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Autoimmunity versus Tolerance: can dying cells tip the balance?

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Abstract

Apoptosis is a physiological process of self-destruction for cells that are damaged or programmed to die. Apoptosis occurs through a series of regulated events that allow cellular debris to be contained and efficiently phagocytosed without initiating a pro-inflammatory immune response. Recent data have linked physiological apoptosis and the uptake of apoptotic cells by macrophages and some subsets of dendritic cells to the maintenance of peripheral immune tolerance. However, when cells die through necrosis, spilling their intracellular contents, or are infected with various pathogens, activation of antigen-presenting cells and induction of an immune response can occur. Receptors for extrinsic pathogen-associated structures, such as membrane bound Toll-like receptors (TLR's) or intracellular Nod-like receptors (NLR's) can also respond to cross-reactive host molecules from dying cells and may focus autoimmune responses onto these antigens. Defective apoptosis of immune cells leads to autoimmunity, as in autoimmune lymphoproliferative syndrome (ALPS) associated with mutations in the death receptor Fas. Defective clearance of apoptotic cell debris can also lead to autoantibody production. We discuss how cell death and apoptotic cell clearance may affect the finely tuned balance between peripheral immune tolerance and autoimmunity.

Overview

Apoptosis, or programmed cell death, is a physiological process occurring in cells that are damaged or no longer useful, including embryogenesis and tissue homeostasis. During apoptosis the cell is degraded by a series of regulated steps that allow rapid uptake and removal of cellular debris by phagocytes without causing inflammation. By contrast, cells that die by necrosis spill their cellular contents including molecules that stimulate inflammation and dendritic cell activation that can prime adaptive immune responses against self-antigens. Apoptotic cell clearance has an important role in embryonic development, tissue homeostasis, and the maintenance of self-tolerance. Signals from the dying cell such as the exposure of phosphatidylserine on the exterior surface of the plasma membrane, are recognized by multiple receptors on the surface of phagocytic cells, providing a swift and efficient disposal system for dying cells. Defects in apoptosis or the uptake of dying cells by macrophages and dendritic cells have been shown to play a role in several pathological conditions, including cancer, neurodegenerative diseases, and autoimmunity. We will discuss recent data on the of macrophages and dendritic cells in apoptotic cell clearance, and describe how the mode of cell death and dead cell uptake may influence the finely tuned balance between maintaining

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immune tolerance and initiation of an potentially pathogenic autoimmune or autoinflammatory response.

Cell Death: General Mechanisms

Apoptosis, also referred to as programmed cell death, is a morphologically identifiable form of cell death characterized by a complex series of processes that adhere to a strict timeline (Figure 1). The initiating signals for cell death are integrated by a number of mechanisms, including interactions between pro and anti-apoptotic members of the bcl-2 protein family. If a critical threshold is reached, the mitochondrial outer membrane becomes permeable to large molecules (MOMP). Mitochondria release cytochrome c, which primes the apoptosome, a cytoplasmic protein complex of caspase-9 and the upstream activator APAF-1 to cleave caspase-9 into its active form. Caspase-9 cleaves caspases-3 and 7, which in turn cleave specific substrates, activating a defined cellular program of plasma membrane blebbing, cytoplasmic and organelle contraction and shrinkage, nuclear chromatin condensation, DNA and RNA degradation by specific nucleases, and cytoskeletal reorganization. (reviewed in [1,2]) One of the earliest changes at the plasma membrane is the display of phosphatidylserine (PS), a membrane lipid that is usually restricted to the inner plasma membrane leaflet, on the external face of the plasma cell membrane. PS provides a platform for recruiting phagocytic cells to vicinity of dying cells, and the resulting apoptotic bodies are rapidly ingested by neighboring cells and resident tissue macrophages and dendritic cells (DCs), via receptor-mediated mechanisms. (reviewed in [3]). Although the ‘flipping’ of membrane lipids to allow PS exposure is caspase dependent, PS can become accessible in any cell losing membrane integrity and is thus not a signal that only marks apoptotic cells.

Necrosis occurs when cell death is ‘accidental’ rather than programmed. Necrosis is often associated with mechanical tissue damage, or certain infectious agents. It is characterized by cell swelling, total cellular breakdown, early loss of plasma membrane integrity, and release of intracellular contents. Whether active signaling pathways play a role in necrosis or if it is simply a passive response to external catastrophe is not clear at this time. The early breakdown of the plasma membrane in necrosis facilitates spilling of intracellular contents, which can contain immunostimulatory molecules such as heat shock proteins [4]. Undegraded mammalian DNA and RNA may also be immunostimulatory through cross-reactive interactions with cellular receptors for pathogen-derived nucleic acids. A comparison of some of the features of apoptosis and necrosis is shown in Table 1.

Alternative Cell death pathways: Autophagic cell death, Anoikis

It has recently become clear that there are other mechanisms of programmed cell death distinct from caspase-mediated apoptosis. In certain circumstances, stimulation of cells in the presence of caspase blockade with agents that would normally induced apoptosis or other cellular responses results in cell death that has biochemical and morphological features resembling autophagy, a process whereby organelles or long-lived proteins are digested in double-membrane enclosed lysosome like structures. In other cells, a necrosis-like phenotype with rapid cell lysis is observed. [5,6] A distinct mechanism of cell death termed ‘Anoikis’ has also been described following detachment of adherent cells from extracellular matrix substrates [7]

Engulfment of dying cells and presentation of self-antigens

In order for activation of the adaptive immune response by antigens derived from dying cells, efficient presentation and costimulation by antigen-presenting cells is necessary. (Figure 2) Macrophages and particular subsets of dendritic cells are responsible for clearance of dying cells in the tissues. Once engulfed, antigens derived from the dead cells are processed and

presented at the cell surface associated with the major histocompatibility complex (MHC). Classically, MHC class II molecules, known to associate with peptides derived from exogenous antigens, present antigens derived from engulfed apoptotic cells. (Reviewed in [8]) However, exogenous antigens can also enter the MHC class I presentation pathway for presentation to CD8⁺ T cells, a process called “cross-presentation”, at which dendritic cells are particularly adept. [9] Both classical exogenous antigen presentation by MHC class II and MHC class I restricted antigen cross-presentation are closely linked to the maturation of dendritic cells, which requires additional signals beyond phagocytosis of apoptotic cells.

