Immunity
Review

Cell Death in the Maintenance and Abrogation of Tolerance: The Five Ws of Dying Cells

Thomas S. Griffith[1,*] and Thomas A. Ferguson[2,*]
[1]Departments of Urology and Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455, USA
[2]Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, MO 63110, USA
*Correspondence: tgriffit@umn.edu (T.S.G.), ferguson@vision.wustl.edu (T.A.F.)
DOI 10.1016/j.immuni.2011.08.011

The mammalian immune system continually faces death in the form of its own dead and dying cells that arise during normal tissue turnover, infections, cellular damage, and cancer. Complex decisions must then be made that will permit a protective response to pathogens, while at the same time destroying tumors but not attacking vital systems of the host that could lead to autoimmunity. By using an investigative technique termed the five Ws (who, what, when, where, and why), we will examine how the immune system responds to antigens generated via cell death. This analysis will give us a better understanding of the molecular differences fundamental to tolerogenic or immunogenic cell death, the cells that sense and react to the dead cells, and the consequences of these fundamental elements on the maintenance or abrogation of tolerance.

Introduction

A variety of pathways are used by multicellular organisms to orchestrate cell death during development and morphogenesis to control cell numbers and eliminate damaged cells (Penaloza et al., 2006). It is estimated that up to 10^6 cells die in the human body every second, most as a result of normal tissue turnover (Green et al., 2009). There is also cell death in response to infection that may represent a primitive defense mechanism to prevent pathogen replication by removing the infected or damaged cells. Moreover, the same cell death pathways are needed to control the number of effector cells generated during an immune response and then eliminate the majority of them once the pathogen is cleared (Parish et al., 2009; Pellegrini et al., 2003; Barreiro et al., 2004). Thus, one of the major challenges for the immune system is to react to foreign pathogens within the context of this constant antigenic "noise" derived from the dead and dying cells, yet not respond to the self-antigens that can be presented to the immune system in far greater (and uncontrollable) amounts. Simultaneously, it is advantageous to retain the ability to direct immune responses toward the "self" antigens expressed by tumor cells. Thus, the immune system is faced with the important task of being responsive to pathogens (foreign invaders) while destroying tumors (derived from self) and not attacking vital systems of the host (self-antigens).

Understanding how these complex immunological decisions are made has been intensely investigated over the past 20 years, although it has been difficult, at times, to get the complete picture. One reason for this is that each study focused on only a few criteria that may not apply in every situation. For example, the general mechanism by which a cell died was proposed to influence the type of immune response. This concept arose from some of the original descriptions of apoptosis as being a "silent death" and tolerogenic, whereas necrosis was a "violent death" that released a number of immunostimulatory molecules (Green et al., 2009; Thompson, 1995). In some instances this has proven true as demonstrated by studies comparing the tolerogenic and immunogenic properties of apoptotic and necrotic cells (Griffith et al., 1996, 2007; Shi et al., 2003). It is now recognized, however, that apoptotic cells can be highly immunogenic, eliciting protective immune responses (Kepp et al., 2009; Ullrich et al., 2008; Zitvogel et al., 2004). In an effort to explain this disparity, some studies have characterized the molecular composition of dying cells, suggesting that factors released at the time of cell death could determine the resultant immune response. For example, the release of cytokines or damage-associated molecular patterns (DAMPs) from a dying cell can influence immunity (Bianchi, 2007; Chen et al., 2001; Gao et al., 1998; Millar et al., 2003). Although these studies are compelling, the release of factors from dying cells does not always dictate the type of immune response, because DAMPs can be modified by the cell death pathway to promote either tolerance or immunity (Kazama et al., 2008). Another approach has considered the activation of phagocytic cells as the deciding factor in the generation of tolerance or immunity to the antigens associated with the eaten dead cells. These data suggest that dead cells (apoptotic or necrotic) can inhibit or increase antigen presentation by the antigen-presenting cell (APC) (Albert et al., 2001; Dhodapkar et al., 2001; Sauter et al., 2000). However, it is important to keep in mind that the maturation state of the phagocyte does not always dictate its ability to induce tolerance or immunity (Ferguson et al., 2002; Kazama et al., 2008). To reconcile some of these disparities, the influence of pathogen-associated molecular patterns (PAMPs) derived from infectious agents has also been considered, because bacterial products or viral nucleic acids perceived by phagocytic cells in the presence of dead cells can dictate the resultant immune response through activation of APC (Medzhitov and Janeway, 2002; Torchinsky et al., 2009). This hypothesis, too, has been questioned by observations where the death of transformed cells (Obeid et al., 2007) and in some cases nontransformed cells (Rock and Kono, 2008; Shi et al., 2003) elicited immune responses in the absence of infection. In addition, debris from nontransformed cells in some settings can stimulate general or organ-specific autoimmune responses (Gaipl et al., 2007). Thus, a simple explanation based on any of these criteria cannot

apply to all aspects of tolerogenic and immunogenic cell death. As important as it is to consider the factors listed above, it is equally important to consider those criteria that are not typically discussed in this context—including variations in the type of dead cell (e.g., lymphocyte, fibroblast, epithelial cell), cell status (transformed, nontransformed, activated, naive), immune response examined (humoral or cellular), organ or organ system explored (e.g., gut, eye, skin), availability of T cell help, and even the desired result (e.g., graft acceptance, antitumor immunity, autoimmunity, immune deviation). With the addition of these many considerations (and caveats), it becomes even more difficult to apply general principles to every situation. We certainly cannot consider all of these variables in this review, but we think it is important to keep them in mind as we discuss the effects of cell death on the immune response. Consequently, for our discussion we have applied (and slightly modified) the old journalism maxim called the "five Ws." This is a classical concept in news style, research, and police investigations such that for a report to be considered complete it must answer a checklist of questions—who, what, when, where, why, and how. Thus, the nature of the immune response that develops in the face of dead cells depends on *who* dies, *what* it releases, *when* it dies, *where* it dies, and *why* it dies. The answers to these questions lead us to an understanding of *how* immunity is regulated. With these interrogative questions we can perhaps get a better understanding of the molecular differences fundamental to tolerogenic or immunogenic cell death, the cells that sense and react to the dead cells, and the consequences of these fundamental elements on the maintenance or abrogation of tolerance. Variations in these factors can have consequences that range from effective antipathogen or antitumor immune responses to autoimmune pathology.

Who Dies: Characteristics of the Dying Cell

Just as different types of living cells are highly specialized to perform unique functions, the type of cell dying can dramatically influence the resultant immune response. For example, bortezomib-induced apoptotic myeloma cells expose the chaperone HSP90 on their surface, facilitating their recognition by dendritic cells (DCs) and the subsequent induction of immunity (Spisek et al., 2007). Similarly, in response to some chemotherapeutics (such as anthracyclins), but not others (such as mitomycin C or etoposide), tumor cells expose complexes formed by the chaperone calreticulin and disulphide isomerase eRp57 on their cell surface. This occurs at a proapoptotic stage and facilitates the uptake of dying cells by DCs (Obeid et al., 2007; Panaretakis et al., 2008). Exposure of these complexes strongly correlates with immunogenicity, and anthracyclin-treated dying tumor cells that expose calreticulin can be used as a cancer vaccine. Because this cell death is caspase dependent and apoptotic, this is an example where apoptotic death can prime for immunity. In contrast, Ronchetti et al. (1999a, 1999b) found that although apoptotic tumor cells could prime, they were much less efficient than nonreplicating live cells. The immunogenicity of the apoptotic cells was proportional to the number of cells injected and correlated with the serum concentration of interleukin-10 (IL-10) and interleukin-1 beta (IL-1β). Another study found that DCs (but not macrophages [Mϕ]) pulsed with apoptotic tumor cells were efficient at cross-priming (immunity) (Miyake et al., 2007), highlighting the importance of the cell phagocytizing the dead cells (see "Where" below). Interestingly, γ-irradiated tumor cells can also be tolerogenic based on their ability to suppress cytotoxic T lymphocyte (CTL) responses and antitumor immunity via the induction of $CD8^+$ T cell anergy and $CD4^+$ regulatory T (Treg) cell responses (Xie et al., 2009). Thus, apoptotic tumor cells can prime or tolerize depending on other factors (see "What" below) or the experimental system (or tumor type) employed. In contrast, a recent study suggests that in some situations live tumor cells may represent a more efficient method of priming the immune system compared to dead cells (Matheoud et al., 2010). These authors found that DCs internalized cytosolic and membrane material into vesicles from metabolically labeled live cells, leading to enhanced cross-priming (immunity).

