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Review

Phagocytosis of apoptotic cells and the resolution of inflammation

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Abstract

Clearance of apoptotic cells by phagocytic cells plays a significant role in the resolution of inflammation, protecting tissue from harmful exposure to the inflammatory and immunogenic contents of dying cells. Apoptosis induces cell surface changes that are important for recognition and engulfment of cells by phagocytes. These changes include alterations in surface sugars, externalization of phosphatidylserine and qualitative changes in the adhesion molecule ICAM-3. Several studies have contributed to clarify the role of the receptors on the surface of phagocytes that are involved in apoptotic cell clearance. The phagocytic removal of apoptotic cells does not elicit pro-inflammatory responses; in contrast, apoptotic cell engulfment appears to activate signals that suppress release of pro-inflammatory cytokines. Therefore, clearance of apoptotic leucocytes is implicated in the resolution of inflammation and mounting evidence suggests that defective clearance of apoptotic cells contributes to inflammatory and autoimmune diseases. Defining the ligands on apoptotic cells and the corresponding receptors on phagocytes with which they engage, is likely to lead to the development of novel anti-inflammatory pro-resolution drugs. In this article, we will review the recognition and signaling mechanisms involved in the phagocytosis of apoptotic cells as well as the role of endogenous compounds that play a relevant role in the modulation of inflammation. We will also discuss what is currently known about diseases that may reflect impaired phagocytosis and the consequences on inflammation and immune responses.

Keywords: Apoptotic cell; Phagocytosis; Resolution of inflammation

1. Introduction

Phagocytosis is a phylogenetically ancient process that is an essential feature of the immune response. It was first observed by Elie Metchnikoff [1], a Russian biologist who in the late 19th century observed that 'microphages' were englobed by macrophages. Since the recognition by Metchnikoff of the biological importance of phagocytosis, investigators have strived to unravel the molecular basis of this process. Today phagocytosis is defined as the cellular engulfment of large particles (>0.5 μm).

Apoptosis, or programmed cell death, is a critical process in natural tissue homeostasis and results in immediate removal of the dying cell. As cells undergo apoptosis, they are rapidly phagocytosed by professional phagocytes, such as macrophages (Mϕ) and dendritic cells (DCs) [2–4] or by semi-professional phagocytes in the surrounding tissue such as mesangial cells [5,6]. In this way, despite the high turnover of cells on a daily basis, tissues are protected from harmful exposure to the inflammatory or immunogenic contents of dying cells by phagocytic clearance. Should cells die by necrosis and disintegrate in situ, release of their contents may exacerbate the local inflammatory response and trigger further leukocyte influx. Therefore, phagocytic removal of apoptotic leukocytes is a prerequisite to restore normal tissue function and plays a critical role in the resolution of inflammation [2–4,7,8]. In addition to removing cells before they undergo lysis, it is proposed that ingestion of apoptotic cells results in potent anti-inflammatory and immunosuppressive effects through the production of anti-inflammatory cytokines such as TGF-β1 and PGE_2 and the suppression of release of pro-inflammatory mediators, including IL-8, TNF-α and TXA_2, from activated Mϕ. This proposal is based on in vitro studies [9,10]. More recently, in vivo models of inflammation have been utilized to demonstrate that clearance of

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apoptotic cells in inflammatory lesions such as thioglycollate-stimulated peritonea and endotoxin-stimulated lungs leads to accelerated resolution of inflammation, mediated by the increased production of TGF-β1 [11]. In addition, apoptotic cell instillation in endotoxin-stimulated lungs reduced pro-inflammatory chemokine levels in the bronchoalveolar lavage fluid [11].

The initial event in phagocytosis is the recognition of the target. Successful engulfment requires that apoptotic cells expose an ''eat me signal'' on their cell surface [3]. The phagocyte recognizes this signal and transduces it to the cell machinery required for engulfment. Recognition of apoptotic cells by phagocytes depends on rearrangement of the lipid portion of the plasma membrane on the target cells. Apoptosis leads to disruption of the normal asymmetric distribution of phospholipid across the plasma membrane that generates ligands on the cell surface [12], facilitating recognition by specific receptors on the phagocytes. Phagocytes show significant redundancy in recognition strategies and are able to use many receptors at the same time [2,4]. Subsequent to recognition mediated through tethering, specific cellular responses culminating in the transduction of a phagocytic signal are generated [4]. These signals target the submembranous cytoskeleton facilitating changes that lead to engulfment, followed by the process of internalization [13]. Finally, the ingested particle enters the lysosomal system in the phagocyte where it is degraded.

