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Phagocytosis of Apoptotic Cells and Immune Regulation

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Abstract

The termination of the apoptotic programme occurs in most cases via recognition and clearance by phagocytes, especially the professional phagocytes, such as macrophages and immature dendritic cells. Engulfed cells do not simply disappear from the midst of living tissues. The fine-defined presentation of yielded self-antigens to T cells is a central event in the induction or the maintenance of the peripheral immune tolerance to self. Conversely, abnormality in this pathway may contribute to the pathogenesis of systemic and organ-specific autoimmune diseases. We herein reviewed the relationship between phagocytosis of apoptotic cells and immune regulation, especially the effects of engulfed apoptotic cells on immune tolerance and autoimmune diseases.

Introduction

Cell death is an integral cellular process that occurs by two major mechanisms. Apoptosis, or programmed cell death, is an active, energy-dependent process that causes typical cellular morphological changes including cell shrinkage, nuclear condensation, DNA fragmentation and membrane alterations. Apoptosis usually affects scattered cells in a tissue and is triggered by stimuli in both physiologic and pathologic settings. In contrast, necrosis, or accidental cell death, is a pathologic process induced by physical or chemical stimuli and is characterized by cellular disintegration and the release of toxic components. In addition to morphologically and biochemically distinguished changes, cells dying by apoptosis and necrosis showed significant difference in their immunological effects [1]. When cells die they are either lost directly to the environment, or more commonly, are engulfed by their adjoining neighbours or by specialized phagocytes, including macrophages, dendritic cells (DC) or neutrophils. Typically, cells dying purposefully by programmed cell death or apoptosis are thought to be phagocytosed by mechanisms that fail to incite inflammatory or immune reactions [2], and that some of them are even actively anti-inflammatory and tolerogenic [3]. By contrast, cells that die accidentally and catastrophically by thermodynamically downhill processes, namely necrosis, are capable of activating pro-inflammatory and immunostimulatory responses [4]. It is gradually accepted that the acquisition of necrotic properties by apoptotic cells because of failed or inefficient clearance has pro-inflammatory consequences that may lead to the development of autoimmune diseases [5]. Thus, the termination of the apoptotic programme via recognition and clearance by phagocytes is very critical in bodies.

Engulfed cells do not simply disappear from the midst of living tissues. Constituents of the corpse indeed survive the intracellular processing and are recycled to the membrane of the phagocyte [3, 5]. The presentation of yielded antigens to T cells is a central event in the induction and the maintenance of peripheral immune tolerance [3]. Conversely, errors in this pathway contribute to the pathogenesis of systemic and organ-specific autoimmune diseases [6, 7]. Here, we only review the relationship between phagocytosis of apoptotic cells by macrophages or DC and the immune regulation, focusing especially on the effects of engulfed apoptotic cells on immune tolerance and autoimmune diseases.

Phagocytosis of apoptotic cells

Clearance of apoptotic cells is mediated by professional and amateur phagocytes. Professional phagocytes, such as macrophages and immature DC (imDC), are highly phagocytic and mobile cells capable of infiltrating a wide variety of tissues [8].

Although these cells are believed to get rid of most of the apoptotic corpses, it is becoming increasingly evident that essentially any cells can take up neighbouring dying cells [9]. However, these amateur phagocytes or less mobile 'resident' cell types typically engulf with much slower kinetics than professional phagocytes [10]. It is currently well accepted that DC could phagocytose some antigens as well as cells undergoing apoptotic cells, although their phagocytosis is less efficient than that of macrophages. Nevertheless, imDC appear to efficiently phagocytose antigens or cells for both major histocompatibility complex (MHC) class II and I presentation [11].

Distinct characteristics distinguish apoptotic cells from living cells [5]. On the surface of normal healthy cells, some signals are continuously used by the immune system to distinguish foreign invaders from host cells. For instance, CD47, on red blood cells, functions as a safe marker by sending a negative engulfment signal to splenic red pulp macrophages through the SIPR-α receptor. Living cells can display signals, such as CD31 (platelet-endothelial cell adhesion molecule-1), which stimulate the detachment of phagocytes and the inhibition of uptake on apoptotic cells [12, 13]. Thus, the negative regulation of phagocytosis might be one of the general mechanisms used to actively prevent ingestion of nonapoptotic cells [14]. During apoptosis, however, this detachment signal is somehow disabled, thus leading to the removal of dying cells. This removal process might be facilitated by CD31 functioning as a tethering molecule on the apoptotic cells [15]. Phosphatidylserine (PtdSer, PS) and annexin-I (Anx-I) may translocate and colocalize in discrete patches on the surface of the outer membrane (possibly with other undescribed ligands) where they function as signals to trigger phagocyte recognition and uptake [9]. In addition, CD31 is converted on apoptotic cells to facilitate phagocyte binding and uptake [16, 17].