It has been suggested that the death of tissue cells during normal cell turnover followed by their uptake by resident DCs is involved in the maintenance of peripheral tolerance (Luckashenak et al., 2008). Several studies have addressed this issue with systems where model antigens were overexpressed in defined cells from birth such that they were considered "self-antigens" by the immune system. In these instances, potent tolerance could be induced to these experimental self-antigens (Adler et al., 1998; Kurts et al., 1998). Similarly, experiments with nontransformed cells such as pancreatic islets demonstrate that apoptotic death can make these cells efficient tolerogens. Young nonobese diabetic (NOD) mice injected with a single low dose of streptozotocin exhibited impaired T cell responses and mice were protected from spontaneous diabetes. β-cell apoptosis was necessary for this tolerance, because streptozotocin did not protect rat insulin promoter-cytokine response modifier A (RIP-CrmA) transgenic NOD mice (Hugues et al., 2002). Similarly, DCs fed apoptotic islet cells induced potent tolerance and prevented the development of diabetes in the recipients when injected (Marin-Gallen et al., 2010). The death of T cells during activation-induced cell death (AICD) can also induce potent tolerance, not just by deleting the reactive cells but also by generating active regulatory cells (Herndon et al., 2005; Gurung et al., 2010). In this situation, tolerance was directed only to T cells of the same specificity, perhaps to control potential autoimmunity during the contraction phase of an immune response where large numbers of antigen-reactive T cells were deleted. In a similar manner, treatment of normal mice with intact antibody to CD3 (which induces tolerance) increases systemic TGF-β produced by Mϕ and immature DC phagocytes exposed to apoptotic T cells leading to immune suppression (Perruche et al., 2008). In contrast, in vitro anti-CD3-activated normal T cells induced to undergo apoptosis are immunogenic, not tolerogenic—an effect mediated by CD154 expression on the activated T cell (Gurung et al., 2009). Similarly, activated γ-irradiated peripheral blood mononuclear cells (PBMC) will mature human DCs, leading to the production of proinflammatory cytokines (Johansson et al., 2007). The question then becomes why do apoptotic T cells in some cases induce tolerance but in others promote immunity? The answer may lie with the timing of the T cell death (see "When" below). Perhaps when small numbers of dying cells arise during normal turnover they are ignored by the immune system. Regardless, different types of dying cells can induce dramatically different immune responses, making it

difficult to predict the outcome and leading us to consider *what* are the dying cells releasing as the next important factor.

What It Releases: Tolerogenic and Immunogenic Factors Produced by Dying Cells

Several mechanisms have been proposed to explain the intrinsic tolerogenic and immunogenic potential of dead cells, including the elaboration of cytokines, DAMPs, and other cellular proteins. Apoptotic lymphocytes produce the immunosuppressive cytokines IL-10 (Gao et al., 1998) and TGF-β (Chen et al., 2001) as they die. In contrast, DAMPs, such as HMGB1 (Kazama et al., 2008; Scaffidi et al., 2002), heat shock proteins (e.g., HSP70) (Millar et al., 2003), uric acid (Shi et al., 2003), mammalian DNA, RNA, IFN-α (Matzinger, 2002), and CD154 (Gurung et al., 2009), released by dead cells are immunogenic. Recognition of DAMPs (e.g., HMGB1) by pattern recognition receptors (PRR) such as the receptor for advance glycation end products (RAGE) or toll-like receptors (TLRs) is thought to mature DCs and stimulate immunity (Bianchi, 2007). In addition, necrotic cells can activate the inflammasome (immunity), as shown by the cleavage of caspase 1 and release of mature IL-1β and IL-18 (Lamkanfi and Dixit, 2010; Li et al., 2009). Thus, the different mechanisms of death along with the production and/or release of various pro- and anti-inflammatory molecules as a result of the death process all contribute to whether immunological tolerance or immunity is initiated against the antigens associated with the dead cells.

Interestingly, the release of "factors" is not always predictive because the cell death process itself can alter the immunogenicity of the molecules released. All apoptotic cells can release DAMPs (Choi et al., 2004; Kazama et al., 2008), but the process of apoptosis can modify these immunostimulatory molecules to promote tolerance instead of immunity (Kazama et al., 2008). For example, intravenously injected necrotic cells promote antigen-specific immune responses through a mechanism that involves the release of HMGB1; in the absence of active HMGB1, these cells induce tolerance. Similarly, apoptotic cells can be immunogenic rather than tolerogenic if caspase activation is blocked or caspase-3 and -7 are absent. This seems to contradict the requirement for caspase activation in immunogenic cell death by anthracyclin-treated tumor cells (Zitvogel et al., 2004) (discussed above), but perhaps factors such as the location of the dying cells have an effect (see "Where" below). This caspase requirement was explained by examining the consequences of caspase activation to the cell. During apoptosis, a loss in mitochondrial membrane potential allows for the release of cytochrome c, which further facilitates caspase activation. The active effector caspases then cleave a component of complex I in the electron transport chain (NADH dehydrogenase [ubiquinone] Fe-S protein 1, 75 kDa [NDUFS1]) in the permeabilized mitochondria. The resulting inhibition of complex I function induces the production of reactive oxygen species (ROS) (Ricci et al., 2004), which oxidizes a key cysteine residue in HMGB1 and neutralizes its ability to stimulate immunity. Mutation of the caspase cleavage site in NDUFS1 does not block apoptosis, but apoptotic cells that express this mutant protein promote immunity rather than tolerance. Thus, one important factor in determining whether tolerance or immunity will ensue is whether HMGB1 (and perhaps other DAMPs) is (are) modified in the dying cells (Kazama et al., 2008). One prediction would be that during immunogenic cell death (e.g., after some types of chemotherapy [Apetoh et al., 2007]), ROS is not produced and DAMPs remain unmodified; however, this hypothesis remains to be tested. It is not currently known how HMGB1 modification influences its ability to bind receptors or other molecules. HMGB1 has been shown to bind to several cytokines (IL-1β, TNF-α, and IFN-γ) as well as free nucleic acids and the PRR mentioned above (RAGE, TLR) (Ferguson et al., 2011). It is possible that redox modification of HMGB1 influences its ability to bind one of these molecules. However, these data highlight the importance of caspase activation in determining tolerogenicity or immunogenicity. In support of this it was suggested that caspase-enhanced presentation could be important for the pathogenesis of human immunodeficiency virus-1 (HIV-1) because a high frequency of effector $CD8^+$ T cells that recognize caspase-cleaved epitopes are in the peripheral blood of HIV-1-infected individuals, and the frequency of these effector $CD8^+$ T cells correlates with the frequency of apoptotic $CD4^+$ T cells (Rawson et al., 2007). Thus, simply considering the factors released from dying cells is also an inadequate way to predict their tolerogenic or immunogenic potential, leading us to consider *when* a cell dies.

When It Dies: Influence of the Timing of Cell Death on Tolerance and Immunity

As mentioned earlier, the activation state of the cell when it dies (especially T cells) can dramatically influence its immunogenicity or tolerogenicity. Anti-CD3-activated T cells express CD154, which can change a normally tolerogenic naive apoptotic T cell into a potent immune stimulator (Gurung et al., 2009). In these studies, CD154 expression induced DC production of IL-12 and resulted in immunity. This observation contrasts with the tolerogenic nature of T cells that undergo AICD (Gurung et al., 2010; Herndon et al., 2005). Perhaps early during immune activation the balance between the $CD154^+$ and the $CD154^-$ T cells dictates the fate of the response. For instance, during an acute infection, the high numbers of CD154-expressing T cells may help maintain a high threshold of inflammation and immune responses required to clear the pathogen. However, during AICD, which occurs toward the conclusion of an immune response, the T cells may no longer express CD154 and tolerance ensues. This permits removal of reactive cells and suppression of potential anti-self immune responses. Figure 1 is a representation of the differential effects of naive versus activated apoptotic T cells on the immune response. It should be noted that this tolerance is relatively short lived (~60 days) and the system can then respond to future antigen challenge (Gurung et al., 2010). It remains to be determined whether immunological memory occurs in this situation.

Another consideration is the stage of cell death *when* the corpses encounter the immune system. Rapid removal of early apoptotic cells prevents immune stimulation, and failure to remove dead cells can lead to autoimmunity (Asano et al., 2004; Hanayama et al., 2004; Ip and Lau, 2004). Apoptotic cells need to be promptly recognized and cleared to avoid potential leakage of inflammatory cytoplasmic contents. When dying cells encounter DCs very early in the cell death process (<2 hr after induction of apoptosis), they are immunogenic, as are dead cells that have progressed to late stage apoptosis (>12 hr; a.k.a.