In this article, we will review the recognition and signaling mechanisms involved in phagocytosis of apoptotic cells as well as the role of endogenous compounds that play a role in the modulation of inflammation. We will also discuss the potential of impaired phagocytosis in the pathogenesis of inflammation and immune disorders.

2. Recognition of the target by the phagocyte

2.1. Exposure of phosphatidylserine

Amongst the multiple changes on the surface of the apoptotic cells that facilitate their recognition, the best-characterized is the loss of phospholipid asymmetry and subsequent exposure of phosphatidylserine (PS) [14–17]. In viable cells, maintenance of the phospholipid asymmetry is attributed largely to an activity of the aminophospholipid translocase [18]. In the early stages of apoptosis, an inhibition of this translocase, in part due to an elevation in intracellular Ca^{2+} and activation of a lipid scramblase, allows the appearance of PS on the surface of the cells [19]. The exposure of PS is facilitated also by the ATP-binding cassette 1 (ABC1) transporter, that plays a role in both phagocytic cells and target cells for efficient engulfment [20–22]. Deletion of the transporter gene or down-regulation of its products reduce exposure of PS during cellular activation and apoptosis [20,21].

PS on apoptotic cells is recognized by a phagocyte PS receptor [16]. The PS receptor is conserved across evolution and, differently from the other molecules involved in phagocytosis, possesses stereospecificity of interaction with PS. However, there are a number of PS-binding proteins that can act as a bridge between apoptotic cells and phagocytes such as plasma-protein β_2-glycoprotein I, the product of growth arrest-specific gene 6 (Gas6) that binds to the Mer kinase and the protein milk-fat globule epidermal growth factor 8 that bridge with vitronectin receptor integrin ($\alpha v\beta 3$) [23–25] and protein S that binds PS [26]. Interestingly data from apoptotic lymphocytes indicate that oxidative stress inhibits phagocytosis of apoptotic cells that have externalized PS, suggesting that PS exposure is necessary but not sufficient to target apoptotic cells for phagocytosis [27]. The ligation of PS receptor has been proposed as the primary mechanism to block the release of pro-inflammatory cytokine in vitro and in vivo [11,15,16].

2.2. Other surface alterations and ligand expression on apoptotic cells

Carbohydrate changes on the surface of apoptotic cells may be important in triggering recognition as demonstrated by studies of lectin-like receptors in a wide range of phagocytes, both professional and semi-professional [28,29]. The mannose receptor of Mϕ and liver endothelial cells is the best-characterized member of a family of surface lectin receptors that mediates binding and internalization of mannose and fucose [28,29]. It has been suggested that the asialoglycoprotein and the galactose-specific receptors of healthy hepatocytes and sinusoidal liver cells are implicated in the engulfment of apoptotic hepatocytes, likely in cooperation with other hepatic carbohydrate-specific receptor systems [29].

The collectins are a family of complex proteins that include surfactant-binding proteins A and D, the mannose-binding lectin and the first component of the complement cascade (C1q). The finding that C1q and mannose-binding lectin participate in apoptotic cell clearance in vitro [30] and in vivo [31,32] followed the initial observation that C1q binds to blebs on the surface of apoptotic cells [33]. The collectins coat apoptotic cells via their globular heads and initiate uptake by interacting with phagocyte receptors through their structurally homologous collagenous tail groups [30]. This interaction requires the recognition of a tail group of calreticulin [34]. However, calreticulin lacks a transmembrane domain, therefore, it is likely that it associates with a transmembrane receptor, CD91 [35].

ICAM-3, a highly glycosylated Ig-superfamily member, constitutively expressed on leukocytes, undergoes a qualitative change during apoptosis which alters its receptor-binding properties and provides a signal engulfment by Mϕ [36]. Apoptosis, in fact, results in the exposure of a carbohydrate group that could bind to CD14, a multifunc-

tional receptor known originally for its role as the lipopolysaccharide (LPS) receptor [37].