The present molecular picture of the interaction between macrophage and apoptotic cell is bewildering [18]. Largely drawn from the *in vitro* studies, a broad array of phagocyte receptors, apoptotic cell-associated ligands and intermediate molecules have emerged (Table 1). Included on the phagocytes are integrins (α_v β3 *et al.*) [19], scavenger receptors (CD36 *et al.*) [20], immunoglobulin super-family molecules (CD31 *et al.*) [16], receptors for complements (CD14 *et al.*) as well as molecules involved in engagement of sugars and phospholipids [lectins and phosphatidylserine receptor (PSR) *et al.*] [21–23]. Implicated thus far on the apoptotic cells are fewer defined structures, but these encompass lipid, carbohydrate and protein moieties, the most renowned being the exposed anionic phospholipid, PtdSer (PS), which resides normally on the inner leaflet of the plasma membrane [17, 23, 24]. An increasingly wide variety of soluble intermediate factors is emerging whose role is to opsonize apoptotic cells and to create molecular bridges between

Table 1 Phagocyte surface receptors and ligands that have been implicated in the recognition of apoptotic cells

Surface receptor	Ligand	References
Scavenger receptors		
CD36	Oxidized LDL	[65]
CD68	Oxidized phospholipids	[21]
SR-A		[18, 22]
SR-B1		[22]
LOX-1		[19]
Integrins		
CR3, CR4	iC3b	[21, 34]
αvβ3/CD36	Thrombospondin	[50]
αvβ5		[50]
α3β5		[38]
FcγR	IgG, CRP, SAP	[34]
Alreticulin-CD91		[18]
Collectin receptors	C1q, MBL, SP-A, SP-D	[38]
ABC1		[48]
CD14	ICAM-3	[22]
Lectins	Sugars	[63]
PtdSer receptor	PtdSer	[22, 23]
β2-GP1 receptor	β2-GP1	[35]
c-mer	Gas-6	[23]

ABC1, ATP-binding cassette transporter1; β2-GP1, β2-glycoprotein 1; CRP, C-reactive protein; gas-6, growth arrest-specific gene-6 product; iC3b, inactivated C3b; ICAM-3, intercellular adhesion molecule-3; LDL, low-density lipoprotein; LOX-1, lectin-like oxidized LDL receptor-1; MBL, mannose-binding lectins; MFG-EB, milk fat globule-epidermal growth factor (EGF)-factor B; PtdSer, phosphatidylserine; SAP, serum amyloid P; SP-A, surfactant protein-A; SR-A, scavenger receptor-A.

components of the apoptotic cells and phagocyte surfaces (Fig. 1). Phagocytosized mechanisms of apoptotic cells mainly include three kinds of pathways, namely Fc receptor-mediated, complement-dependent and pattern recognition molecular controlled pathways [24, 25]. Numerous molecules including CD14, CD91/calreticulin, C1q, C3bi, collectins and pentraxins have been implicated in the clearance of apoptotic cells by macrophages [26]. This has led to the proposal that apoptotic cells can present conserved molecular patterns to innate immune molecules to mediate or facilitate the clearance processes [27, 28]. Such apoptotic cell-associated molecular patterns may share structural homology with pathogen-associated molecular patterns, conserved structures decorating microorganisms that are recognized by innate immune receptors and pattern recognition receptors. For the opsonization of apoptotic debris *in vivo*, the innate immune proteins are available in plasma. Several receptors have been postulated to have a role in the complement-mediated uptake of apoptotic cells by phagocytes. Although several potential C1q receptors have been described, including complement receptor 1, C1qRp (CD93) and cC1qR (calreticulin), their roles in the clearance of apoptotic debris were incompletely defined [29, 30]. Fc receptors for IgG (FcγRs) were expressed on phagocytic cells. Several groups have shown that binding