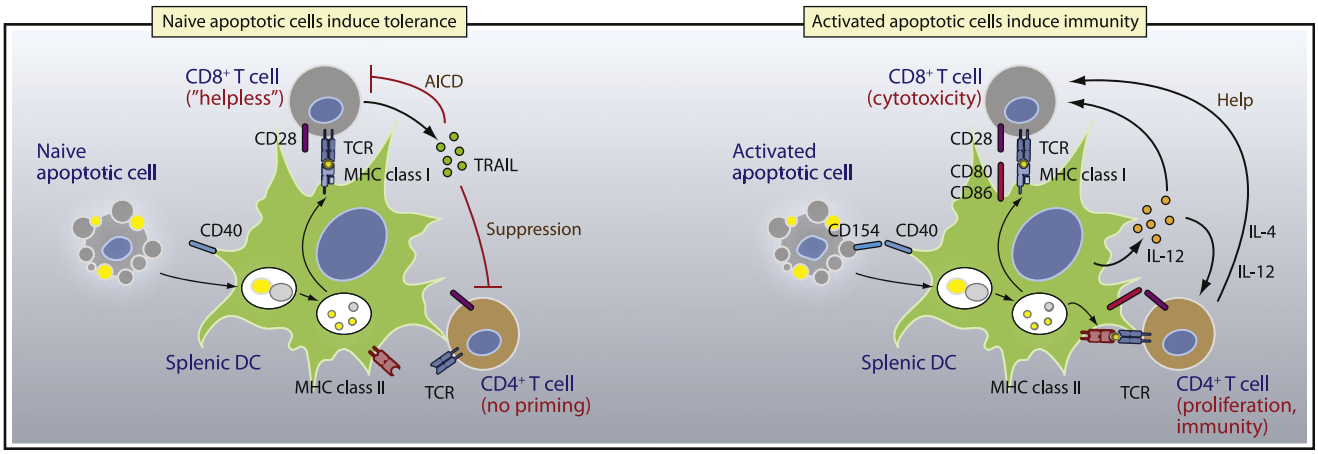

Figure 1. Proposed Mechanism of Tolerance or Immunity Induced by Naive or Activated Apoptotic T Cells
Left: Induction of tolerance by naive apoptotic T cells. Naive apoptotic T cells are taken up by DCs, which remain in an immature state. The antigens derived from these apoptotic T cells are cross-presented on MHC class I to $CD8^+$ T cells in the absence of costimulatory molecules and $CD4^+$ T cell help. These "helpless" $CD8^+$ T cells upregulate TRAIL expression and then go on to suppress subsequent immune responses mediated by $CD4^+$ T cells. Right: Induction of immunity by activated apoptotic T cells. CD154-expressing, activated apoptotic T cells activate DCs to upregulate costimulatory molecules (CD80, CD86) and produce proinflammatory cytokines such as IL-12. Antigens derived from the activated apoptotic T cells are cross-presented on MHC class I and directly presented on MHC class II on matured DCs, resulting in the priming of both $CD8^+$ and $CD4^+$ T cells. Consequently, the proinflammatory cytokines and $CD4^+$ T cell help the $CD8^+$ T cells to fully differentiate into effector CTL instead of a helpless, TRAIL-expressing, $CD8^+$ Treg cells.

secondary necrosis) because they can leak HMGB1 (Scaffidi et al., 2002). For optimum tolerogenicity, DCs must encounter apoptotic corpses approximately 4–8 hr after the induction of death; presumably this is due to the need for caspase activation, expression of "find me and eat me" signals, and DAMP modification by ROS.

Where It Dies: Influence of the Anatomical Location of Death on Immunity and Tolerance

Although *who*, *what*, and *when* are important considerations in our discussion, *where* the corpses are removed from the body can directly determine the APC and/or phagocytic cell involved in stimulating the immune system. Apoptotic cells are typically phagocytized by Mϕ and DCs, and apoptotic cells elaborate a variety of "find me and eat me" signals that direct phagocyte attraction and engulfment (Green et al., 2009). Phosphatidylserine (PS) present on apoptotic cells play important roles in their efficient clearance, and a number of cellular receptors expressed by phagocytes, such as CD36, T cell immunoglobulin domain, mucin-like domain (Tim)-1, and Tim-4, facilitate the uptake of apoptotic cells (Miyanishi et al., 2007; Miyasaka et al., 2004; Peng et al., 2007). Other receptors, including the integrins $\alpha v\beta 3$ and $\alpha v\beta 5$, Class B scavenger receptors, ATP-binding cassette transporter (ABC1), CD14, receptor tyrosine kinases c-MER, TYRO, and Axl, CD91 (receptor for heat shock protein gp96), and signal regulatory protein (SIRP)-α, have also been shown to play various roles in the recognition and clearance of apoptotic cells (Peng et al., 2007). A complete discussion of these molecules is found in an accompanying review (Ravichandran, 2011, this issue) and these issues will not be discussed further. However, it is clear that the exposure of these molecules is the result of the cell death process (e.g., caspase activation) and is critical for apoptotic cell clearance, and that failure to remove apoptotic bodies typically leads to inflammation and/or autoimmunity. Indeed, autoimmunity results when Mϕ uptake of apoptotic cells is compromised by interfering with specific "dead cell" receptors (e.g., Tim4 or milk fat globule-EGF factor 8 [MFG-E8]). Interestingly, only autoantibodies were induced in these cases of autoimmunity and anti-self T cell responses were not detected (Hanayama et al., 2004; Miyanishi et al., 2007; Miyasaka et al., 2004). This unexpected outcome may be related to evidence showing that Mϕ are incapable of cross-priming despite their profound phagocytic capabilities (Albert et al., 1998). Thus, it would appear that the consequences of dead cell phagocytosis on Mϕ or DCs may be quite different immunologically.

DCs that engulf apoptotic cells can cross-present self-antigens to T cells and induce tolerance (a.k.a. "cross-tolerance"), such that cross-tolerance has become a central concept in self tolerance (Albert, 2004). Evidence suggests that several DC subsets exist that perform different functions depending on their lineage or regional localization (i.e., where they reside). For example, splenic $CD8\alpha^+$ DCs promote tolerance whereas $CD8\alpha^-$ DCs promote immunity (den Haan et al., 2000). Yet, $CD8\alpha^+$ DCs are also potent inducers of antiviral immunity (Allan et al., 2003), and antiviral T cell responses to herpes simplex virus-1 (HSV-1) infection are more robust when antigens derived from the infected apoptotic cells are cross-presented on major histocompatibility complex (MHC) class I rather than when those antigens are presented by direct infection of the DC (Bosnjak et al., 2005). Such observations suggest that the location of the uptake of dying cells, rather than the type of DC, may better dictate the immunological outcome. For example, $CD8\alpha^+$ DCs in the skin could promote immunity (cross-priming) because these cells typically migrate to the regional lymph nodes whereas these same DCs that reside in the spleen stimulate tolerance (cross-tolerance). Interestingly, the spleen is not the only location that appears to be involved in tolerance induction by dying cells.

Lymphoid cells that die in vivo tend to accumulate in the liver (Huang et al., 1994), and this organ has also been implicated as a site for tolerance induction (Crispe et al., 2006).

Although many studies investigating the uptake of apoptotic cells and their influence on immune function have focused on Mϕ (Miyake et al., 2007), DCs can phagocytize apoptotic cells (Albert et al., 2001) and apoptotic cells can certainly suppress DC production of proinflammatory cytokines such as IL-12 (Kim et al., 2004). That is why it is essential to consider the region of the body *where* the dead cells are engulfed because Mϕ and DCs are preferentially concentrated in different anatomical locations. For example, the absence of splenic marginal zone Mϕ delays the clearance of apoptotic cells and promotes immunity via DC antigen presentation (Miyake et al., 2007), perhaps by overwhelming immunostimulatory DCs with cellular debris. It is also well established that antigen-coupled cells injected intravenously induce a state of immune tolerance (Battisto et al., 1980; Conlon et al., 1980) through a process that involves apoptosis of the injected cells (Ferguson et al., 2002). However, subcutaneous injection of the same cells induces immunity (Greene and Benacerraf, 1980), and most studies of immunogenic apoptosis involve injection by this route. Subcutaneous injection of cells leads to their engulfment by skin-derived DCs that ultimately traffic to LN to induce an immune response. This may mimic the effect of tumors that are implanted into subcutaneous sites and undergo apoptosis after chemotherapy (Apetoh et al., 2007; Chaput et al., 2007).

Although the main player for tolerance or immunity is the DC, there are no studies (to our knowledge) describing how the "find me and eat me" signals promote DC uptake of dead cells and then participate in the induction of tolerance or immunity. In fact, although phagocytosis of dead cells by DCs has been studied, the requirement for DC phagocytosis via one of the known PS receptors for tolerance is also largely unexplored. Deletion of the MFG-E8 receptor in mice results in autoantibody production but also leads to enhanced $CD8^+$ CTL cross-priming (i.e., immunity) (Peng and Elkon, 2011). This puzzling observation, as well as the role of "find me and eat me" signals for tolerance or immunity mediated through DCs, requires further investigation.

It is also noteworthy that there appears to be a difference in antigen processing intrinsic to DC subsets that is associated with increased expression of proteins involved in MHC processing (Dudziak et al., 2007). $CD8\alpha^+$ DCs tend to process antigens for presentation via MHC class I molecules, whereas $CD8\alpha^-$ DCs preferentially present antigens via MHC class II. This suggests that for tolerance, $CD4^+$ T cell immunity may be diminished whereas $CD8^+$ T cell immunity is promoted, resulting in "helpless" CTL induction (see below). In one study, $CD8\alpha^+$ DCs preferentially phagocytized apoptotic cells, again suggesting a tolerogenic role for this DC subpopulation (Iyoda et al., 2002). However, there are other data showing that $CD8\alpha^+$ DCs are no better at phagocytizing apoptotic cells than are $CD8\alpha^-$ DCs (Schnorrer et al., 2006), suggesting that phagocytosis cannot be the sole criterion for tolerance or immunity.

There are also a number of other consequences for the DC after an encounter with apoptotic cells that can have implications for the type of immune response induced. It is generally accepted that DC maturation through interaction with PAMPs or DAMPs, as measured by increased MHC class II and costimulatory molecule (e.g., CD80, CD86) expression, is critical for the induction of immunity. Several reports have documented that apoptotic cells can prevent DC maturation, keeping them immature and in a tolerance-inducing state (Albert et al., 2001; Sauter et al., 2000). This is a compelling idea, but it should be noted that this is not always the case because mature DCs can also induce tolerance subsequent to engulfment of apoptotic cells (Ferguson et al., 2002; Kazama et al., 2008). Thus, simple maturation cannot be the determining factor and this may be related to other factors discussed here such as DC localization (*where*) and the properties of apoptotic cells (*what*).