Dendritic cell maturation involves changes in cellular morphology and trafficking of antigens that transform these cells from phagocytes to efficient antigen-presenting cells. Immature dendritic cells are present in the circulation and a number of organs, and have been shown to be over five-fold more efficient than mature DCs at phagocytosing apoptotic cells. [10] Maturation signal(s) reprogram dendritic cells to become highly efficient antigen presenting cells, a differentiation step that involves changes in the structure of secretory organelles and antigen trafficking to increase antigen presentation, upregulation of surface molecules including CD40, CD80, and CD86, as well as MHC class II. Maturation signals also trigger dendritic cell migration to draining lymph nodes, where antigen presentation to T cells takes place. [10–12]. Mature dendritic cells are uniquely efficient at activating naïve T cells, making them ideal cells to initiate immune responses to foreign antigens, but also able to activate self-reactive T cells present in the periphery that have escaped negative selection in the thymus. However, as we will discuss, consensus has emerged that antigens from dying cells are presented in a tolerogenic fashion unless other signals are provided that cause maturation of antigen presenting cells or synthesis of cytokines that prime the adaptive immune system.

Immunogenicity vs. Tolerance to antigens from dying cells

Since apoptosis occurs physiologically in many tissues, one proposed model for inducing peripheral tolerance is that immature DCs constantly survey their immunological milieu through endocytosis and phagocytosis mediated by scavenger receptors and uptake of apoptotic cell debris. Which subset of DC's takes up apoptotic cells is under study: In the mouse, CD8⁺ ‘lymphoid’ dendritic cells have been found to be particularly efficient [13,14] Normal recirculation of dendritic cells to the lymph node would then allow antigens to be presented in a tolerogenic fashion. Local tissue antigen uptake and trafficking by DCs to lymph nodes to present tissue-specific self-antigen under non-immunostimulatory conditions without inducing active autoimmunity or full T cell deletion has been demonstrated in a number of animal model systems. This suggests that continual transport of apoptotic “self” cells to the lymph nodes may relate to peripheral tolerance via by presentation of self-antigen. (Figure 3)[15,16] Additionally, dendritic cell maturation is required for the efficient formation of MHC class II-peptide complexes and trafficking of these complexes to the cell surface in dendritic cells. [17]. Nevertheless, peripheral non-mature dendritic cells have been shown to be effective at inducing clonal deletion and anergy in autoreactive CD8⁺ T cells. [18,19].

For skin-derived antigens the situation may be different, in that epidermal Langerhans cells were recently shown to present a self-antigen derived peptide and activate naïve antigen-specific T cells and induce a chronic skin disease without any apparent exogenous stimuli. [20] This work suggests that, unlike other DCs, Langerhans cells may spontaneously mature and initiate immunogenic responses, and that additional mechanisms must control autoreactivity to skin-derived self-antigens. Physiologically, tissue macrophages are likely to be the cell type that phagocytoses most apoptotic cells *in vivo*. After apoptotic cell uptake, macrophages produce cytokines such as TGF- β , PGE2, and PAF that dampen activation of adaptive immune cells. Thus, macrophages may actively suppress immune responses rather than present antigen in a tolerogenic manner. [21]

Two theories were initially proposed as to how innate immune cells are activated. The first, proposed by the late Charles Janeway in 1989 [22], suggested the existence of an innate pattern-recognition system for common structures of antigens from “strangers” (e.g. bacterial components). In 1997 the discovery of Toll-like receptors validated Janeway’s theory. [23] At least 13 Toll-like receptors (TLRs) that recognize extracellular “foreign” antigens, such as bacterial cell walls, bacterial DNA motifs, and viral DNA and RNA exist in mammals. Examples of this are the activation of DCs via TLR5 in response to bacterial flagellum proteins or TLR3 in response to virally infected cells.[24,25]. Importantly, TLRs can in some cases be triggered by cross-reacting host molecules, such as in the case of TLR9-mediated B cell costimulation which can apparently be triggered by mammalian chromosomal DNA released from dying cells [26] A more specific demonstration of the involvement of TLR9 in autoantibody production was made by crossing TLR9^{-/-} mice to a lupus-prone MRL^{lpr/lpr} background. In the absence of TLR9, but not TLR3, anti-dsDNA and anti-chromatin antibody production was completely ablated. However, other lupus-associated autoantibodies, such as anti-Smith antigen (anti-Sm), were maintained or even increased in TLR9^{-/-} mice. Surprisingly, these mice still developed immune-complex nephritis, suggesting that although anti-dsDNA autoantibodies may predict nephritis in SLE, they are not the exclusive pathogenic specificity [27]. TLR7 (or TLR8 in humans), which binds ssRNA are may regulate production of autoantibodies against RNA, which may compensate for the lack of dsDNA antibodies in these mice. Another possibility is that the anti-chromatin antibodies present in TLR9 sufficient mice may actually be protective by mediating clearance of immune complexes under conditions of high antigenic burden. [28,29].