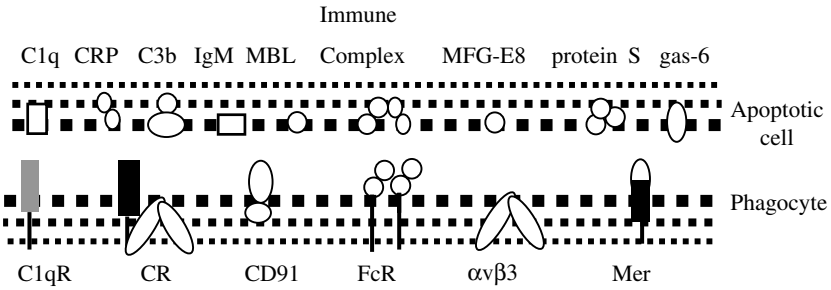

Figure 1 Potential opsonins bind to the surface of the apoptotic cells (top), leading to recognition by phagocyte receptors (bottom).

of C-reactive protein (CRP) to FcγRI (CD64) and FcγRII (CD32) and that serum amyloid P additionally interacts with FcγRIII (CD16). However, others question the interpretation of these data and suggest that the binding to FcγR is influenced by contamination of CRP with other proteins, as well as the interference of the Fc part of the detecting antibodies with FcγR [19] (Fig. 1).

The immune regulatory effects of engulfed apoptotic cells

Clearance of apoptotic cells by phagocytes results in either anti-inflammatory and immunosuppressive effects or prostimulatory consequences through presentation of cell-associated antigens to T cells [5, 7]. The differences in outcome are because of the conditions under which apoptosis is induced, the type of phagocytic cells, the nature of the receptors involved in apoptotic cell capture and the milieu in which phagocytosis of apoptotic cells takes place [31]. Preferential ligation of specific receptors on professional antigen-presenting cells (APC, such as macrophages and DC) has been proposed to induce potentially tolerogenic signals [6, 32]. On the contrary, macrophages and DC can efficiently process and present antigens from pathogen-infected apoptotic cells to T cells [33] (Table 2).

Effects of engulfed apoptotic cells on macrophages

Macrophages play important roles in the clearance of dying and dead cells. Typically, and perhaps simplistically, they are viewed as the professional phagocytes of apoptotic cells [32]. Clearance by macrophages of cells undergoing apoptosis is a nonphlogistic phenomenon that is often associated with actively anti-inflammatory phagocyte response [34]. By contrast, macrophage responses to necrotic cells, including secondarily necrotic cells derived from uncleaned apoptotic cells, are perceived as pro-inflammatory [19, 35]. Indeed, persistence of apoptotic cells because of defective apoptotic-cell clearance has been found to be closely associated with the pathogenesis of autoimmune diseases [36–38].

Commonly, the induction of pathogenic immune responses may be dependent on the immune system receiving 'danger' signals resulting from tissue damage, rather than tolerogenic stimuli associated with normal cell turnover [39]. Barker *et al.* [40] tested this hypothesis by comparing the effects of the uptake of necrotic and apoptotic cells on the ability of APC (macrophages) to stimulate immune responses *in vitro*. Murine bone marrow-derived macrophages were pulsed with neutrophils that had been rendered apoptotic or necrotic, and tested for the ability to induce T-cell responses. The macrophages that had taken up

Table 2 Immune regulatory effects of apoptotic cell phagocytosis on macrophages and immature DC (dendritic cell)

	Macrophages		Immature DC	
	Apoptotic cells	Necrotic cells	Apoptotic cells	Necrotic cells
Cytokine secretion	Anti-inflammatory cytokine secretion: TGF-β, IL-10, IL-13 secretion	Pro-inflammatory cytokine secretion; nitrogen monoxidum (NO) etc.	Anti-inflammatory cytokine secretion: TGF-β, IL-10, IL-13	Pro-inflammatory cytokine secretion: NO etc.
Costimulatory molecular expression	CD40L, CD40 etc. downregulation	CD40L, CD40 etc. upregulation	CD40L, CD40 etc. downregulation	CD40L, CD40 etc. upregulation
Effects of LPS	None	Activation	None	Maturation
Functional changes	Immune suppression; altered regulatory response for pathogen	Present exogenous antigens to both $CD8^+$ and $CD4^+$ T cells	Immune tolerance; silencing $CD4^+$ and $CD8^+$ T cells	Present exogenous antigens to both $CD8^+$ and $CD4^+$ T cells