Why It Dies: Influence of Infection and Tissue Damage

Why cells die can have a strong influence on the subsequent immune response, especially if the cells are dying as the result of an infection. Phagocytosis of apoptotic cells in the presence of TLR ligands (PAMPs) derived from infectious agents can convert the tolerogenic signals from the apoptotic cells to immunogenic ones by increasing the activation status of phagocytic cells and changing the inflammatory cytokines they elaborate. For example, infected apoptotic cells are a critical component of the innate immune signals instructing Th17 cell differentiation (Torchinsky et al., 2009), suggesting that pathogens particularly adept at triggering apoptosis might preferentially induce T cell-mediated immunity. Similarly, apoptotic vesicles from mycobacterial-infected Mϕ stimulate $CD8^+$ T cell immunity in vivo. In this system, the apoptotic vesicles displayed potent adjuvant activity by stimulating protection against *M. tuberculosis* infection via TLR (Winau et al., 2006). Likewise, *Histoplasma*-specific $CD8^+$ T cell immunity could also be induced by DCs that present exogenous Histoplasma antigens, either through direct ingestion of the yeast cells or through uptake of apoptotic Mϕ-associated fungal antigens (Lin et al., 2005).

Inflammation and cell death also occur during tissue damage under sterile conditions, leading to the release of cytoplasmic contents (i.e., DAMPs) and the activation of immunity. The best examples of this are the recent studies examining uric acid (Shi et al., 2003) and ATP release from dying cells. Uric acid is the final product of purine metabolism and its causative role in gout is well established, because this painful inflammatory condition is the result of uric acid crystals precipitating in joints and capillaries (Rock and Kono, 2008). The association between uric acid with other forms of inflammation and immune regulation was not apparent until it was found to serve as an adjuvant in CTL responses against particulate antigens (Shi et al., 2003). Thus, uric acid released from damaged cells, without an associated pathogen, can alert the body to danger and stimulate inflammation. Similarly, the release of nucleotides (including ATP and UTP) from damaged neurons in mice attracts microglial cells (Mϕ-like phagocytes in the brain) to sites of tissue damage (Hanley et al., 2004; Koizumi et al., 2007). In addition, ATP released from dying cells is now recognized as an important "find me" signal for phagocytic cells (Elliott et al., 2009). ATP also stimulates the inflammasome and in association with TLR ligand stimulation can result in the release of IL-1β, IL-18, and HMGB1 (Lamkanfi and Dixit, 2010). Attraction of phagocytic cells and inflammasome activation result in the induction of immunity.

It should be noted that the presence of PAMPs (as well DAMPs) is not always predictive of robust immunity.

Inflammatory cells responding to a viral infection in the eye undergo apoptosis (Griffith et al., 1995), but even in the presence of viral antigens, nucleic acids, and ample DCs for presentation (McMenamin, 1999; Steptoe et al., 2000), these dead cells induce systemic immune tolerance to the viral antigens (Griffith et al., 1996). In this case, apoptosis and tolerance function to protect the delicate structures of the eye from the damaging effects of inflammation, thereby preserving vision. Similar tolerogenic effects of apoptotic cells were observed in an experimental model of polymicrobial sepsis where sepsis-induced lymphocyte apoptosis decreased the survival of the infected host. In this model, preventing apoptosis via caspase inhibitors or transgenic *Bcl-2* expression significantly improved survival (Hotchkiss et al., 1999a, 1999b, 2000). Further studies revealed that the presence of infection-induced apoptotic cells also induced potent immune tolerance for a subsequent T cell response (Unsinger et al., 2010). Thus, it would seem that the immunostimulatory potential of the PAMPs elicited by the infection can be overcome by the presence of large numbers of apoptotic cells. This could be considered a detrimental effect of apoptotic cell-induced tolerance because immunosuppressed septic patients are prone to secondary nosocomial infections (Hotchkiss et al., 1999b).

How Is Immunity Regulated: A Synthesis of the Five Ws

The immune system has devised multiple means by which immunological tolerance is maintained. Thymic selection removes the vast majority of developing thymocytes that express a TCR with high specificity for self-peptide:MHC complexes, but some self-reactive T cells still make it through the selection process (Sprent and Kishimoto, 2002). The elimination of peripheral cells capable of responding to self-antigens or antigens associated with apoptotic cells is one means by which peripheral tolerance can be established, with FasL-dependent AICD and Bim-mediated death being key pathways of establishing and/or maintaining peripheral tolerance (Green et al., 2003; Hildeman et al., 2002; Pellegrini et al., 2003). The timely elimination of lymphocytes—especially those immune cells that can respond to self-antigen—is one way to prevent their accumulation and persistence. Autoimmune lymphoproliferative syndrome (ALPS) exemplifies how disruption of the Fas signaling pathway leads to the massive accumulation of autoreactive lymphocytes that would normally be eliminated via deletion and the development of a variety of autoimmune pathologies (Straus et al., 1999). One would expect, though, that tolerance resulting from lymphocyte deletion would be transient because the deleted cells should eventually be replenished. Indeed, deletional tolerance wanes over time, as seen in models of soluble peptide antigen-induced peripheral T cell deletion, but the process of T cell expansion and contraction after the injection of soluble peptide antigen also generates Treg cells that can maintain tolerance for an extended period—even after a second infusion of antigen-specific effector T cells (Gurung et al., 2010; Herndon et al., 2005).

Work by Gershon and Kondo (1970, 1971) pioneered the idea that immune tolerance was actively mediated by regulatory or suppressive populations of T cells that could also transfer tolerance to a naive individual (termed "infectious immunologic tolerance"). Interestingly, the immune cells responsible for transferring immunologic tolerance in these studies were $CD8^+$ T cells (Cantor et al., 1976; Gershon et al., 1972); however, the inability to successfully isolate and identify these $CD8^+$ Treg cells led to skepticism about their true existence despite the consistent reproducible observation of the suppressive activity of these cells. The description of $CD4^+CD25^+Foxp3^+$ Treg cells in the 1990s revived the idea that T cells are potent regulators of immunity (Takahashi et al., 1998). There have been numerous reports describing the basic characteristics of $CD4^+CD25^+Foxp3^+$ Treg cells, and immune regulation by Treg cells has been implicated in numerous experimental model systems, including transplantation and autoimmunity (reviewed in Rudensky, 2011; Sakaguchi et al., 2006). Apoptotic cell infusion or the in vivo depletion of T cells via CD3-specific mAb results in the expansion of $CD4^+CD25^+Foxp3^+$ Treg cells associated with the production of TGF-β from Mϕ and DCs that phagocytize the apoptotic cells (Kleinclauss et al., 2006; Perruche et al., 2008). The increase in $CD4^+CD25^+Foxp3^+$ Treg cells in this system is also related to the selective depletion of $CD4^+Foxp3^-$ conventional T cells (Penaranda et al., 2011). In contrast, the recognition of apoptotic cells in the context of inflammation can direct helper T cell differentiation in a very different direction that supports productive (and sometimes pathogenic) immunity. Specifically, phagocytosis of bacterially infected apoptotic cells (i.e., TLR ligand-expressing apoptotic cells) drives IL-6, IL-23, and TGF-β production by DCs leading to the induction of Th17 cells. Blocking apoptosis prevents the development of a Th17 cell-mediated immune response needed to clear the infection (Torchinsky et al., 2009). This finding is very important when one considers that self-reactive Th17 cells are prominent players in a number of autoimmune diseases (Hu et al., 2011; Jäger and Kuchroo, 2010), making it tempting to speculate that the autoimmunity was triggered by substantial apoptotic death during a time of infection or inflammation. This contrasts the tolerogenic effects of "substantial" apoptosis during sepsis (Hotchkiss et al., 1999b; Unsinger et al., 2010), indicating that further study of these mechanisms is required.

DCs can cross-present antigens associated with apoptotic cells by MHC class I molecules to $CD8^+$ T cells (cross-priming) (Albert et al., 1998). Perhaps paradoxically, the induction of tolerance by apoptotic cells also depends on MHC class I and can involve the deletion of $CD8^+$ T cells as well as immune suppression by $CD8^+$ Treg cells (cross-tolerance) (Ferguson et al., 2002; Heath and Carbone, 2001; Steinman et al., 2003). Reconciling cross-priming and cross-tolerance provides insights into one mechanism of immunity or tolerance induction by dying cells. After antigen recognition by $CD8^+$ T cells and their development into CTL, the long-term fate of these cells is determined by additional signals provided by DCs, which must be "licensed" by a previous CD40-CD154-mediated interaction with activated $CD4^+$ T cells (Schoenberger et al., 1998). Without this additional signal, the activated helpless CTL function as primary effector T cells but with a short lifespan (Sun and Bevan, 2003) or they die as a result of AICD after subsequent exposure to antigen (Janssen et al., 2005, 2006). AICD in this instance is mediated by TNF-related apoptosis-inducing ligand (TRAIL), which triggers apoptosis in the helpless CTL and other activated T cells. The relationship between these observations and the induction of tolerance (cross-tolerance) by apoptotic cells became clear

in a recent series of experiments. Specifically, DCs that had engulfed necrotic cells could present antigen to both $CD4^+$ and $CD8^+$ T cells, but those that engulfed apoptotic cells presented antigen only to $CD8^+$ T cells (Griffith et al., 2007). Consequently, the $CD8^+$ T cells produced TRAIL after re-exposure to antigen, which inhibited the induction of a cell-mediated immune response mediated by $CD4^+$ T cells—a phenomenon we have noted in other experimental models of tolerance (Griffith et al., 2011; Gurung et al., 2010). Thus, exposure of the immune system to apoptotic cells can shift the development of classical "helped" CTL-mediated immune responses to those dominated by tolerogenic, helpless CTL that produce TRAIL after re-exposure to antigen (see Figure 1). Although one effect of apoptotic cells on DC function is to prevent activation of $CD4^+$ T cell help for CTL, in some cases DCs that have engulfed apoptotic cells can drive $CD4^+$ T cell differentiation toward the Th2 cell type via the production of IL-10 (Gao et al., 1998). We do not know whether such Th2 cells can license DCs to promote $CD8^+$ T cell immunity, but if not, such polarization may further promote the generation of helpless $CD8^+$ T cells to suppress immune responses.