There may also be TLR-independent mechanisms by which cells can respond to foreign and cross-reactive autoantigens. Three groups recently reported a myeloid cell activation pathway stimulated by mammalian DNA that is independent of the TLR adapter molecules MyD88 and TRIF, but dependent on the adaptor protein IPS-1/VISA/MAVS. This innate inflammatory response is characterized by interferon (IFN)-beta induction, as well as TNFα and several chemokines, and a pattern of gene upregulation unique to this novel sensing mechanism. [30–32]. Whether TLR-independent sensing of chromatin also plays a role in the pathogenesis of autoimmunity is not known.

Matzinger’s group has suggested an alternative concept of dendritic cell activation in which endogenous ‘danger’ signals from stressed or dying cells may activate host innate immune cells in addition to exogenous ‘stranger’ signals. Several groups have demonstrated that necrotic cells or their supernatants can trigger maturation of immunostimulatory DCs, whereas apoptotic cells did not. [10,33] However, simply pipetting DCs to dissociate cell interactions or transferring the resting dendritic cells to fresh plates was found to activate dendritic cells, which demonstrates the difficulties in studying this phenomenon *in vitro*. [33] This suggests that there may be several different pathways of triggering the activation of immunostimulatory dendritic cells. The molecular identity of endogenous “danger” signals has been elusive, because it is often unclear whether, for example, a virally infected cell secreting an inflammatory cytokine, IFNα, is doing so in response to cellular stress/damage, or because the viral RNA has triggered an interferon response through TLRs or other receptors. [34].

Some of the molecules that mediate the “danger” signals may in fact be known triggers of inflammation in other settings. In searching for the identity of factors in necrotic cells that stimulated dendritic cell activation and cross-presentation to CD8⁺ T cells, Ken Rock’s group made the surprising observation that uric acid, a byproduct of nucleic acid metabolism, can induce dendritic cell maturation. Uric acid significantly increased cytotoxic CD8⁺ T cell responses when co-injected with antigen *in vivo* at concentrations high enough to form uric acid crystals, [35]. Long known as a proinflammatory molecule when crystallized in the joints in gout, uric acid was also recently shown to stimulate the intracellular NLR protein *ciasl1* to

activate the ‘inflammasome’, a protein complex in which caspase-1 processes the inflammatory cytokine IL1- β into the mature active form [36]. If a “danger” signal was all that was necessary to break immune tolerance, one might expect patients with gout, or those with activating *cias1* mutations (associated with a number of familial autoinflammatory syndromes) to suffer from autoimmunity. However in both these cases the disease remains strictly confined to symptoms resulting from innate immune system activation. In addition, patients with genetic autoinflammatory diseases in which the inflammasome is hyperactivated through genetic mutations rarely develop autoantibody or lymphocyte-mediated pathology. This suggests that while TLR-mediated or other innate signals may provide ‘fuel for the fire’ of systemic autoimmunity by focusing adaptive autoimmune responses on particular antigens, but they seem unlikely to initiate autoimmune disease alone. In support of this idea, it was found that to activate dendritic cells sufficiently to trigger autoimmune myocarditis, both CD40, a T cell derived activation signal, and TLR signals were required [37].

Mechanisms of Apoptotic Cell Recognition and Uptake

A better understanding of how antigens from dying cells may or may not trigger autoimmunity will come from elucidating the molecular pathways by which phagocytes sense and take up these cells, and how these processes impact the maturation and antigen-presenting capability of these cells. There has been significant recent progress in identifying the molecules involved in apoptotic cell recognition and uptake. Most of these systems involve indirect recognition of the PS on the extracellular surface of the plasma membrane on the dying cell through various ‘bridge’ molecules. (Figure 4) Opsonization via the classical and lectin complement pathways has also been demonstrated to play a role in the phagocytosis of apoptotic cells as well as its established role in clearing many viruses, bacteria and fungi. Strikingly, impairment of many of these pathways in both and genetic human diseases strongly predisposes towards systemic autoimmunity.

Mer, Tyro3 and Axl are members of a family of receptor tyrosine kinases that have recently been demonstrated to play roles in the regulation of macrophage and NKT cell responses and cytokine production. [38,39] These receptors have also been shown to mediate recognition and initiate phagocytosis of apoptotic cells. Mer mRNA was found to be expressed in innate immune cells (Macrophages, DCs, NKs, and NKT cells), but not in B or T cells. [39] Philip Cohen and colleagues have demonstrated that Mer is required for apoptotic cell engulfment *in vitro* by macrophages, but not by dendritic cells. Tyro3 and Axl are not yet as well understood. Together, these three kinases, along with their ligands, growth-arrest-specific gene 6 (GAS 6) and Protein S, both of which associate with PS, constitute one system of recognition of apoptotic cells.

CD36 is a scavenger receptor that associates with phosphatidylserine (PS) via **Thrombospondin-1 (TSP-1)**, which acts as a bridge molecule between PS on the apoptotic cell and CD36 on the macrophage. This receptor has been suggested to work synergistically with the vitronectin receptor, a $\alpha v \beta 3$ integrin, on the surface of macrophages to mediate the uptake of apoptotic cells. [40–42] The vitronectin receptor may mediate phagocytosis primarily in resting macrophages whereas CD36 may be the major mediator of uptake in activated macrophages. The vitronectin receptor may also be important in recognition of apoptotic cells through MFG-E8 (see below).

Milk fat globule-epidermal growth factor (EGF)-factor 8 (MFG-E8) is another bridge molecule that links PS on the apoptotic cell to the vitronectin receptor. MFG-E8 is a glycoprotein secreted by activated macrophages, especially in germinal centers of the spleen and lymph nodes, as well as by immature dendritic cells. MFG-E8 specifically binds to

phosphatidylserine on apoptotic cells, facilitating their engulfment, and plays a critical role in facilitating the clearance of apoptotic B cells in germinal centers.[43,44].