IL, interleukin; LPS, lipopolysaccharide; TGF, transforming growth factor.

necrotic, but not apoptotic, cells were able to stimulate recall proliferation by ovalbumin-specific T cells. Furthermore, the response to the mitogen concanavalin A (Con A) was more than six times higher when macrophages had been pulsed with necrotic, in comparison with apoptotic cells. In control experiments, macrophages that had not been exposed to dying neutrophils stimulated weak responses to ovalbumin and Con A. To determine why the uptake of apoptotic and necrotic cells exert opposing effects on the ability of macrophages to stimulate T cells, the expression of costimulatory molecules by treated macrophages, and their production of potentially immunomodulatory cytokines were measured. Flow cytometry revealed that macrophages that had taken up necrotic, but not apoptotic, neutrophils expressed significantly increased levels of CD40 compared with untreated controls within 4 h. Macrophages pulsed with apoptotic cells secreted higher levels of transforming growth factor-β1 (TGF-β1) than those ingesting necrotic cells or untreated controls. So, these results indicated that macrophages that have taken up necrotic cell debris present antigens to T lymphocytes with greater efficiency because of transient CD40 upregulation, whereas those that have ingested apoptotic cells are ineffective APC because they secrete inhibitory cytokines [8, 41, 42].

The apoptotic cells cannot only fail to stimulate but can actively suppress pro-inflammatory mediator release from engulfing macrophages *in vitro* [5, 43]. A convincing body of evidence shows that mononuclear phagocytes responding to apoptotic cells release anti-inflammatory cytokines, including interleukin (IL)-10 and TGF-β1, which may act as key local autocrine or paracrine anti-inflammatory factors and immunosuppressants [44]. While the mechanisms that determine anti-inflammatory mediator release have yet to be detailed, the process appears to be PS-dependent, and it has been suggested that the PSR may play an important signalling role in this pathway, particularly with respect to TGF-β1 [45]. Nationally, as Anx-I can stimulate IL-10 production, this could provide an additional mechanism whereby IL-10 is secreted by mononuclear phagocytes engaging with apoptotic cells via Anx-I-dependent mechanisms [41].

Direct suppression of pro-inflammatory responses may follow apoptotic cell interaction via mechanisms that do not require soluble intermediates [34]. One such signalling pathway may emanate from SHPS-1 following its ligation by CD47, because it is known that pro-inflammatory cytokine production by imDC is inhibited by SHPS-1 signalling [12]. However, SHPS-1 activation is also known to cause inhibition of phagocytosis in macrophages. Like SHPS-1, CD31 possesses intracellular immunoreceptor tyrosine-based inhibitory motifs, suggesting that it also could inhibit pro-inflammatory macrophage responses [16]. Furthermore, the kinase domain of Mer has the potential to directly promote the anti-inflammatory effects on the macrophage responses to apoptotic cells possibly through its ability to engage PS on apoptotic cells via the product of the growth-arrest-specific gene, gas 6 (Fig. 1) [46–48].

Additional mechanisms may also contribute to the anti-inflammatory relationship between the phagocyte and the apoptotic cell. First, apoptotic cells could themselves produce immunomodulatory factors such as IL-10 and TGF-β1. Each of these anti-inflammatory mediators is capable of upregulating the capacity of macrophages to clear apoptotic cells [41]. Furthermore, by making contact with apoptotic neutrophils, activated macrophages can become switched from a pro-inflammatory to an anti-inflammatory state [49]. Thus, the interplay between apoptotic cells and mononuclear phagocytes appears to create a microenvironment that not only suppresses immune and inflammatory responses but also facilitates efficient apoptotic cell clearance [5, 19, 34].