Can This Information Be Exploited for Therapeutic Purposes?

One of the most important considerations subsequent to our discussion of the five Ws is how can we take what we have learned to date and apply it therapeutically. In some cases, it will be critical to break the tolerance induced by the apoptotic cells to restore immunity. One recent example that fits this concept used the mouse model of sepsis induced by cecal ligation and puncture, which leads to the induction of tolerance, but also included a secondary heterologous bacterial infection subsequent to sepsis initiation. Septic mice had a reduced ability to control the secondary infection, which was paralleled by suppressed T cell responses, versus sham-treated control mice. Administration of a blocking TRAIL mAb to the septic WT mice restored the ability to control the secondary infection and generate Ag-specific $CD8^+$ T cell responses like those seen in sham-treated mice (Gurung et al., 2011). A second example is the use of apoptotic tumor cells in cancer vaccines, which is becoming more accepted as a viable immunotherapy option in some cancer patients. The GVAX platform of cancer immunotherapy utilizes a vaccine consisting of irradiated whole tumor cells that have been modified to secrete granulocyte macrophage-colony stimulating factor (GM-CSF), which helps recruit and mature the APC that phagocytize the injected apoptotic tumor cells (Hege et al., 2006).

It may also be desirable to use apoptotic cells to deliberately establish tolerance. Organ transplantation has long appreciated the powerful tolerogenic potential of apoptotic cells (Kleinclauss et al., 2003, 2006; Li et al., 2006; Morelli and Larregina, 2010), and it is clear that the alterations in APC function and the generation of regulatory cells occurs in transplant recipients given an infusion of apoptotic cells therapeutically. The use of apoptotic cells to prevent autoimmunity has also been reported. Specifically, intravenous injection of myelin oligodendroglial glycoprotein (MOG)-expressing apoptotic cells reduced MOG-specific T cell responses and prevented the development of experimental autoimmune encephalomyelitis (EAE) (Miyake et al., 2007). Similar results were found in another model of experimentally induced disease (Smith and Miller, 2006). The therapeutic use of apoptotic cells in this fashion is very exciting, but it is important to remember that the apoptotic cells were given prophylactically (prior to the induction of EAE), and it remains to be seen whether the same therapeutic benefit would be seen in settings where autoimmunity was already established.

Extracorporeal photopheresis has been used clinically for almost 20 years as an approved therapy for the treatment of cutaneous T cell lymphoma (Dupont and Craciun, 2009). For this treatment, peripheral blood is treated ex vivo with a photoactivatable compound (8-methoxypsoralen) and UVA light and immediately returned to the patient. A similar strategy is being tested for the treatment of graft-versus-host disease (Hannani et al., 2010). Although mechanisms are not completely clear, tolerance is thought to be the result of apoptosis in the treated leukocytes followed by uptake by the patient's phagocytes leading to modulation of the immune response and decreased severity of the disease.

Another area of potential therapeutic intervention is related to the recent findings with HMGB1 in which the redox status of the protein determines its immunogenicity. When HMGB1 is released from apoptotic cells, it is oxidized and tolerance to antigen associated with the apoptotic cells develops. In contrast, when HMGB1 was reduced by treatment of the apoptotic cells with antioxidants or when a form of HMGB1 was used that could not be oxidized (changing HMGB1 Cys106 to Ser), it promoted immunity to the same antigen (see above discussion and Kazama et al., 2008). Because HMGB1 is thought to mediate inflammation in a number of pathogenic processes including septic shock (Bianchi, 2007), perhaps this is related to its redox status. The oxidative conditions generated during sepsis (Roth et al., 2004) may oxidize HMGB1 such that in the presence of apoptotic cells, tolerance ensues. Thus, treatment of immunosuppressed individuals with a nonoxidizable form of HMGB1 might be a method to overcome the immunosuppression induced by apoptotic cells promoting beneficial adaptive immune responses.

Concluding Thoughts

It is safe to say that in all aspects of life, organisms must deal with death. Although there is no immunological consequence associated with death at the end of life, from birth our immune systems must deal with continuous exposure to dying and dead cells. Therefore, understanding how the mammalian immune system deals with the myriad of antigens associated with dying and dead cells can have important implications for the study of cancer biology, infectious diseases, tissue injury, and autoimmunity. At this point we have to ask ourselves why the effects of cell death on the immune system are so complex. When the complexity of the vertebrate immune system was increased to deal with pathogens, it also increased the chances of eliciting autoimmune reactions (Green et al., 2009). Failing (or delaying) to remove dying cells can be highly inflammatory and activate autoimmunity, but the mechanisms of dead cell removal are highly conserved in animals with no adaptive immune system. Because pathogens evolved in parallel to avoid detection, the immune system once again adapted to deal with these invaders by incorporating the more primitive pathogen-sensing mechanisms into the adaptive

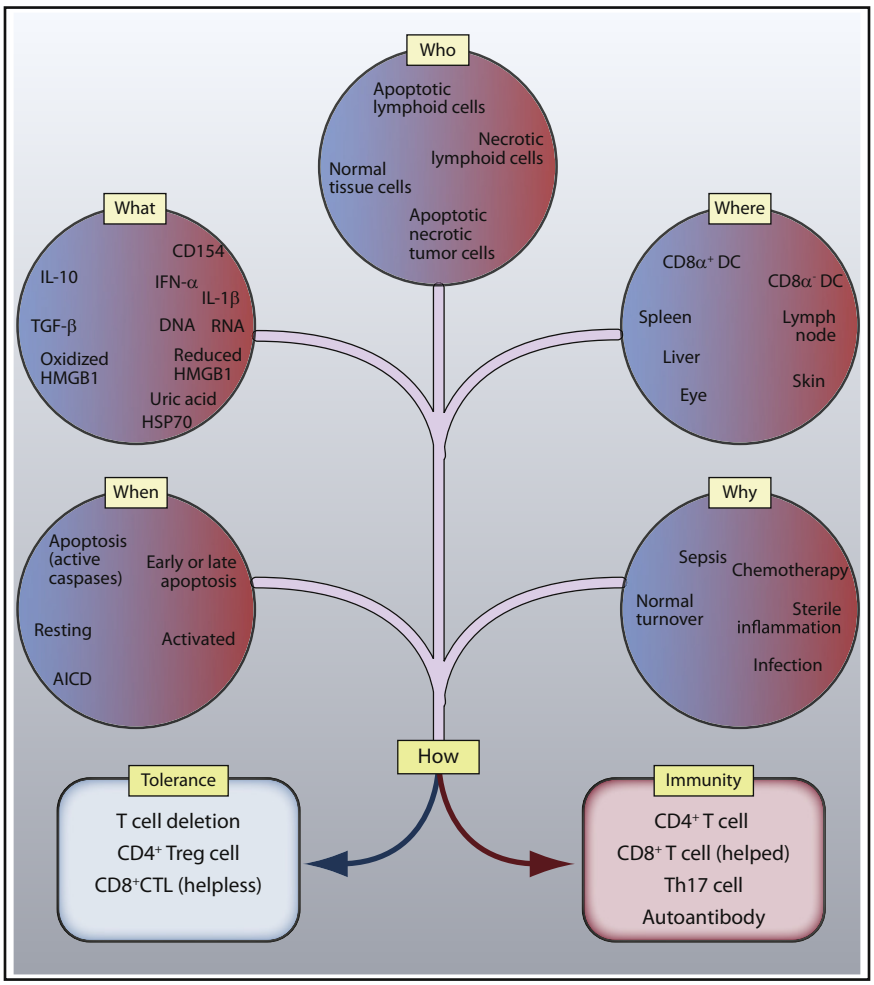

Figure 2. Predicting the Immunological Outcome via the Five Ws

Each petal represents one of the five Ws and contains some of the factors considered in this review. The color gradient indicates a spectrum from tolerogenic (blue) to immunogenic (red). It is important to note that any element in a particular petal may be linked to any element in the other petals, resulting in a very large number of potential permutations. For example, a resting (*when*) lymphocyte (*who*) induced to die during sepsis (*why*) releases IL-10 (*what*) and is then engulfed by a splenic CD8α^+ DC (*where*) and will induce immune tolerance via a helpless CD8$^+$ T cell that makes TRAIL (*how*). Also consider another example: an apoptotic (*when*) normal tissue cell (*who*) infected with a virus (*why*) releases reduced HMGB1 (*what*) as it dies. The infected apoptotic cell is then engulfed by a CD8α^+ DC in the skin (*where*), leading to cross-priming and activation of virus-specific CTL-mediated immunity (*how*).

immune response (Medzhitov and Janeway, 2002). However, dead cells cannot always be immunogenic because this might lead to autoimmunity, and they certainly cannot always be tolerogenic because this might prevent adaptive responses against the invaders. Thus, the immune system is constantly evaluating cell death based on many of the criteria discussed here to stay ahead of infectious agents, promoting survival of the organism.