2.3. Scavenger receptors

Clearance of apoptotic cells can also be mediated by scavenger receptors (SR) including the class SR-A, SR-B1, oxidized low-density lipoprotein receptor-1 and CD36 [38–40]. CD36, one of the first Mϕ receptors to be implicated in the recognition of apoptotic cells [39,41], was first characterized in the context of lipoprotein metabolism, foam cell formation and atherosclerosis [42]. The role of CD36 in phagocytosis was demonstrated by studies using anti-CD36 antibody and by the observation that its ectopic expression gives phagocytic ability to non-phagocytic cells [41,43]. The bridging molecule thrombospondin (TPS) binds apoptotic PMNs and link them to CD36 and the phagocyte vitronectin receptor [39]. CD36 has been identified as a necessary cofactor in PS-mediated recognition of apoptotic cells [44].

CD36 was shown to play a role in the clearance of photoreceptor rod outer segment by retinal pigment epithelium [45]. In addition, glial cells, the resident macrophages in the central nervous system, express SR [46]. SR-A mediates the binding and phagocytosis of apoptotic cells that express PS by neonatal microglia [46] and SR-B seems to participate in astrocyte clearance of apoptotic cells in vivo [47].

Although its activities are well-defined in vitro, the contributions of SR-A in vivo have been difficult to determine. It has been demonstrated that apoptotic thymocyte clearance in SRA-deficient mice is apparently normal [48].

The ability of SR to mediate recognition of apoptotic cells is widely distributed in phylogeny. The hemocytes of *Drosophila melanogaster* remove cells undergoing apoptosis during embryogenesis, through a receptor called Croquemort (Crq), a homologue of the mammalian CD36 [49].

2.4. CD31

Recent data provide evidence for the presence of a repulsive signal between Mϕ and leukocytes. Under flow conditions, it has been demonstrated that Mϕ extends processes that palpate leukocytes and if a leukocyte is viable, it rapidly detaches from Mϕ. However, apoptotic leukocytes remain in contact and phagocytosis is the eventual consequence of such contact [50]. Brown et al. [50] suggested that apoptosis switches the repulsive signals that are received by leukocytes CD31 to adhesive signals. Ligation of CD31 on viable leukocytes promotes their detachment, whereas in dying cells the signaling through CD31 is disrupted, so the cell is not repelled. It has been proposed that prolonged attachment per se may be necessary but not sufficient for phagocytosis and that engagement of PS receptor is required for the complete ingestion of dying cells [2].

3. Signaling mechanisms in apoptotic cell uptake

Phagocytosis is triggered by the interaction of the particles to be internalized with specific receptors on the surface of the phagocyte. These receptors include the Fc receptors of immunoglobulins and the complement receptors that bind to complement on opsonised particles. Fc receptor-mediated phagocytosis occurs by a zippering process in which repeated interactions between ligands on the target particle and receptors on the phagocyte cell are required until complete internalization of the particle is achieved within a specialized structure, the phagosome [51–53]. In complement-mediated phagocytosis, the particles sink into the phagocyte with minimal membrane disturbance [13].

Uptake of apoptotic cells has been suggested to involve two separate steps. Individual or multiple engagements of many receptors including CD14, CD68, CD36 and $\alpha_v\beta_3$ integrin result in the binding of the particles that are not ingested until PS is present [4]. Therefore, the engulfment of apoptotic cells involves an initial tethering event followed by a PSR-mediated uptake by a process akin to macropinocytosis [54]. In macropinocytosis, local membrane ruffling is associated with enclosure of extracellular fluid followed by internalization in a so-called ‘‘tether and tickle’’ mechanism [54]. Adhesion ligands lead to attachment of the apoptotic cell (tethering), but they are not able to trigger uptake. Engagement of signaling receptors (the ‘tickle’ component) then leads to initiation of uptake signals providing more opportunities for regulation and specificity. This mechanism requires Rac-1 and Cdc42, members of the Rho family of GTPases [55].

The assembly and disassembly of peripheral actin filaments is used to promote localized changes in the structure of membranes during phagocytosis. Members of the RhoGTPases (RhoA, Rac and Cdc42) regulate many signal transduction pathways linking plasma membrane receptors to the assembly of distinct filamentous actin structures [56]. Differential involvement of specific Rho family members has been demonstrated in phagocytosis in response to stimulation of Fc or complement receptors. Rac and Cdc42 have been shown to regulate actin reorganization during Fc receptor-mediated phagocytosis by promoting pseudopod extension and phagosome closure [57–60], whereas complement-mediated phagocytosis, a process not associated with the release of pro-inflammatory molecules, requires the activation of RhoA [58]. Phagocytosis of apoptotic cells shares some of the characteristics of both mechanisms. Similar to the Fc receptor-mediated phagocytosis, internalization of the apoptotic cells is due to extension of the phagocyte membrane around the cell to be engulfed [61] and require Rac and Cdc42, whereas inhibition of Rho enhanced phagocytosis [62]. Rac and Cdc42 may exert their action through the Wiskott–Aldrich syndrome protein (WASp). WASp is activated by Cdc42 to stimulate actin polymerization through the Arp2/3 complex [63].