C1q and C4 are important early components of the classical pathway of complement and are two of the strongest disease susceptibility genes for the development of systemic lupus erythematosus (SLE) known in humans. Cell clearance is reported to be dependent on C1q both through direct recognition of apoptotic cells and activation of the classical complement pathway. C4 has also been suggested as a mediator of apoptotic cell uptake, possibly through a different mechanism. Studies suggest that cellular uptake can be mediated by complement receptors on macrophages. [45,46] One proposed mechanism of uptake via C1q is through interaction with CD91 on the phagocyte. Receptors mediating C4-dependent uptake by phagocytes have remained elusive. [47] Mannose-binding lectin and soluble IgM, which can bind C1q and activate complement have also been implicated in apoptotic cell clearance, suggesting the involvement of both the classical and mannose-binding lectin branches of the complement pathway.

Phosphatidylserine Receptor (PSR)

PSR was discovered in a screen for genes that conferred PS-specific binding and uptake of apoptotic cells [48]. It was proposed to directly recognize PS on apoptotic cells. However, from gene knockout studies in mice it has become clear that PSR may act more indirectly. PSR^{-/-} mice have defects in mammalian organogenesis, erythropoiesis and T-lymphopoiesis, and some groups reported impaired clearance of apoptotic cells in embryonic liver and thymus. However, this did not induce the upregulation of inflammatory cytokines. [49–51] Rather than being a cell surface receptor, PSR appears to be a nuclear protein, and may not have a bona fide transmembrane domain [52] Further sequence analysis suggests that the proposed PSR could in fact be a transcription factor that promotes upregulation of genes required for phagocytosis of PS⁺ cells, perhaps explaining the original transfection experiments that identified this molecule.

When uptake goes wrong

Under normal circumstances, apoptotic cells are rapidly cleared by phagocytes [53]. However, if uptake is impaired, or cell death enhanced, apoptotic material may build up to a point that overwhelms the uptake systems discussed above. In these circumstances, it has been suggested that self-tolerance breaks down due to the priming of autoreactive lymphocytes by more efficient antigen presenting cells that would then take up apoptotic material. If uptake of apoptotic cells is delayed, secondary necrosis may ensue, resulting in the exposure of pro-inflammatory intracellular molecules. Mice deficient in a number of the molecules necessary for apoptotic cell recognition and uptake manifest similar autoimmune phenotypes (Table 2)

Triple knockout mice for the related Tyro3/Axl/Mer receptors have profound defects in apoptotic cell clearance, blindness (due to failure of retinal epithelial cells to clear shed outer segment layers resulting in the death of photoreceptor cells), and neurological abnormalities. Additionally, these mice develop splenomegaly and spontaneous antibodies against nuclear antigens, swollen joints with lymphocyte infiltration, as well as IgG deposits in the kidney leading to glomerulonephritis, a common complication of SLE. [54] Macrophages from mer^{-/-} mice were reported to be ~90% less efficient in phagocytosis of apoptotic cells compared to macrophages from wild type mice. [55] and also develop elevated levels of antinuclear antibodies. However, the genesis of autoimmunity in these mice may also be linked to excessive dendritic cell activation by Toll-like receptors, as Mer^{-/-} macrophages have increased expression of costimulatory surface molecules and increased production of pro inflammatory cytokines such as TNF α in response to LPS.

MFG-E8^{-/-} mice develop splenomegaly with the formation of numerous germinal centers, and suffer from glomerulonephritis due to autoantibody production. Macrophages from the MFG-E8 deficient mouse demonstrated a four-fold decrease in their efficiency of apoptotic cell uptake compared to wild type macrophages. However, engulfment of microspheres was not affected in the MFG-E8^{-/-} mice. This mouse model suggests that MFG-E8 has a critical role in clearing apoptotic B cells from germinal centers and failure to remove these cells can lead to autoimmunity. Notably, it was found that MFG-E8 is expressed in immature DCs such as Langerhans cells but not in thymic macrophages and thus not involved in clearance of apoptotic thymocytes, suggesting that different tissues may use different recognition systems for the uptake of apoptotic cells. [44] This group has also shown that MFG-E8 carrying a mutation in the second EGF domain, designated D89E, inhibited the phagocytosis of apoptotic cells both *in vitro* and *in vivo* by a variety of phagocytes. This mutation masks PS on apoptotic cells. Injection of mutated MFG-E8 into mice induced autoantibody production. This effect was exacerbated by co-injecting apoptotic thymocytes. The authors suggest that the perturbation of apoptotic cell clearance and the consistent presentation of self-antigens may be enough to disrupt peripheral immune tolerance. [56]

Mice deficient in even seemingly unrelated molecules can develop autoimmunity if apoptotic cell uptake is impaired. For example, Cytochrome P450 1B1 (CYP1B1) enzyme-deficient mice were found to have evidence of systemic autoimmunity, manifested by antinuclear and anti-DNA antibodies as well as immune-complex deposition in the kidney and glomerulonephritis. CYP1B1 is highly expressed in the mononuclear phagocyte lineage. Peritoneal macrophages from CYP1B1 deficient mice were impaired in the phagocytosis of apoptotic, necrotic and opsonized cells, suggesting that CYP1B1 may have an unexpected role in macrophage phagocytic function that is important for maintenance of immunological self-tolerance.[57] Macrophages from patients with systemic lupus erythematosus have also been found to have phagocytic defects[58–60], although in these human studies, it is difficult to determine if this is a cause or consequence of the disease.