To better understand the issues involved in determining the immunogenic or tolerogenic nature of dead cells, we have applied the investigative technique called the five Ws. With this formula we have tried to get the "full story" to help us predict the outcome of the encounter between dead cells and the immune system. The principle underlying the maxim is that each question should elicit a factual answer, and importantly none of these questions can be answered with a simple "yes" or "no." The result of an encounter between dead cells and the immune system depends on factors related to *who* dies, *what* it releases, *when* it dies, *where* it dies, and *why* it dies to collectively determine *how* immunity is regulated. Figure 2 is our interpretation of a five Ws flower petal diagram commonly used in this analysis. We hope this diagram can make some sense out of the complexities involved by determining the contribution of each petal (the five Ws) and applying the result to the immune response (*how*). Because of space limitations, we have limited our discussion to the effects of cell death on T cell responses; however, we hope that these ideas can be applied to understating the nature of B cell response and factors that induce B cell tolerance. Although we probably don't have enough information to be precise in every case, we can keep these in mind as we continue to study this important area of immunology. We are reminded of Mark Twain's comment on naming constellations in *Following the Equator: A Journey Around the World* (1897): "Constellations have always been troublesome things to name. If you give one of them a fanciful name, it will always refuse to live up to it; it will always persist in not resembling the thing it has been named for." Hopefully with more study, we can be more precise in determining the constellation of immunological responses in the presence of cell death.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health grants AI077565 (T.S.G.), CA109446 (T.S.G.), EY06765 (T.A.F.), EY015570 (T.A.F.), and EY02687 (Department of Ophthalmology and Visual Science Core Grant, Washington University, St. Louis, MO). Additional support was obtained from a University of Iowa Carver College of Medicine Medical Research Initiative Grant (T.S.G.) and a Department of Ophthalmology and Visual Science grant from Research to Prevent Blindness (New York, NY) (T.A.F.) and the Macular Vision Research Foundation (West Conshohocken, PA) (T.A.F.). The authors wish to express their sincere thanks to J. Choi and P. Gurung for designing the figures.

REFERENCES

Adler, A.J., Marsh, D.W., Yochum, G.S., Guzzo, J.L., Nigam, A., Nelson, W.G., and Pardoll, D.M. (1998). CD4+ T cell tolerance to parenchymal self-antigens requires presentation by bone marrow-derived antigen-presenting cells. J. Exp. Med. *187*, 1555–1564.

Albert, M.L. (2004). Death-defying immunity: do apoptotic cells influence antigen processing and presentation? Nat. Rev. Immunol. *4*, 223–231.

Albert, M.L., Pearce, S.F., Francisco, L.M., Sauter, B., Roy, P., Silverstein, R.L., and Bhardwaj, N. (1998). Immature dendritic cells phagocytose apoptotic cells via alphavbeta5 and CD36, and cross-present antigens to cytotoxic T lymphocytes. J. Exp. Med. *188*, 1359–1368.

Albert, M.L., Jegathesan, M., and Darnell, R.B. (2001). Dendritic cell maturation is required for the cross-tolerization of CD8+ T cells. Nat. Immunol. *2*, 1010–1017.

Allan, R.S., Smith, C.M., Belz, G.T., van Lint, A.L., Wakim, L.M., Heath, W.R., and Carbone, F.R. (2003). Epidermal viral immunity induced by CD8alpha+ dendritic cells but not by Langerhans cells. Science *301*, 1925–1928.

Apetoh, L., Ghiringhelli, F., Tesniere, A., Obeid, M., Ortiz, C., Criollo, A., Mignot, G., Maiuri, M.C., Ullrich, E., Saulnier, P., et al. (2007). Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat. Med. *13*, 1050–1059.

Asano, K., Miwa, M., Miwa, K., Hanayama, R., Nagase, H., Nagata, S., and Tanaka, M. (2004). Masking of phosphatidylserine inhibits apoptotic cell engulfment and induces autoantibody production in mice. J. Exp. Med. *200*, 459–467.

Barreiro, R., Luker, G., Herndon, J., and Ferguson, T.A. (2004). Termination of antigen-specific immunity by CD95 ligand (Fas ligand) and IL-10. J. Immunol. *173*, 1519–1525.

Battisto, J.R., Butler, L.D., and Wong, H.L. (1980). Use of hapten-altered self moieties to probe the cell-mediated lympholytic response and immunotolerance interface. Immunol. Rev. *50*, 47–70.

Bianchi, M.E. (2007). DAMPs, PAMPs and alarmins: all we need to know about danger. J. Leukoc. Biol. *81*, 1–5.

Bosnjak, L., Miranda-Saksena, M., Koelle, D.M., Boadle, R.A., Jones, C.A., and Cunningham, A.L. (2005). Herpes simplex virus infection of human dendritic cells induces apoptosis and allows cross-presentation via uninfected dendritic cells. J. Immunol. *174*, 2220–2227.

Cantor, H., Shen, F.W., and Boyse, E.A. (1976). Separation of helper T cells from suppressor T cells expressing different Ly components. II. Activation by antigen: after immunization, antigen-specific suppressor and helper activities are mediated by distinct T-cell subclasses. J. Exp. Med. *143*, 1391–40.

Chaput, N., De Botton, S., Obeid, M., Apetoh, L., Ghiringhelli, F., Panaretakis, T., Flament, C., Zitvogel, L., and Kroemer, G. (2007). Molecular determinants of immunogenic cell death: surface exposure of calreticulin makes the difference. J. Mol. Med. *85*, 1069–1076.

Chen, W., Frank, M.E., Jin, W., and Wahl, S.M. (2001). TGF-beta released by apoptotic T cells contributes to an immunosuppressive milieu. Immunity *14*, 715–725.

Choi, J.J., Reich, C.F., 3rd, and Pisetsky, D.S. (2004). Release of DNA from dead and dying lymphocyte and monocyte cell lines in vitro. Scand. J. Immunol. *60*, 159–166.

Conlon, P.J., Miller, S.D., and Claman, H.N. (1980). The induction of tolerance to DNFB contact sensitivity by using hapten-modified lymphoid cells. III. Effects of hapten concentration on the ability of MLS-disparate cells to induce rapid unresponsiveness. J. Immunol. *125*, 807–813.

Crispe, I.N., Giannandrea, M., Klein, I., John, B., Sampson, B., and Wuensch, S. (2006). Cellular and molecular mechanisms of liver tolerance. Immunol. Rev. *213*, 101–118.

den Haan, J.M., Lehar, S.M., and Bevan, M.J. (2000). CD8(+) but not CD8(-) dendritic cells cross-prime cytotoxic T cells in vivo. J. Exp. Med. *192*, 1685–1696.

Dhodapkar, M.V., Steinman, R.M., Krasovsky, J., Munz, C., and Bhardwaj, N. (2001). Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. J. Exp. Med. *193*, 233–238.

Dudziak, D., Kamphorst, A.O., Heidkamp, G.F., Buchholz, V.R., Trumpfheller, C., Yamazaki, S., Cheong, C., Liu, K., Lee, H.W., Park, C.G., et al. (2007). Differential antigen processing by dendritic cell subsets in vivo. Science *315*, 107–111.

Dupont, E., and Craciun, L. (2009). UV-induced immunosuppressive and anti-inflammatory actions: mechanisms and clinical applications. Immunotherapy *1*, 205–210.

Elliott, M.R., Chekeni, F.B., Trampont, P.C., Lazarowski, E.R., Kadl, A., Walk, S.F., Park, D., Woodson, R.I., Ostankovich, M., Sharma, P., et al. (2009). Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature *461*, 282–286.

Ferguson, T.A., Herndon, J., Elzey, B., Griffith, T.S., Schoenberger, S., and Green, D.R. (2002). Uptake of apoptotic antigen-coupled cells by lymphoid dendritic cells and cross-priming of CD8(+) T cells produce active immune unresponsiveness. J. Immunol. *168*, 5589–5595.

Ferguson, T.A., Choi, J., and Green, D.R. (2011). Armed response: how dying cells influence T-cell functions. Immunol. Rev. *241*, 77–88.

Gaipl, U.S., Munoz, L.E., Grossmayer, G., Lauber, K., Franz, S., Sarter, K., Voll, R.E., Winkler, T., Kuhn, A., Kalden, J., et al. (2007). Clearance deficiency and systemic lupus erythematosus (SLE). J. Autoimmun. *28*, 114–121.

Gao, Y., Herndon, J.M., Zhang, H., Griffith, T.S., and Ferguson, T.A. (1998). Antiinflammatory effects of CD95 ligand (FasL)-induced apoptosis. J. Exp. Med. *188*, 887–896.

Gershon, R.K., and Kondo, K. (1970). Cell interactions in the induction of tolerance: the role of thymic lymphocytes. Immunology *18*, 723–737.

Gershon, R.K., and Kondo, K. (1971). Infectious immunological tolerance. Immunology *21*, 903–914.

Gershon, R.K., Cohen, P., Hencin, R., and Liebhaber, S.A. (1972). Suppressor T cells. J. Immunol. *108*, 586–590.

Green, D.R., Droin, N., and Pinkoski, M. (2003). Activation-induced cell death in T cells. Immunol. Rev. *193*, 70–81.

Green, D.R., Ferguson, T., Zitvogel, L., and Kroemer, G. (2009). Immunogenic and tolerogenic cell death. Nat. Rev. Immunol. *9*, 353–363.

Greene, M.I., and Benacerraf, B. (1980). Studies on hapten specific T cell immunity and suppression. Immunol. Rev. *50*, 163–186.