Signaling through the integrin $\alpha_v\beta_5$ heterodimer results in recruitment of the p130^{cas} CrkII–DOCK 180 molecular complex which in turn triggers Rac 1 activation and phagosome formation [64], a process analogous to the homologous CED2–CED5–CED10–CED12 complex of engulfment genes defined in the nematode *Caenorhabditis elegans* [65–67]. In *C. elegans* apoptotic cells are engulfed by non-professional, neighbouring cells. On the basis of their genetic interactions, the engulfment genes fall into two partially redundant sets, ced-1, ced-6, ced-7 on the one hand and ced-2, ced-5, ced-10 and ced-12, on the other [68,69]. Candidate orthologs in mammals exist for all of these and have been defined on the basis of the sequence or of the functional conservation. Ced-1, ced-6 and ced-7 encode a transmembrane protein similar to scavenger-receptor, a protein-binding domain containing an adaptor protein and an ABC transporter, respectively [70–72]. The ABC transporter CED-7 promotes cell corpse recognition by CED-1, possibly by exposing a phospholipid ligand on the surfaces of cell corpses [70]. The distribution of CED-1 at the plasma membrane of the engulfing cell and in particular in the vicinity of the apoptotic target strongly suggest that it acts as a receptor for some ligands in the recognition process [70]. Interestingly, in the nematode, a functional ced-7 gene is required in both target and phagocytic cell for an effective engulfment [72], a situation also observed for ABC1 in mammalian cells [21].

CED-2, CED-5, CED-10, and CED-12 encode homologues of mammalian CrkII, Dock180, Rac, and ELMO, respectively, and function in phagocytic cells to transduce another unknown cell corpse signal to the actin cytoskeleton of the phagocytic cell [64,66,67,70]. In mammalian cells, Ced12/ELMO1 functionally cooperate with CrkII and DOCK 180 to function upstream of CED-10/Rac1, leading to cytoskeletal rearrangement [67].

4. Role of dendritic cells in phagocytosis

Uptake of apoptotic cells by the most potent antigen-presenting cells, DCs, plays a significant role in the immune responses. Immature DCs in peripheral tissue can take up antigens and process them. DCs then migrate to secondary lymphoid organs and become competent to present antigen to T lymphocytes, thus initiating antigen-specific immune responses [73]. Ingestion of certain necrotic cells is capable of inducing DC maturation, while DCs that have ingested apoptotic cells have a reduced capacity to stimulate T cells [74,75]. However, such DCs are capable of responding to strong external stimuli, to mature and present antigens derived from the ingested apoptotic cells to T cells [76,77]. The ingestion of apoptotic cells by immature DCs can inhibit their maturation and antigen presentation with suppression of the secretion of IL-12, which has autocrine and/or paracrine effects on cell maturation [74,75]. Interestingly, the ingestion of apoptotic cells by macrophages generates an active anti-inflammatory response through the production of TGF-β1 and other anti-inflammatory molecules and down-regulates subsequent release of pro-inflammatory cytokines [44]. Therefore, a controlled mechanism of DC maturation is necessary to regulate an autoimmune response due to the generation of autoantigens [78]. The ability of DCs to trigger immune responses to antigens expressed by ingested apoptotic cells, however may be a useful mechanism in tumour therapy. DCs loaded with apoptotic tumour cells initiate immune responses against the antigens expressed by dying cells, making then an attractive target for anti-neoplastic immunisation [79].

5. Endogenous regulators of phagocytosis of apoptotic cells

Given that recognition and engulfment of apoptotic cells is an important process in the resolution of inflammation, a positive regulation of the capacity of Mϕ for phagocytosis of dying cells represents a potential therapeutic target in the control of inflammatory disease. In recent years, the role of endogenous anti-inflammatory mediators in the modulation of phagocytosis of apoptotic cells has emerged.