Another important mouse model for systemic lupus erythematosus (SLE) is the C1q-deficient mouse. Homozygous deficiency in C1q is associated with severe SLE in humans. C1q deficient mice have a defect in phagocytic uptake of apoptotic cells by activated macrophages as well as resident peritoneal macrophages. C1q-deficient mice develop glomerulonephritis with an excess of glomerular apoptotic bodies. Mice deficient in C4, another member of the classical complement pathway, show a less severe disease phenotype than C1q^{-/-} mice, and no impairment of resident peritoneal macrophage phagocytic uptake. C3 deficient mice do not develop autoimmunity, suggesting a hierarchical role for classical complement proteins in apoptotic cell clearance. [45] Similarly, patients with C4 deficiencies generally have less severe SLE, and those with C3 and C2 deficiencies are often non-symptomatic. Additionally, low levels of mannose-binding lectin (which plays the same role as C1q in the lectin pathway) have also been associated with SLE. In conjunction with these findings, deficiencies in molecules that can bind C1q and activate the classical pathway, such as soluble IgM, have also been linked to increased incidence of autoimmunity in humans and mouse models [61].

Supporting the hypothesis that ordered degradation of DNA reduces its immunogenicity, mice deficient in DNase I also develop systemic autoimmunity. Additionally, data implicating apoptotic cell debris as immunogens in SLE has come from DNase I deficient mice. DNase I^{-/-} mice develop anti-chromatin antibodies similar to those in human SLE patients, as well as glomerulonephritis due to the deposition of immune complexes in glomeruli [62]. Two human SLE patients were also reported to have heterozygous nonsense mutations in exon 2 of the gene encoding DNase I [63]. DNase I acts together with C1q to efficiently degrade necrotic cell-derived chromatin. [64] A significant reduction of DNase I activity in sera from SLE patients and patients with rheumatoid arthritis (RA) compared with sera from healthy donors

has been observed. However, only SLE sera showed a strongly reduced capacity to degrade necrotic cell-derived chromatin[65]. Administration of DNase has been studied as a therapeutic modality in patients with SLE, and was well tolerated, but did not produce clinical improvement in a small phase I study[66]. Unlike other models mentioned above, macrophages from DNase I deficient mice are not defective in apoptotic cell clearance. Instead, these data support the idea that non-degraded DNA can lead to enhanced immunogenicity. Whether TLR or other innate immune sensors contribute to autoimmunity in DNase deficient mice is not known.

Unlike cell death in non-immune cells, which may fuel autoimmunity, death of immune cells is generally a negative signal for immune responses to pathogens as well as self-antigens. A large amount of cell death occurs in peripheral T and B cells after antigenic activation, and approximately 90% of antigen-specific lymphocytes die after initial activation. Some of this cell death is under the control of the BH3-family protein Bim, which initiates mitochondrial-dependent apoptosis. In addition to accumulation of peripheral T and B cells, Bim deficient mice develop systemic autoimmunity on some genetic backgrounds [67]. In humans with the autoimmune lymphoproliferation syndrome (ALPS) due to mutations in the death receptor Fas/CD95, or *lpr/lpr* mice homozygous for loss of function mutations in Fas, multiple immune cell types are resistant to apoptosis induced through ligation of this receptor by Fas ligand, which occurs particularly in chronically restimulated T and B cells [68]. Unlike sporadic SLE, Fas deficiency also produces the accumulation of an unusual subset of CD3/CD4 ‘double negative’ T cells, which can be useful for diagnostic purposes. Autoantibody production is common in ALPS and *lpr* mice but nephritis is rare except on susceptible genetic backgrounds. Additionally, a recent report suggests that the death of terminally differentiated DCs may also contribute to immune tolerance. Mice engineered to express the baculovirus caspase inhibitor p35 in dendritic cells developed late onset systemic autoimmunity with nephritis on susceptible genetic backgrounds [69].

Conclusion

Defects in apoptosis and the clearance of apoptotic cells have been linked to several autoimmune diseases, especially SLE. Many mouse disease models implicate apoptotic cell uptake mediators such as Mer/Tyros3 and MFG-E8 as having critical roles in protecting against autoimmunity by efficiently clearing self-antigens associated with apoptotic cells. As discussed here, the uptake of apoptotic cells by immature dendritic cells promotes tolerance, whereas cell debris in combination with maturation triggers such as danger/stranger signals elicits an inflammatory immune response. This can result in cytotoxic T cell activation via cross-presentation of exogenous self antigens in the context of MHC class I, but additional signals are probably necessary to initiate and maintain systemic autoimmunity. Macrophages are also involved in the maintenance of tolerance by silently phagocytosing apoptotic cells and producing anti-inflammatory cytokines. Thus, it may not be the dying cell itself that tips the balance from tolerance to autoimmunity so much as how the dying cell is perceived; through “eat me” signals from the state of the dying cell, the signals and debris it releases, and how long it persists before phagocytosis occurs.

References

1. Aigner T. Apoptosis, necrosis, or whatever: how to find out what really happens? *J Pathol* 2002;198:1–4. [PubMed: 12210056]
2. Hengartner MO. The biochemistry of apoptosis. *Nature* 2000;407:770–776. [PubMed: 11048727]
3. Roos A, Xu W, Castellano G, Nauta AJ, Garred P, Daha MR, van Kooten C. Mini-review: A pivotal role for innate immunity in the clearance of apoptotic cells. *Eur J Immunol* 2004;34:921–929. [PubMed: 15048702]