Griffith, T.S., Brunner, T., Fletcher, S.M., Green, D.R., and Ferguson, T.A. (1995). Fas ligand-induced apoptosis as a mechanism of immune privilege. Science *270*, 1189–1192.

Griffith, T.S., Yu, X., Herndon, J.M., Green, D.R., and Ferguson, T.A. (1996). CD95-induced apoptosis of lymphocytes in an immune privileged site induces immunological tolerance. Immunity *5*, 7–16.

Griffith, T.S., Kazama, H., VanOosten, R.L., Earle, J.K., Jr., Herndon, J.M., Green, D.R., and Ferguson, T.A. (2007). Apoptotic cells induce tolerance by generating helpless $CD8^+$ T cells that produce TRAIL. J. Immunol. *178*, 2679–2687.

Griffith, T.S., Brincks, E.L., Gurung, P., Kucaba, T.A., and Ferguson, T.A. (2011). Systemic immunological tolerance to ocular antigens is mediated by TRAIL-expressing CD8+ T cells. J. Immunol. *186*, 791–798.

Gurung, P., Kucaba, T.A., Ferguson, T.A., and Griffith, T.S. (2009). Activation-induced CD154 expression abrogates tolerance induced by apoptotic cells. J. Immunol. *183*, 6114–6123.

Gurung, P., Kucaba, T.A., Schoenberger, S.P., Ferguson, T.A., and Griffith, T.S. (2010). TRAIL-expressing CD8+ T cells mediate tolerance following soluble peptide-induced peripheral T cell deletion. J. Leukoc. Biol. *88*, 1217–1225.

Gurung, P., Rai, D., Condotta, S.A., Babcock, J.C., Badovinac, V.P., and Griffith, T.S. (2011). Immune unresponsiveness to secondary heterologous bacterial infection after sepsis induction is TRAIL dependent. J. Immunol. *187*, 2148–2154.

Hanayama, R., Tanaka, M., Miyasaka, K., Aozasa, K., Koike, M., Uchiyama, Y., and Nagata, S. (2004). Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. Science *304*, 1147–1150.

Hanley, P.J., Musset, B., Renigunta, V., Limberg, S.H., Dalpke, A.H., Sus, R., Heeg, K.M., Preisig-Müller, R., and Daut, J. (2004). Extracellular ATP induces oscillations of intracellular Ca2+ and membrane potential and promotes transcription of IL-6 in macrophages. Proc. Natl. Acad. Sci. USA *101*, 9479–9484.

Hannani, D., Merlin, E., Gabert, F., Laurin, D., Deméocq, F., Chaperot, L., Kanold, J., and Plumas, J. (2010). Photochemotherapy induces a faster apoptosis of alloreactive activated T cells than of nonalloreactive resting T cells in graft versus host disease. Transplantation *90*, 1232–1238.

Heath, W.R., and Carbone, F.R. (2001). Cross-presentation, dendritic cells, tolerance and immunity. Annu. Rev. Immunol. *19*, 47–64.

Hege, K.M., Jooss, K., and Pardoll, D. (2006). GM-CSF gene-modifed cancer cell immunotherapies: of mice and men. Int. Rev. Immunol. *25*, 321–352.

Herndon, J.M., Stuart, P.M., and Ferguson, T.A. (2005). Peripheral deletion of antigen-specific T cells leads to long-term tolerance mediated by CD8+ cytotoxic cells. J. Immunol. *174*, 4098–4104.

Hildeman, D.A., Zhu, Y., Mitchell, T.C., Bouillet, P., Strasser, A., Kappler, J., and Marrack, P. (2002). Activated T cell death in vivo mediated by proapoptotic bcl-2 family member bim. Immunity *16*, 759–767.

Hotchkiss, R.S., Swanson, P.E., Knudson, C.M., Chang, K.C., Cobb, J.P., Osborne, D.F., Zollner, K.M., Buchman, T.G., Korsmeyer, S.J., and Karl, I.E. (1999a). Overexpression of Bcl-2 in transgenic mice decreases apoptosis and improves survival in sepsis. J. Immunol. *162*, 4148–4156.

Hotchkiss, R.S., Tinsley, K.W., Swanson, P.E., Chang, K.C., Cobb, J.P., Buchman, T.G., Korsmeyer, S.J., and Karl, I.E. (1999b). Prevention of lymphocyte cell death in sepsis improves survival in mice. Proc. Natl. Acad. Sci. USA *96*, 14541–14546.

Hotchkiss, R.S., Chang, K.C., Swanson, P.E., Tinsley, K.W., Hui, J.J., Klender, P., Xanthoudakis, S., Roy, S., Black, C., Grimm, E., et al. (2000). Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte. Nat. Immunol. *1*, 496–501.

Hu, Y., Shen, F., Crellin, N.K., and Ouyang, W. (2011). The IL-17 pathway as a major therapeutic target in autoimmune diseases. Ann. N.Y. Acad. Sci. *1217*, 60–76.

Huang, L., Soldevila, G., Leeker, M., Flavell, R., and Crispe, I.N. (1994). The liver eliminates T cells undergoing antigen-triggered apoptosis in vivo. Immunity *1*, 741–749.

Hugues, S., Mougneau, E., Ferlin, W., Jeske, D., Hofman, P., Homann, D., Beaudoin, L., Schrike, C., Von Herrath, M., Lehuen, A., and Glaichenhaus, N. (2002). Tolerance to islet antigens and prevention from diabetes induced by limited apoptosis of pancreatic beta cells. Immunity *16*, 169–181.

Ip, W.K., and Lau, Y.L. (2004). Distinct maturation of, but not migration between, human monocyte-derived dendritic cells upon ingestion of apoptotic cells of early or late phases. J. Immunol. *173*, 189–196.

Iyoda, T., Shimoyama, S., Liu, K., Omatsu, Y., Akiyama, Y., Maeda, Y., Takahara, K., Steinman, R.M., and Inaba, K. (2002). The CD8+ dendritic cell subset selectively endocytoses dying cells in culture and in vivo. J. Exp. Med. *195*, 1289–1302.

Jäger, A., and Kuchroo, V.K. (2010). Effector and regulatory T-cell subsets in autoimmunity and tissue inflammation. Scand. J. Immunol. *72*, 173–184.

Janssen, E.M., Droin, N.M., Lemmens, E.E., Pinkoski, M.J., Bensinger, S.J., Ehst, B.D., Griffith, T.S., Green, D.R., and Schoenberger, S.P. (2005). CD4+ T-cell help controls CD8+ T-cell memory via TRAIL-mediated activation-induced cell death. Nature *434*, 88–93.

Janssen, E., Tabeta, K., Barnes, M.J., Rutschmann, S., McBride, S., Bahjat, K.S., Schoenberger, S.P., Theofilopoulos, A.N., Beutler, B., and Hoebe, K. (2006). Efficient T cell activation via a Toll-Interleukin 1 Receptor-independent pathway. Immunity *24*, 787–799.

Johansson, U., Walther-Jallow, L., Smed-Sörensen, A., and Spetz, A.L. (2007). Triggering of dendritic cell responses after exposure to activated, but not resting, apoptotic PBMCs. J. Immunol. *179*, 1711–1720.

Kazama, H., Ricci, J.E., Herndon, J.M., Hoppe, G., Green, D.R., and Ferguson, T.A. (2008). Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box-1 protein. Immunity *29*, 21–32.

Kepp, O., Tesniere, A., Schlemmer, F., Michaud, M., Senovilla, L., Zitvogel, L., and Kroemer, G. (2009). Immunogenic cell death modalities and their impact on cancer treatment. Apoptosis *14*, 364–375.

Kim, S., Elkon, K.B., and Ma, X. (2004). Transcriptional suppression of interleukin-12 gene expression following phagocytosis of apoptotic cells. Immunity *21*, 643–653.

Kleinclauss, F., Perruche, S., Cahn, J.Y., Tiberghien, P., and Saas, P. (2003). Administration of donor apoptotic cells: an alternative cell-based therapy to induce tolerance? Transplantation *75* (9, Suppl), 43S–45S.

Kleinclauss, F., Perruche, S., Masson, E., de Carvalho Bittencourt, M., Biichle, S., Remy-Martin, J.P., Ferrand, C., Martin, M., Bittard, H., Chalopin, J.M., et al. (2006). Intravenous apoptotic spleen cell infusion induces a TGF-beta-dependent regulatory T-cell expansion. Cell Death Differ. *13*, 41–52.

Koizumi, S., Shigemoto-Mogami, Y., Nasu-Tada, K., Shinozaki, Y., Ohsawa, K., Tsuda, M., Joshi, B.V., Jacobson, K.A., Kohsaka, S., and Inoue, K. (2007). UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. Nature *446*, 1091–1095.

Kurts, C., Heath, W.R., Kosaka, H., Miller, J.F., and Carbone, F.R. (1998). The peripheral deletion of autoreactive CD8+ T cells induced by cross-presentation of self-antigens involves signaling through CD95 (Fas, Apo-1). J. Exp. Med. *188*, 415–420.

Lamkanfi, M., and Dixit, V.M. (2010). Manipulation of host cell death pathways during microbial infections. Cell Host Microbe *8*, 44–54.

Li, A., Ojogho, O., and Escher, A. (2006). Saving death: apoptosis for intervention in transplantation and autoimmunity. Clin. Dev. Immunol. *13*, 273–282.

Li, H., Ambade, A., and Re, F. (2009). Cutting edge: Necrosis activates the NLRP3 inflammasome. J. Immunol. *183*, 1528–1532.