5.1. Glucocorticoids

Glucocorticoids are powerful anti-inflammatory agents that suppress many phlogistic responses including inflammatory cell recruitment and activation [80]. Pretreatment with glucocorticoids results in an increased clearance by Mϕ and renal mesangial cells of apoptotic leukocytes from different lineages without promoting the release of pro-inflammatory cytokines in vitro [81]. Long-term exposure of monocytes to dexamethasone reprograms them to a more phagocytic phenotype with changes in adhesion-dependent reorganization of the Mϕ cytoskeletal elements coupled to activation of Rac [82]. These steroid reprogrammed Mϕ present a reduced tyrosine phosphorylation of the components of adhesion contacts, paxillin and pyk2 proteins and loss of expression of p130^{cas}, a mediator of adhesion signaling [82]. These data suggest that phagocytic clearance of apoptotic leukocytes may be a further mechanism through which glucocorticoids exert their anti-inflammatory activities.

5.2. Lipoxins

Lipoxins (LXs) formed by leukocytes during cell–cell interactions under a variety of conditions including inflammation, represent a unique class of lipid mediators with potent anti-inflammatory actions [83–86]. In a cytokine primed milieu, aspirin acetylation of cyclooxygenase type 2 switches the catalytic activity of the enzyme to an *R*-lipoxygenase which initiates the biosynthesis of the 15-epimer lipoxins (aspirin-triggered lipoxins, ATL) which

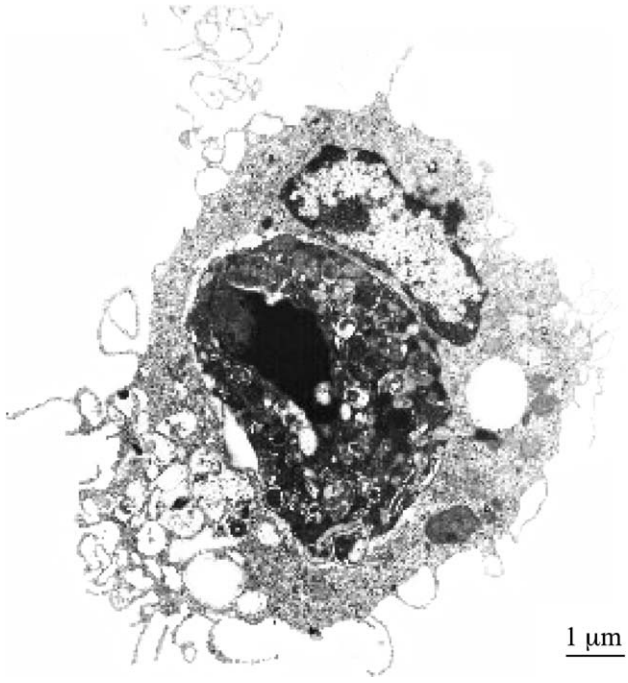

Fig. 1. Electron micrograph of LX-stimulated Mϕ phagocytosis of apoptotic PMN. Mϕ were treated with LXA_4 (1 nM for 15 min) before coincubation with aged PMN for 30 min. Cells were fixed with glutaraldehyde, postfixed in 1% osmium tetroxide and embedded in Epon using standard methods. Sections were stained with uranyl acetate and lead citrate and examined in a JEOL 2000EX microscope.

share many of the bioactions of the non-epimeric forms [87].

LXA_4 and its stable analogues bind to a G protein-coupled receptor (ALXR) [83,85] and inhibit recruitment of PMNs in vitro and in vivo models of inflammation [88–95]. LX can directly modulate the cytokine composition in a local inflammatory milieu thereby participating in a negative feedback loop opposing inflammatory cytokine-induced cell activation [96,97]. It is noteworthy that the ALXR can bind pleiotropic ligands including peptide agonists [98], prion protein [99], serum amyloid A [100] and the glucocorticoid-inducible protein annexin-1 [101]. Intriguingly, recent data have shown that annexin-1 exposure on the surface of apoptotic cells facilitates phagocytic clearance on colocalization with PS [102].