4. Manjili MH, Park J, Facciponte JG, Subjeck JR. HSP110 induces “danger signals” upon interaction with antigen presenting cells and mouse mammary carcinoma. *Immunobiology* 2005;210:295–303. [PubMed: 16164037]
5. Denecker G, Vercammen D, Steemans M, Vanden Berghe T, Brouckaert G, Van Loo G, Zhivotovsky B, Fiers W, Grooten J, Declercq W, Vandenabeele P. Death receptor-induced apoptotic and necrotic cell death: differential role of caspases and mitochondria. *Cell Death Differ* 2001;8:829–840. [PubMed: 11526436]
6. Yu L, Alva A, Su H, Dutt P, Freundt E, Welsh S, Baehrecke EH, Lenardo MJ. Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. *Science* 2004;304:1500–1502. [PubMed: 15131264]
7. Zhan M, Zhao H, Han ZC. Signalling mechanisms of anoikis. *Histol Histopathol* 2004;19:973–983. [PubMed: 15168359]
8. Rock KL, York IA, Saric T, Goldberg AL. Protein degradation and the generation of MHC class I-presented peptides. *Adv Immunol* 2002;80:1–70. [PubMed: 12078479]
9. Albert ML, Sauter B, Bhardwaj N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature* 1998;392:86–89. [PubMed: 9510252]
10. Sauter B, Albert ML, Francisco L, Larsson M, Somersan S, Bhardwaj N. Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. *J Exp Med* 2000;191:423–434. [PubMed: 10662788]
11. Steinman RM, Turley S, Mellman I, Inaba K. The induction of tolerance by dendritic cells that have captured apoptotic cells. *J Exp Med* 2000;191:411–416. [PubMed: 10662786]
12. Delamarre L, Holcombe H, Mellman I. Presentation of Exogenous Antigens on Major Histocompatibility Complex (MHC) Class I and MHC Class II Molecules Is Differentially Regulated during Dendritic Cell Maturation. *J Exp Med* 2003;198:111–122. [PubMed: 12835477]
13. Ferguson TA, Herndon J, Elzey B, Griffith TS, Schoenberger S, Green DR. Uptake of apoptotic antigen-coupled cells by lymphoid dendritic cells and cross-priming of CD8 (+) T cells produce active immune unresponsiveness. *J Immunol* 2002;168:5589–5595. [PubMed: 12023355]
14. Schulz O, Reis e Sousa C. Cross-presentation of cell-associated antigens by CD8alpha+ dendritic cells is attributable to their ability to internalize dead cells. *Immunology* 2002;107:183–189. [PubMed: 12383197]
15. Scheinecker C, McHugh R, Shevach EM, Germain RN. Constitutive presentation of a natural tissue autoantigen exclusively by dendritic cells in the draining lymph node. *J Exp Med* 2002;196:1079–1090. [PubMed: 12391019]
16. Huang FP, Platt N, Wykes M, Major JR, Powell TJ, Jenkins CD, MacPherson GG. A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J Exp Med* 2000;191:435–444. [PubMed: 10662789]
17. Inaba K, Turley S, Iyoda T, Yamaide F, Shimoyama S, Reis e Sousa C, Germain RN, Mellman I, Steinman RM. The formation of immunogenic major histocompatibility complex class II-peptide ligands in lysosomal compartments of dendritic cells is regulated by inflammatory stimuli. *J Exp Med* 2000;191:927–936. [PubMed: 10727455]
18. Davey GM, Kurts C, Miller JF, Bouillet P, Strasser A, Brooks AG, Carbone FR, Heath WR. Peripheral deletion of autoreactive CD8 T cells by cross presentation of self-antigen occurs by a Bcl-2-inhibitable pathway mediated by Bim. *J Exp Med* 2002;196:947–955. [PubMed: 12370256]
19. Lambolez F, Jooss K, Vasseur F, Sarukhan A. Tolerance induction to self antigens by peripheral dendritic cells. *Eur J Immunol* 2002;32:2588–2597. [PubMed: 12207343]
20. Mayerova D, Parke EA, Bursch LS, Odumade OA, Hogquist KA. Langerhans cells activate naive self-antigen-specific CD8 T cells in the steady state. *Immunity* 2004;21:391–400. [PubMed: 15357950]
21. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J Clin Invest* 1998;101:890–898. [PubMed: 9466984]
22. Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol* 1989;54:1–13. [PubMed: 2700931]

