, but not from apoptotic cells. This protein is classified in some instances as a danger-associated molecular pattern (DAMP) and is responsible for activating local immune cells by interacting with innate immune receptors such as TLRs.[94] Thus, many innate immune proteins serve the purpose of preparing local phagocytes for efferocytosis or designating cells for clearance by this mechanism, or activating immune responses in a situation considered to be immunologically dangerous.

Concluding remarks and future directions

Clearance of dying cells and their components is crucial to the healthy maintenance of tissues. Dying cells are usually cleared effectively by a wide variety of cells but accumulate temporarily in the tissues during many acute inflammatory diseases. Pattern recognition proteins, such as the major collectins SP-A, SP-D and MBL, which recognize carbohydrate moieties and DNA, can bind to apoptotic cells and direct their clearance by macrophages. Ficolins, pentraxins and antibodies in association with complement protein can also bind to patterned targets on apoptotic cells and direct their clearance. In organs such as lungs, inflammation can be critically compromising; hence, the inflammation-related complement-mediated apoptotic clearance may be the last resort. It is important to recognize that recent developments have highlighted alternative ways that apoptotic cells can be recognized. The identification of a putative ligand for IgM on apoptotic cells illustrates that the adaptive immune system can contribute to complement-mediated cell clearance. The adaptation of innate immune pattern recognition proteins to recognize many different patterns present on pathogenic material and apoptotic cells highlights their importance in immunity. Most importantly, in diseases where accumulation or clearance impairment of dying cells contributes to compromising pathology, such as in inflammatory lung diseases, cystic fibrosis or SLE, soluble pattern recognition proteins should help to alleviate inflammatory immune responses and direct the subsequent resolution of inflammation or prevention of autoimmunity.

Importantly, the pattern recognition proteins that reside predominantly in mucosal tissues enhance clearance of apoptotic cells without requiring or activating inflammatory complement pathway. The sPRPs typically found in the serum will help clear apoptotic cells particularly by activating complement. This key difference of properties amongst mucosal- or serum-derived pattern recognition proteins underscores the tissue-specific importance of apoptotic cell clearance. Moreover, since the sPRPs of mucosal tissue behave uniquely in comparison to non-mucosal tissue, it is reasonable to consider the unique means by which apoptotic cells are cleared by mucosal phagocytes. The formation and clearance of apoptotic blebs and microparticles in mucosal tissues, such as the lungs, in contrast to the engulfment of full apoptotic cells in non-mucosal tissue, appears to be a distinguishing feature of mucosal tissue. The release of these small particles by apoptotic cells represents a means by which phagocytic cells can readily initiate efferocytosis prior to direct contact with apoptotic cells. This alternative is likely important for enhancing the rate by which efferocytosis is completed and ensuring an efficient resolution of inflammation in mucosal tissues. Understanding the role that different sPRPs play in facilitating the clearance of these small particles will clarify this valuable relationship. The future direction of research in this field should concern the applications of these innate immune sPRPs in the induction of different forms of cell death, the clearance of apoptotic cell derived blebs/microparticles and the resolution of tissue inflammation. Different types of programmed cell deaths display different ligands. Hence, identifying the ligands for sPRPs present on the different types of dying cells at different stages of deaths is also important. Moreover, it has recently been shown that the collectin pattern recognition proteins can also interact and bind to A2M, a protease inhibitor that can protect collectins from enzymatic degradation while enhancing their innate immune function.[95,96] These points highlight the therapeutic potential of specific soluble innate immune pattern recognition proteins in different tissues and organs. Phagocytic cell surface receptors for many of these proteins are not clearly established. Hence, a significant amount of work is needed to establish the identity of these receptors. Finally, the mechanisms devised by the innate immune system to clear apoptotic cells from all tissues are substantial and continued examination of the growing repertoire of sPRPs and their ligands and receptors such as Myo18A and integrins must continue into the new decade.

Several other soluble proteins also bind apoptotic cells (Figs 1 and 2). These proteins are present in different tissues. Identifying precise ligands and specific functions relevant to the clearance of dying cells and their components are also necessary to understand fully the

importance of these soluble proteins. Recent studies have uncovered several different types of immune cell death pathways (*e.g*. NETosis, EETosis) that deliberately release toxic cellular components into the tissues. Studying the soluble proteins that can identify these components for effective clearance should also be studied to devise appropriate treatment strategies to limit tissue injury and inflammation.

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