LXs are potent activators of monocytes, stimulating their chemotaxis and adherence without causing degranulation or release of reactive species [103,104]. We have recently demonstrated that LXs and ATL promote another important step in the resolution phase of inflammation, namely non-phlogistic phagocytosis of apoptotic PMN by human monocyte-derived Mϕ in vitro (Figs. 1 and 2) [105]. LXs stimulate phagocytosis of exogenously administered excess apoptotic PMN in a murine model of thioglycollate-induced peritonitis in vivo, suggesting that LX rapidly promote the clearance of apoptotic leukocytes within an inflammatory milieu [106]. This effect of LXs on phagocytosis of apoptotic PMNs by Mϕ can be blocked by antibodies to several macrophage surface proteins known to contribute to the recognition of apoptotic leukocytes such CD36, αvβ3 and CD11b/CD18. The non-phlogistic LX-stimulated phagocytosis is mediated by protein kinase C and PI-3-kinase and is associated with increased production of TGF-1β [106] (Fig. 3). A modulatory role for cAMP is suggested by the observation that LX-induced phagocytosis is inhibited by a cell permeant cAMP analogue and mimicked by a PKA inhibitor [105]. Interestingly, these data suggest that LX-stimulated phagocytosis

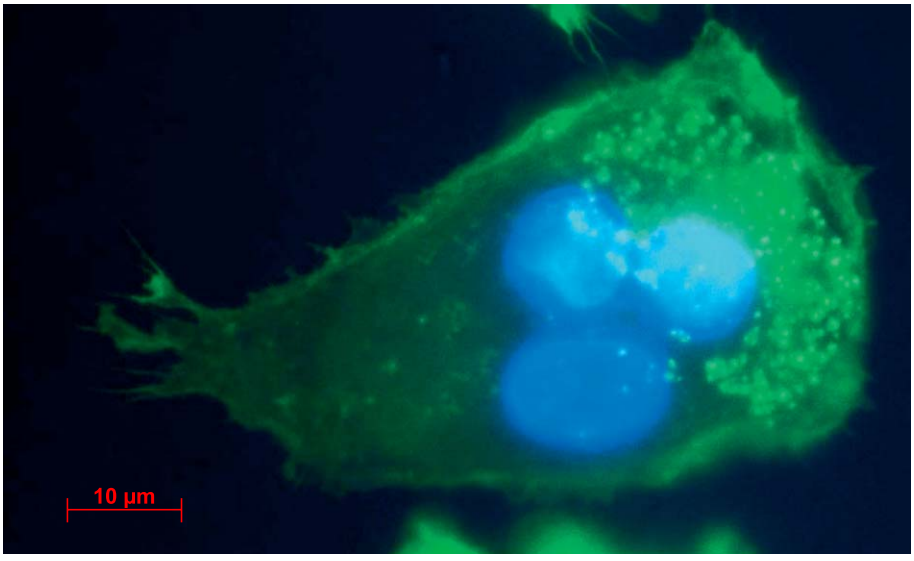

Fig. 2. Fluorescence micrograph of LX-stimulated Mϕ phagocytosis of apoptotic PMN. Mϕ were treated with LXA_4 (1 nM for 15 min) before coincubation with aged PMN for 30 min. Cells were fixed with paraformaldehyde. Localization of actin was determined using Oregon Green phalloidin and nuclei were stained with Hoechst 33258. Images were visualized by fluorescence microscopy using a ×100 oil objective. A Mϕ with a polarized shape and with pseudopodia has ingested an apoptotic PMN.