In addition, engulfed apoptotic cells may influence macrophage differentiation. Cells infected with influenza virus (IV) undergoing apoptosis were phagocytosed by macrophages in an apoptosis-dependent and PS-mediated manner; these reactions subsequently induced antigen presentation to T lymphocytes and the abortion of virus growth *in vitro* [50]. To examine the differentiation of monocytes to macrophages, we employed an adhesion assay using the human monocytic leukemia THP-1 cell line. THP-1 cells became adherent to a substrate by incubation with the culture supernatant of IV-infected chorion cells but not with that of amnion cells. The spreading THP-1 cells were morphologically characteristic of macrophages, and they phagocytosed latex particles. Reverse transcription-polymerase chain reaction analysis revealed that the expression of class A scavenger receptor mRNA was induced in THP-1 cells by incubation with the culture supernatant of IV-infected chorion cells. These results suggested that monocytic THP-1 cells were morphologically and functionally differentiated to macrophages by IV-infected apoptotic cells because of a soluble factor released from the apoptotic cells [50, 51]. But, Brucella virus (Brucella genus) can prevent macrophage apoptosis [52, 53]. Jimenez *et al.* have shown that *B. suis* infection inhibits the spontaneously occurring apoptosis in human monocytes/macrophages [52].

Macrophages are the most efficient scavenger cells. They can also act, upon activation by pro-inflammatory stimuli, as APC, and present exogenous antigens to both $CD8^+$ and $CD4^+$ T cells [46]. It has been demonstrated that apoptotic RMA cells, a murine T lymphoma, induced by the *Rauscher* virus, can be efficiently phagocytosed by peritoneal and bone marrow-derived macrophages, and that the apoptotic material leaks within their cytosol, which may represent for presentation on MHC class I molecules of exogenous peptides [54, 55]. Indeed, exogenous antigens need to reach the cytosol, from where they are translocated as antigenic peptides to the endoplasmic reticulum by transporters associated with antigen presentation

(TAP) proteins, to be eventually complexed with the heavy and light chains of MHC class I molecules. Processing of phagocytosed apoptotic RMA cells indeed yields antigens that access the MHC class I pathway of engulfing macrophages via a TAP-dependent mechanism and are recognized by RMA-specific cytotoxic T lymphocytes (CTL).

Effects of engulfed apoptotic cells on DC

Recently, there has been great interest in understanding how apoptotic cells affect the ability of DC to induce an immune response [44, 50, 56]. DC, particularly the imDC, are able to phagocytose apoptotic cells, although not as efficiently as macrophages. The data on whether a DC can mature and present antigens derived from apoptotic cells are conflicting [22]. At least *in vitro*, several data suggest that DC can phagocytose apoptotic virally infected or tumour cells and present virus- or tumour-derived antigens via cross-presentation to $CD8^+$ cytotoxic T cells, and that the activated CTL can efficiently kill the target cells at least *in vitro* [44, 57, 58]. A large amount of studies showed this outcome results from delayed clearance, which may reflect the relative inefficiency of the DC to engulf apoptotic cells. However, examination of the methodology used to induce apoptosis suggests that in some cases the apoptotic cells consist of a mix of apoptotic and necrotic cells or that the apoptotic cells were stressed by heat shock or infected with virus, thus providing promaturation signals [44, 50, 59]. Some studies showed that the engulfment of purely apoptotic populations by apoptotic cell receptors on DC is nonstimulatory, whereas engulfment of necrotic cells, particularly those derived from tumours, strongly stimulate the DC to mature and activate T cells [50]. Most intracellular proteins, including heat-shock proteins, have been reported to activate DC, suggesting that cells must lyse before they can promote an immune response [58].

Takahashi *et al.* [1, 60] have demonstrated that phagocytosis of late apoptotic cells by mouse macrophages leads to the production of proinflammatory cytokines, notably macrophage inflammatory protein (MIP)-2, and therefore, a yet unknown mechanism should keep our body free of inflammation. In this study, Takahashi *et al.* [61] examined the effect of the addition of imDC to a coculture of macrophages and apoptotic cells on MIP-2 production and phagocytosis by macrophages. The addition of imDC to the coculture system significantly reduced MIP-2 production but unexpectedly enhanced the phagocytosis by macrophages. Other study revealed that the reduction of MIP-2 production was dependent on cell-to-cell contact partly involving the β-integrin family Mac-1 (CD11b) molecules [2, 58]. In addition, anti-inflammatory cytokines, IL-10 and TGF-β, were involved in the reduction of MIP-2 production, as antibodies against these cytokines recovered MIP-2 production [44, 62]. Both cytokines were expressed by imDC more significantly than macrophages at the mRNA levels, although they were hardly detected in the supernatant at the protein levels, suggesting that minute amounts of these anti-inflammatory cytokines were produced mainly by imDC to block MIP-2 production in a cell-to-cell contact-dependent manner [58]. Thus, these studies reveal a new mechanism. On the contrary, no agreement has been yet reached on the effects exerted by apoptotic cells on DC differentiation [22, 50, 63].