23. Medzhitov R, Preston-Hurlburt P, Janeway CA Jr. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 1997;388:394–397. [PubMed: 9237759]
24. Schulz O, Diebold SS, Chen M, Naslund TI, Nolte MA, Alexopoulou L, Azuma YT, Flavell RA, Liljestrom P, Reis e Sousa C. Toll-like receptor 3 promotes cross-priming to virus-infected cells. *Nature* 2005;433:887–892. [PubMed: 15711573]
25. Sporri R, Reis e Sousa C. Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4+ T cell populations lacking helper function. *Nat Immunol* 2005;6:163–170. [PubMed: 15654341]Epub 2005 Jan 2016
26. Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-Rothstein A. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* 2002;416:603–607. [PubMed: 11948342]
27. Christensen SR, Kashgarian M, Alexopoulou L, Flavell RA, Akira S, Shlomchik MJ. Toll-like receptor 9 controls anti-DNA autoantibody production in murine lupus. *J Exp Med* 2005;202:321–331. [PubMed: 16027240]
28. Clarkson AB Jr, Mellow GH. Rheumatoid factor-like immunoglobulin M protects previously uninfected rat pups and dams from *Trypanosoma lewisi*. *Science* 1981;214:186–188. [PubMed: 7025211]
29. Coulie P, Van Snick J. Rheumatoid factors and secondary immune responses in the mouse. II. Incidence, kinetics and induction mechanisms. *Eur J Immunol* 1983;13:895–899. [PubMed: 6641787]
30. Ishii KJ, Coban C, Kato H, Takahashi K, Torii Y, Takeshita F, Ludwig H, Sutter G, Suzuki K, Hemmi H, Sato S, Yamamoto M, Uematsu S, Kawai T, Takeuchi O, Akira S. A Toll-like receptor-independent antiviral response induced by double-stranded B-form DNA. *Nat Immunol* 2006;7:40–48. [PubMed: 16286919]
31. Okabe Y, Kawane K, Akira S, Taniguchi T, Nagata S. Toll-like receptor-independent gene induction program activated by mammalian DNA escaped from apoptotic DNA degradation. *J Exp Med* 2005;202:1333–1339. [PubMed: 16301743]
32. Stetson DB, Medzhitov R. Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. *Immunity* 2006;24:93–103. [PubMed: 16413926]
33. Gallucci S, Lolkema M, Matzinger P. Natural adjuvants: endogenous activators of dendritic cells. *Nat Med* 1999;5:1249–1255. [PubMed: 10545990]
34. Heath WR, Carbone FR. Immunology: dangerous liaisons. *Nature* 2003;425:460–461. [PubMed: 14523425]
35. Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* 2003;425:516–521. [PubMed: 14520412]Epub 2003 Sep 2007
36. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 2006;440:237–241. [PubMed: 16407889]
37. Eriksson U, Ricci R, Hunziker L, Kurrer MO, Oudit GY, Watts TH, Sonderegger I, Bachmaier K, Kopf M, Penninger JM. Dendritic cell-induced autoimmune heart failure requires cooperation between adaptive and innate immunity. *Nat Med* 2003;9:1484–1490. [PubMed: 14625544]
38. Heiring C, Dahlback B, Muller YA. Ligand recognition and homophilic interactions in Tyro3: structural insights into the Axl/Tyro3 receptor tyrosine kinase family. *J Biol Chem* 2004;279:6952–6958. [PubMed: 14623883]Epub 2003 Nov 2007
39. Behrens EM, Gadue P, Gong SY, Garrett S, Stein PL, Cohen PL. The mer receptor tyrosine kinase: expression and function suggest a role in innate immunity. *Eur J Immunol* 2003;33:2160–2167. [PubMed: 12884290]
40. Fadok VA, Warner ML, Bratton DL, Henson PM. CD36 is required for phagocytosis of apoptotic cells by human macrophages that use either a phosphatidylserine receptor or the vitronectin receptor (alpha v beta 3). *J Immunol* 1998;161:6250–6257. [PubMed: 9834113]
41. Grimsley C, Ravichandran KS. Cues for apoptotic cell engulfment: eat-me, don't eat-me and come-get-me signals. *Trends Cell Biol* 2003;13:648–656. [PubMed: 14624843]
42. Savill J, Hogg N, Ren Y, Haslett C. Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. *J Clin Invest* 1992;90:1513–1522. [PubMed: 1383273]

43. Hanayama R, Tanaka M, Miwa K, Shinohara A, Iwamatsu A, Nagata S. Identification of a factor that links apoptotic cells to phagocytes. *Nature* 2002;417:182–187. [PubMed: 12000961]
44. Hanayama R, Tanaka M, Miyasaka K, Aozasa K, Koike M, Uchiyama Y, Nagata S. Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. *Science* 2004;304:1147–1150. [PubMed: 15155946]
45. Taylor PR, Carugati A, Fadok VA, Cook HT, Andrews M, Carroll MC, Savill JS, Henson PM, Botto M, Walport MJ. A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells in vivo. *J Exp Med* 2000;192:359–366. [PubMed: 10934224]
46. Botto M, Dell'Agnola C, Bygrave AE, Thompson EM, Cook HT, Petry F, Loos M, Pandolfi PP, Walport MJ. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat Genet* 1998;19:56–59. [PubMed: 9590289]
47. Manderson AP, Botto M, Walport MJ. The role of complement in the development of systemic lupus erythematosus. *Annu Rev Immunol* 2004;22:431–456. [PubMed: 15032584]
48. Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM. A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* 2000;405:85–90. [PubMed: 10811223]
49. Kunisaki Y, Masuko S, Noda M, Inayoshi A, Sanui T, Harada M, Sasazuki T, Fukui Y. Defective fetal liver erythropoiesis and T lymphopoiesis in mice lacking the phosphatidylserine receptor. *Blood* 2004;103:3362–3364. [PubMed: 14715629] Epub 2004 Jan 3308
50. Li MO, Sarkisian MR, Mehal WZ, Rakic P, Flavell RA. Phosphatidylserine receptor is required for clearance of apoptotic cells. *Science* 2003;302:1560–1563. [PubMed: 14645847]
51. Bose J, Gruber AD, Helming L, Schiebe S, Wegener I, Hafner M, Beales M, Kontgen F, Lengeling A. The phosphatidylserine receptor has essential functions during embryogenesis but not in apoptotic cell removal. *J Biol* 2004;3:15. [PubMed: 15345036] Epub 2004 Aug 2023
52. Cui P, Qin B, Liu N, Pan G, Pei D. Nuclear localization of the phosphatidylserine receptor protein via multiple nuclear localization signals. *Exp Cell Res* 2004;293:154–163. [PubMed: 14729065]
53. van Nieuwenhuijze AE, van Lopik T, Smeenk RJ, Aarden LA. Time between onset of apoptosis and release of nucleosomes from apoptotic cells: putative implications for systemic lupus erythematosus. *Ann Rheum Dis* 2003;62:10–14. [PubMed: 12480662]
54. Lu Q, Lemke G. Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family. *Science* 2001;293:306–311. [PubMed: 11452127]
55. Scott RS, McMahon EJ, Pop SM, Reap EA, Caricchio R, Cohen PL, Earp HS, Matsushima GK. Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature* 2001;411:207–211. [PubMed: 11346799]
56. Asano K, Miwa M, Miwa K, Hanayama R, Nagase H, Nagata S, Tanaka M. Masking of phosphatidylserine inhibits apoptotic cell engulfment and induces autoantibody production in mice. *J Exp Med* 2004;200:459–467. [PubMed: 15302904]
57. Ward JM, Nikolov NP, Tschetter JR, Kopp JB, Gonzalez FJ, Kimura S, Siegel RM. Progressive glomerulonephritis and histiocytic sarcoma associated with macrophage functional defects in CYP1B1-deficient mice. *Toxicol Pathol* 2004;32:710–718. [PubMed: 15580705]
58. Baumann I, Kolowos W, Voll RE, Manger B, Gaip U, Neuhuber WL, Kirchner T, Kalden JR, Herrmann M. Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus. *Arthritis Rheum* 2002;46:191–201. [PubMed: 11817590]
59. Herrmann M, Voll RE, Zoller OM, Hagenhofer M, Ponner BB, Kalden JR. Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. *Arthritis Rheum* 1998;41:1241–1250. [PubMed: 9663482]
60. Kalden JR. Defective phagocytosis of apoptotic cells: possible explanation for the induction of autoantibodies in SLE. *Lupus* 1997;6:326–327. [PubMed: 9296780]
61. Manson JJ, Mauri C, Ehrenstein MR. Natural serum IgM maintains immunological homeostasis and prevents autoimmunity. *Springer Semin Immunopathol* 2005;26:425–432. [PubMed: 15611856]
62. Napirei M, Karsunky H, Zevnik B, Stephan H, Mannherz HG, Moroy T. Features of systemic lupus erythematosus in Dnase1-deficient mice. *Nat Genet* 2000;25:177–181. [PubMed: 10835632]