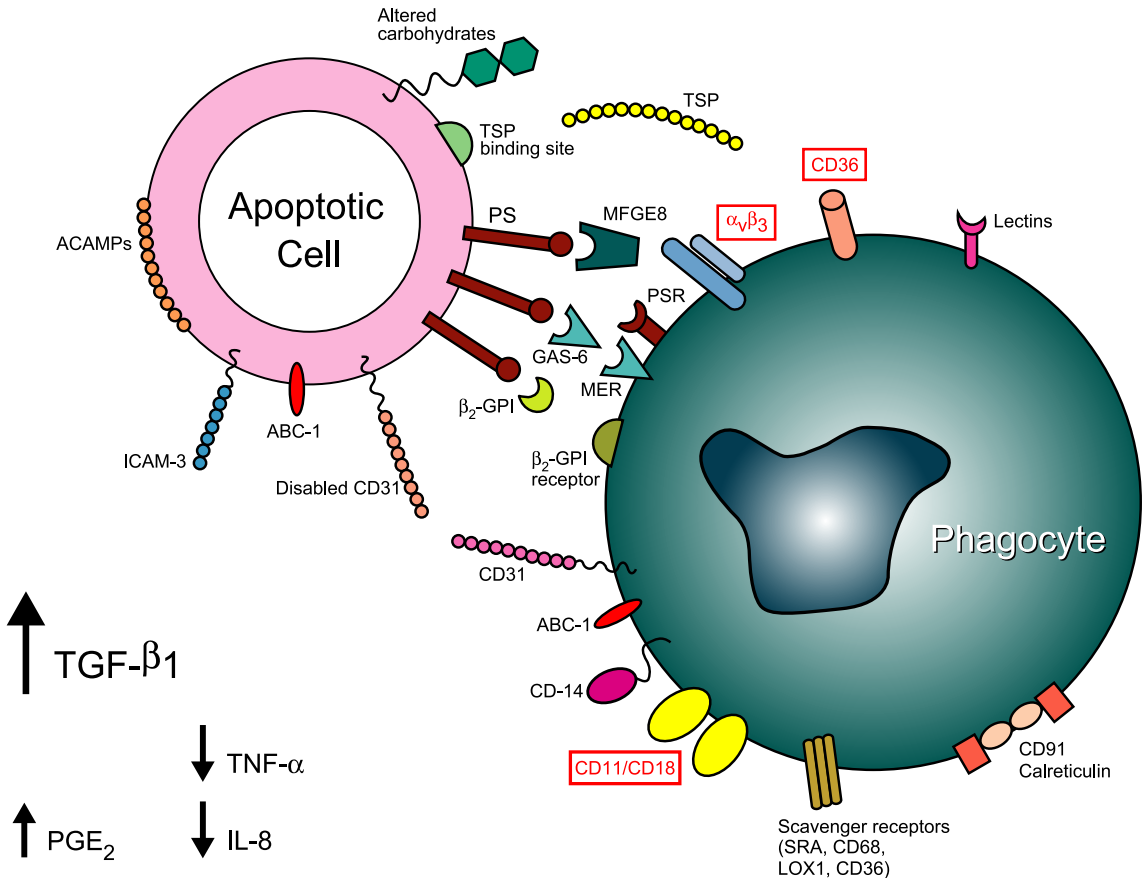

Fig. 3. Phagocyte recognition of apoptotic cells is coupled to the resolution of inflammation. Several receptors are implicated in the uptake of apoptotic cells by phagocytes. These receptors interact with their ligands on the apoptotic cells either directly or via bridging proteins. The role, cooperation and redundancy amongst these receptors are discussed in the text. The mechanisms that are involved in the LX recognition of apoptotic cells are indicated in red. Increased production of TGF-β1 and PGE_2 have been demonstrated in several models following phagocytosis of apoptotic cells, increased expression of such mediators is associated with decreased production of prototypic pro-inflammatory mediators such as TNF-α and IL-8 as discussed in the text.

of apoptotic PMN is through a novel mechanism independent of the PS receptor as it was not blocked by anti-PS receptor antisera or by pre-treatment of Mϕ with β1,3 glucan [106]. These results suggest that LXs promote phagocytosis of apoptotic PMN either by increasing the avidity of αvβ3-CD36 for PMN ligands or by influencing cytoskeletal events. Indeed, LXs induce significant changes in the reorganization of actin in human monocytes and Mϕ resulting in the promotion of cytoplasmic extensions and in the formation of pseudopodia with a mechanism that is dependent on monomeric GTPases RhoA and Rac [107]. Given the role of the Rho family in cell motility and phagocytosis, it may be proposed that LX pretreatment of Mϕ might prime them for chemotaxis [104] and phagocytosis, contributing to the potential role of LXs in the resolution of inflammation.

It is noteworthy that while this paper was in review, evidence for a further lipid mediator involved in phagocytosis of apoptotic cells was reported. Apoptotic cells were demonstrated to release lysophosphatidylcholine which stimulates chemotaxis of phagocytic Mϕ [108].

6. Consequences of impaired phagocytic clearance

The modulation of phagocytic capacity for apoptotic cell clearance represents a potential therapeutic target in the control of inflammatory disease. In this regard, in vivo models, in which a deficiency in the clearance of apoptotic cells is present, are useful to study the consequences of inadequate phagocytosis of dying cells [31,109]. Mounting evidence highlights a prominent role for complement components in the homeostatic modulation of phagocytosis of apoptotic cells, in particular the observation that C1q binds specifically to the surface blebs of apoptotic cells [33]. The importance of C1q in vivo is demonstrated by the fact that inherited deficiencies of classical pathway components, particularly C1q and C4, are inextricably linked with development of systemic lupus erythematosus (SLE) [110]. Elucidation of the mechanisms of association between C1q and autoimmune disorders has been provided by a study using mice with a deficiency in C1q. The mice spontaneously developed anti-nuclear antibodies and displayed major immuno-complexes deposition in the renal