Accordingly, mouse DC pulsed with apoptotic cells were efficiently recognized by both antigen-specific $CD4^+$ and $CD8^+$ T cells [3, 64]. Of note, two of those studies found that cells induced to necrosis by rapid freeze-thaw cycles were efficiently phagocytosed by DC but did not allow their recognition by antigen-specific T lymphocytes, therefore suggesting a unique role of apoptosis in the delivery of antigens for T-cell recognition [35]. Indeed, for efficient antigen presentation and T-cell priming, DC need to undergo a characteristic process of terminal differentiation (maturation) that brings them to overexpress MHC class II as well as costimulatory molecules, and to migrate to lymphoid tissues, where the priming of T lymphocytes usually takes place [22, 61]. Li *et al.* found that imDC efficiently phagocytose both apoptotic and necrosis cells and present antigens from the internalized dying cells to MHC class II-restricted T lymphocytes [22]. On the contrary, mature DC lose the ability to phagocytose dying cells. Of relevance, Dalgaard *et al.* [65] reported that, coincubation with apoptotic tumour cells caused DC to acquire the phenotype of mature and therefore highly efficient APC. The latter finding has been challenged by others, who showed that both murine and human DC do not undergo maturation upon phagocytosis of apoptotic cells. Moreover, Moehler *et al.* [66] showed that cells induced to necrosis by freeze-thaw cycles were, at difference with apoptotic cells, optimal stimuli for DC maturation and good sources of antigens for $CD4^+$ T-cell activation. Finally, Ghoneum *et al.* [61, 67] reported that neither internalized apoptotic nor necrotic cells were able to induce human DC to mature and that only mycoplasma infected cells were able to allow upregulation of MHC and costimulatory molecules on DC. The conflicting findings in all those studies might be explained by the different models or systems used, such as the *in vitro* induction of DC, the number and type of apoptotic cells used for DC loading, and the time for detecting the release of pro-inflammatory materials and so on. The fate and immunogenic potential of DC that have internalized apoptotic cells or their debris remain an open issue in immunology.

Phagocytosis of apoptotic cells and autoimmunity

Apoptosis is most important for the immune system in regulating cell growth and terminating immune responses to ensure that overall the rate of division is balanced by the rate of cell death. It is evident that control of apoptosis is

critical for the homeostasis of many organs like the immune system. A number of investigations ranging from animal models to human pathology lend support to the view that apoptosis plays a significant role in the development of autoimmunity. Some immunologists even recognize the failure to appropriately achieve programmed cell death and to clear apoptotic cell fragments as a key pathogenic factor leading to autoimmunity [5, 68]. The involvement of a defect of apoptosis in the generation of autoimmune phenomena may be twofold. Firstly, it may affect the lymphocyte switch-off system and lead to broken immunological tolerance with survival of B and T clones involved in autoimmune response [3, 41]. Secondly, it may slow down cell apoptosis and/or induce exposure of abnormal amount of apoptosis-related antigens, thus favouring the immune response against them [41].