63. Tsukumo S, Yasutomo K. DNaseI in pathogenesis of systemic lupus erythematosus. *Clin Immunol* 2004;113:14–18. [PubMed: 15380524]
64. Gaipf US, Beyer TD, Heyder P, Kuenkele S, Bottcher A, Voll RE, Kalden JR, Herrmann M. Cooperation between C1q and DNase I in the clearance of necrotic cell-derived chromatin. *Arthritis Rheum* 2004;50:640–649. [PubMed: 14872509]
65. Munoz LE, Gaipf US, Franz S, Sheriff A, Voll RE, Kalden JR, Herrmann M. SLE--a disease of clearance deficiency? *Rheumatology (Oxford)* 2005;44:1101–1107. [PubMed: 15928001]
66. Davis JC Jr, Manzi S, Yarboro C, Rairie J, McInnes I, Averthelyi D, Sinicropi D, Hale VG, Balow J, Austin H, Boumpas DT, Klippel JH. Recombinant human Dnase I (rhDNase) in patients with lupus nephritis. *Lupus* 1999;8:68–76. [PubMed: 10025601]
67. Bouillet P, Metcalf D, Huang DC, Tarlinton DM, Kay TW, Kontgen F, Adams JM, Strasser A. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* 1999;286:1735–1738. [PubMed: 10576740]
68. Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, Strober W, Lenardo MJ, Puck JM. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell* 1995;81:935–946. [PubMed: 7540117]
69. Chen M, Wang YH, Wang Y, Huang L, Sandoval H, Liu YJ, Wang J. Dendritic cell apoptosis in the maintenance of immune tolerance. *Science* 2006;311:1160–1164. [PubMed: 16497935]

Figure 1. Steps in apoptosis and apoptotic cell uptake

Critical stages are shown for the apoptotic cell at bottom, with the phosphatidylserine externalized on the plasma membrane early in apoptosis serving as the main signal for apoptotic cell uptake by a macrophage at top.

Figure 2. Ligands and Receptors involved in apoptotic cell recognition and uptake

The major recognition systems for surface proteins on dying cells are shown. MBP: Maltose Binding Protein, MFG-E8: Milk Fat Globule protein E8, PSR: putative phosphatidylserine receptor.

Figure 3. Influence of apoptotic and other cellular stimuli on activation of antigen-presenting cells
The mode of cell death, kinetics of uptake and presence of other activation signals determine the cell type that clears dying cells and the immunological outcome.

Table 1
Comparing cellular modifications in apoptotic and necrotic death

	Apoptosis	Necrosis
Cellular role	Active	Passive
Distribution	Dispersed	Contiguous
Morphology	Decreased cell volume	Increased cell volume
Cellular membrane	Preserved	Loss of integrity
Induction	Slow (hours)	Rapid (seconds – minutes)
Cell removal	Rapid	Slow
Tissue Inflammation	Absent	Present

Table 2**Phenotypes of mice deficient in apoptotic cell uptake**

Molecule	Phenotype of knockout mouse
C1q/C4	Systemic autoimmunity with autoantibodies and glomerulonephritis Increased numbers of apoptotic cells in glomeruli (C1q only) Delayed clearance of apoptotic cells from the peritoneal cavity
IgM	Autoimmunity to nuclear Ags; renal IgG and complement deposition
Mer/Tyro3/Axl & Mer (single KO)	Autoimmunity to nuclear Ags; renal IgG and complement deposition Defect in clearance of apoptotic cells in the thymus and spleen
MFG-E8	Glomerulonephritis, Splenomegaly, Autoantibodies, Numerous germinal centers with increased apoptotic B cells
PSR (Phosphatidylserine Receptor)	Abnormal development and neonatal lethality Accumulation of apoptotic cells in brain and lungs
CYP1B1	Proliferative immune-mediated glomerulonephritis; Autoantibodies; Histiocytic sarcomas; Defective cellular phagocytosis by peritoneal macrophages
DNaseI	Autoimmunity to chromatin; renal IgG and complement deposition Glomerulonephritis