glomeruli with an associated glomerulonephritis and chronic renal damage [32]. In addition, a marked accumulation of apoptotic bodies in C1q −/− mice was demonstrated. This observation correlated with the proposal that apoptotic cells, when not efficiently cleared, may represent a source of autoantigens that drive the autoimmune response in SLE [111]. In fact, Mϕ from patients with SLE has been shown in vitro to exhibit a reduction in the phagocytic uptake of apoptotic cells [112]. Studies by Taylor et al. [31] have investigated the relative contribution of different complement proteins to the phagocytosis of apoptotic cells by both inflammatory and resident murine Mϕ using novel in vivo peritoneal models of apoptotic cell clearance. C1q- and C4-deficient mice exhibited impaired clearance of apoptotic Jurkat T cells. However, C1q-deficient animals exhibited a more severe defect in inflammatory Mϕ phagocytosis of apoptotic murine thymocytes than C4-deficient mice, indicating hierarchical superiority of classical pathway complement proteins in the phagocytic clearance of apoptotic cells [31]. Also C1q-deficient mice were the only complement-deficient animals to exhibit a defect in the phagocytosis of apoptotic cells by resident peritoneal Mϕ in vivo. This highlights the prominent role of C1q in mediating effective clearance of apoptotic cells in vivo in the absence of inflammation and strengthens the observation that apoptotic cells on their surface blebs express many of the autoantigens of SLE [111].

As already discussed the complex Mer/Gas-6/PS is involved in the interaction between apoptotic cells and phagocytes [23]. The implication of Mer in phagocytosis is emphasized by in vivo studies in which it has been demonstrated that thymi from Mer kinase-deficient mice upon stimulation with dexamethasone, showed a seven-fold accumulation of remnant apoptotic cells compared to wild-type controls [109]. The accumulation of apoptotic cells was not due to increased apoptosis, but to defective clearance by Mϕ and the systemic exposure to apoptotic thymocytes in mice results in the transient production of autoantibodies [109]. Interestingly, the mutation in Mer −/− mice renders them blind and coincidentally human patients with retinitis pigmentosa associated with lack of clearance have been shown to contain mutations in the Mer gene [113]. These studies indicate a role for the Mer receptor as immunoregulator probably due to its ability to down-regulate the production of cytokines such as TNF-α [114].

Cystic fibrosis is a disease characterized by early, protracted inflammation that is associated with a massive influx of inflammatory cells and release of intracellular protease in the lung [115]. An increased number of apoptotic cells has been demonstrated in the airways of patients with cystic fibrosis and non-cystic fibrosis bronchiectasis [116]. It has been speculated that the defective airway clearance of apoptotic cells observed in these pathological conditions may be due to elastase-mediated cleavage of PS receptor on phagocytes that may precipitate an ongoing inflammation condition and progressive airway damage [116]. Given that LX-stimulated phagocytosis appears to be independent of the PS receptor, this suggests a potential therapeutic role for LX in cystic fibrosis lung disease.

SR-A is expressed on microglia in the CNS, raising the possibility that it may be actively involved in the development of some neuronal disease, in particular with the etiology of neurodegeneration in Alzheimer disease [46,117]. Prominent expression of SR-A has been shown on reactive microglia surrounding amyloid plaques [118] and microglial scavenger receptors may be novel targets for therapeutic interventions in Alzheimer's disease. Indeed the ability of the LX receptor to interact with serum amyloid A [100] suggests that it may have a role in phagocytic clearance of amyloid protein, an important strategy in the accumulation of extracellular amyloid, a key feature of neurodegenerative disorders.

Evidence of the anti-inflammatory effects of apoptotic leukocyte clearance in immune-complex mediated arthritis (ICA) are provided from experiments in which injection of apoptotic leukocytes before the induction of ICA were shown to be protective as uptake of the instilled apoptotic leukocytes by synovial lining macrophages significantly reduced subsequent PMN chemotaxis into the joint [119].

7. Conclusions

The uptake of apoptotic cells by professional phagocytes such as Mϕ, immature DCs and brain microglial cells is an important step in the resolution of inflammation. The in vitro mechanisms underlying the phagocytosis of apoptotic cells are well defined but more studies are necessary to clarify the importance of apoptotic cell clearance in vivo. In addition, more studies are needed to define a clear link between impaired phagocytosis of apoptotic cells and the pathogenesis of diseases. These studies will facilitate the development of new therapeutic strategies for autoimmune and inflammatory diseases.

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