Apoptotic cells are a source of autoantigens [46]. The clearance of apoptotic cells by phagocytes not only functions to remove them from the surrounding tissue but also serves to protect against local damage resulting from uncontrolled leakage of noxious contents. Apoptosis is thought to be a tightly regulated mode of cell death that occurs without inflammation. However, when the clearance is disturbed, apoptotic cells can proceed to secondary necrosis, accompanied by swelling and eventually bursting of the cell. Administration of anti-Fas antibodies to mice induced massive apoptosis and was associated with a severe inflammatory response. Apoptotic cells have been suggested to be a source of autoantigens [46, 69, 70]. The autoimmune disease systemic lupus erythematosus (SLE) is characterized by the presence of autoantibodies that recognize nuclear antigens, such as DNA, RNA and chromatin, which are present within apoptotic blebs. Immunizing mice with an overload of apoptotic thymocytes can induce the production of autoantibodies. Macrophages and DC can internalize apoptotic cells and present antigens derived from the processing of these apoptotic cells to T cells [71]. In a normal situation, macrophages or imDC induce tolerance to self-antigens by clearance of apoptotic cells derived from normal turnover of tissues [64, 69]. However, in the case of delayed or inefficient clearance of apoptotic cells, maturation signals might be provided, resulting in a switch from a tolerogenic response to an immunogenicity of apoptotic cells which might be further enhanced by the action of specific enzymes, such as caspases during apoptosis, thereby generating neoantigens that are capable of triggering autoimmune responses [41, 46, 70]. Clearance of apoptotic cells protects against autoantigens; however, disturbances in the recognition and clearance of apoptotic cells could result in injury and autoimmunity [72]. Antibodies against nucleosomes are a serological hallmark of SLE. Apoptotic cells are the unique source of nucleosomes, which are formed through cleavage of chromatin by nucleases. These nucleosomes and other autoantigens targeted in SLE are expressed in apoptotic blebs or at the surface of apoptotic cells. Therefore, it is conceivable that circulating antibodies can influence apoptotic cell clearance. Using an *in vitro* phagocytosis assay, Licht *et al.* [73] analysed the phagocytic efficacy for apoptotic cells of resident peritoneal macrophages from premorbid and diseased lupus mice. The assay was carried out in the presence of autologous serum, using autologous apoptotic thymocytes as targets. Under these conditions, macrophages from diseased MRL/lpr and NZB × NZW (F1) lupus mice and from age-matched NZB mice showed a decreased phagocytic efficacy serum and are not because of an acquired defect of macrophages. In conclusion, during disease progression in murine SLE, apoptotic cell clearance becomes impaired, which might amplify further disease progression. In fact, macrophages from patients with SLE have been shown *in vitro* to exhibit a reduction in the phagocytic uptake of apoptotic cells.

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. An increased number of apoptotic cells have been demonstrated in the airways of patients with cystic fibrosis and noncystic fibrosis bronchiectasis [30, 46]. It has been speculated that the defective airway clearance of apoptotic cells observed in these pathological conditions may be because of elastase-mediated cleavage of PS receptor on phagocytes that may precipitate an ongoing inflammation condition and progressive airway damage [74]. Furthermore, the anti-inflammatory effects of apoptotic leukocyte clearance in immune-complex-mediated arthritis (ICA) were observed. Injection of apoptotic leukocytes before the induction of ICA has shown to be protective, as uptake of the instilled apoptotic leukocytes by synovial lining macrophages significantly reduced subsequent polymorphonuclear neutrophilic leukocyte chemotaxis into the joint [75]. On the contrary, some studies also showed that a defect in the apoptosis of macrophages or DC might be involved in the occurrence of autoimmune diseases [5, 76, 77]. It is plausible that defects in DC apoptosis lead to DC accumulation and develop chronic lymphocyte activation and autoimmune diseases. This suggests that apoptosis in the immune system is critical for maintaining self-tolerance and preventing autoimmunity in different ways.

Closing remarks and outlook

Although a substantial amount of work has produced significant accomplishments in identifying molecules involved in the engulfment of apoptotic cells, the exact role of individual molecules during this process remains unclear [19, 55, 78]. How certain receptors can promote different outcomes, such as inflammation when triggered by microorganisms but anti-inflammatory responses when

recognizing apoptotic cells, remains an important question. Understanding the engulfed mechanisms, process and immune regulatory effects of apoptotic cells on macrophage and DC functions is critical and essential for us to use macrophages and DC as an efficient carrier to treat autoimmune diseases or to induce immune tolerance in clinics. Actually, Kleinclauss *et al.* [79] had administrated donor apoptotic cells to explore an alternative cell-based therapy to induce immune tolerance and to facilitate allogeneic hematopoietic engraftment after a nonmyeloablative conditioning regimen. Moreover, Sun *et al.* [55] reported that without using immunosuppressants, transfusion of apoptotic splenocytes from the donor strain before transplant dramatically prolonged survival of heart allografts in a rat model. These and other studies suggested that infusion of donor apoptotic cells can promote specific allograft acceptance and transplant tolerance. This may prove to be an interesting approach for preventing autoimmune diseases and graft rejection.

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