Lin, J.S., Yang, C.W., Wang, D.W., and Wu-Hsieh, B.A. (2005). Dendritic cells cross-present exogenous fungal antigens to stimulate a protective CD8 T cell response in infection by *Histoplasma capsulatum*. J. Immunol. *174*, 6282–6291.

Luckashenak, N., Schroeder, S., Endt, K., Schmidt, D., Mahnke, K., Bachmann, M.F., Marconi, P., Deeg, C.A., and Brocker, T. (2008). Constitutive crosspresentation of tissue antigens by dendritic cells controls CD8+ T cell tolerance in vivo. Immunity *28*, 521–532.

Marin-Gallen, S., Clemente-Casares, X., Planas, R., Pujol-Autonell, I., Carrascal, J., Carrillo, J., Ampudia, R., Verdaguer, J., Pujol-Borrell, R., Borràs, F.E., and Vives-Pi, M. (2010). Dendritic cells pulsed with antigen-specific apoptotic bodies prevent experimental type 1 diabetes. Clin. Exp. Immunol. *160*, 207–214.

Matheoud, D., Perié, L., Hoeffel, G., Vimeux, L., Parent, I., Marañón, C., Bourdoncle, P., Renia, L., Prevost-Blondel, A., Lucas, B., et al. (2010). Cross-presentation by dendritic cells from live cells induces protective immune responses in vivo. Blood *115*, 4412–4420.

Matzinger, P. (2002). The danger model: a renewed sense of self. Science *296*, 301–305.

McMenamin, P.G. (1999). Dendritic cells and macrophages in the uveal tract of the normal mouse eye. Br. J. Ophthalmol. *83*, 598–604.

Medzhitov, R., and Janeway, C.A., Jr. (2002). Decoding the patterns of self and nonself by the innate immune system. Science *296*, 298–300.

Millar, D.G., Garza, K.M., Odermatt, B., Elford, A.R., Ono, N., Li, Z., and Ohashi, P.S. (2003). Hsp70 promotes antigen-presenting cell function and converts T-cell tolerance to autoimmunity in vivo. Nat. Med. *9*, 1469–1476.

Miyake, Y., Asano, K., Kaise, H., Uemura, M., Nakayama, M., and Tanaka, M. (2007). Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell-associated antigens. J. Clin. Invest. *117*, 2268–2278.

Miyanishi, M., Tada, K., Koike, M., Uchiyama, Y., Kitamura, T., and Nagata, S. (2007). Identification of Tim4 as a phosphatidylserine receptor. Nature *450*, 435–439.

Miyasaka, K., Hanayama, R., Tanaka, M., and Nagata, S. (2004). Expression of milk fat globule epidermal growth factor 8 in immature dendritic cells for engulfment of apoptotic cells. Eur. J. Immunol. *34*, 1414–1422.

Morelli, A.E., and Larregina, A.T. (2010). Apoptotic cell-based therapies against transplant rejection: role of recipient's dendritic cells. Apoptosis *15*, 1083–1097.

Obeid, M., Tesniere, A., Ghiringhelli, F., Fimia, G.M., Apetoh, L., Perfettini, J.L., Castedo, M., Mignot, G., Panaretakis, T., Casares, N., et al. (2007). Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat. Med. *13*, 54–61.

Panaretakis, T., Joza, N., Modjtahedi, N., Tesniere, A., Vitale, I., Durchschlag, M., Fimia, G.M., Kepp, O., Piacentini, M., Froehlich, K.U., et al. (2008). The co-translocation of ERp57 and calreticulin determines the immunogenicity of cell death. Cell Death Differ. *15*, 1499–1509.

Parish, I.A., Rao, S., Smyth, G.K., Juelich, T., Denyer, G.S., Davey, G.M., Strasser, A., and Heath, W.R. (2009). The molecular signature of CD8+ T cells undergoing deletional tolerance. Blood *113*, 4575–4585.

Pellegrini, M., Belz, G., Bouillet, P., and Strasser, A. (2003). Shutdown of an acute T cell immune response to viral infection is mediated by the proapoptotic Bcl-2 homology 3-only protein Bim. Proc. Natl. Acad. Sci. USA *100*, 14175–14180.

Penaloza, C., Lin, L., Lockshin, R.A., and Zakeri, Z. (2006). Cell death in development: shaping the embryo. Histochem. Cell Biol. *126*, 149–158.

Penaranda, C., Tang, Q., and Bluestone, J.A. (2011). Anti-CD3 therapy promotes tolerance by selectively depleting pathogenic cells while preserving regulatory T cells. J. Immunol. *187*, 2015–2022.

Peng, Y., and Elkon, K.B. (2011). Autoimmunity in MFG-E8-deficient mice is associated with altered trafficking and enhanced cross-presentation of apoptotic cell antigens. J. Clin. Invest. *121*, 2221–2241.

Peng, Y., Martin, D.A., Kenkel, J., Zhang, K., Ogden, C.A., and Elkon, K.B. (2007). Innate and adaptive immune response to apoptotic cells. J. Autoimmun. *29*, 303–309.

Perruche, S., Zhang, P., Liu, Y., Saas, P., Bluestone, J.A., and Chen, W. (2008). CD3-specific antibody-induced immune tolerance involves transforming growth factor-beta from phagocytes digesting apoptotic T cells. Nat. Med. *14*, 528–535.

Rawson, P.M., Molette, C., Videtta, M., Altieri, L., Franceschini, D., Donato, T., Finocchi, L., Propato, A., Paroli, M., Meloni, F., et al. (2007). Cross-presentation of caspase-cleaved apoptotic self antigens in HIV infection. Nat. Med. *13*, 1431–1439.

Ravichandran, K.S. (2011). Beginnings of a good apoptotic meal: The find-me and eat-me signaling pathways. Immunity *35*, this issue, 445–455.

Ricci, J.E., Muñoz-Pinedo, C., Fitzgerald, P., Bailly-Maitre, B., Perkins, G.A., Yadava, N., Scheffler, I.E., Ellisman, M.H., and Green, D.R. (2004). Disruption of mitochondrial function during apoptosis is mediated by caspase cleavage of the p75 subunit of complex I of the electron transport chain. Cell *117*, 773–786.

Rock, K.L., and Kono, H. (2008). The inflammatory response to cell death. Annu. Rev. Pathol. *3*, 99–126.

Ronchetti, A., Iezzi, G., Crosti, M.C., Garancini, M.P., Protti, M.P., and Bellone, M. (1999a). Role of antigen-presenting cells in cross-priming of cytotoxic T lymphocytes by apoptotic cells. J. Leukoc. Biol. *66*, 247–251.

Ronchetti, A., Rovere, P., Iezzi, G., Galati, G., Heltai, S., Protti, M.P., Garancini, M.P., Manfredi, A.A., Rugarli, C., and Bellone, M. (1999b). Immunogenicity of apoptotic cells in vivo: role of antigen load, antigen-presenting cells, and cytokines. J. Immunol. *163*, 130–136.

Roth, E., Manhart, N., and Wessner, B. (2004). Assessing the antioxidative status in critically ill patients. Curr. Opin. Clin. Nutr. Metab. Care *7*, 161–168.

Rudensky, A.Y. (2011). Regulatory T cells and Foxp3. Immunol. Rev. *241*, 260–268.

Sakaguchi, S., Ono, M., Setoguchi, R., Yagi, H., Hori, S., Fehervari, Z., Shimizu, J., Takahashi, T., and Nomura, T. (2006). Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol. Rev. *212*, 8–27.

Sauter, B., Albert, M.L., Francisco, L., Larsson, M., Somersan, S., and Bhardwaj, N. (2000). 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. *191*, 423–434.

Scaffidi, P., Misteli, T., and Bianchi, M.E. (2002). Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature *418*, 191–195.

Schnorrer, P., Behrens, G.M., Wilson, N.S., Pooley, J.L., Smith, C.M., El-Sukkari, D., Davey, G., Kupresanin, F., Li, M., Maraskovsky, E., et al. (2006). The dominant role of CD8+ dendritic cells in cross-presentation is not dictated by antigen capture. Proc. Natl. Acad. Sci. USA *103*, 10729–10734.

Schoenberger, S.P., Toes, R.E., van der Voort, E.I., Offringa, R., and Melief, C.J. (1998). T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature *393*, 480–483.

Shi, Y., Evans, J.E., and Rock, K.L. (2003). Molecular identification of a danger signal that alerts the immune system to dying cells. Nature *425*, 516–521.

Smith, C.E., and Miller, S.D. (2006). Multi-peptide coupled-cell tolerance ameliorates ongoing relapsing EAE associated with multiple pathogenic autoreactivities. J. Autoimmun. *27*, 218–231.

Spisek, R., Charalambous, A., Mazumder, A., Vesole, D.H., Jagannath, S., and Dhodapkar, M.V. (2007). Bortezomib enhances dendritic cell (DC)-mediated induction of immunity to human myeloma via exposure of cell surface heat shock protein 90 on dying tumor cells: therapeutic implications. Blood *109*, 4839–4845.

Sprent, J., and Kishimoto, H. (2002). The thymus and negative selection. Immunol. Rev. *185*, 126–135.

Steinman, R.M., Hawiger, D., and Nussenzweig, M.C. (2003). Tolerogenic dendritic cells. Annu. Rev. Immunol. *21*, 685–711.

Steptoe, R.J., McMenamin, P.G., and Holt, P.G. (2000). Resident tissue macrophages within the normal rat iris lack immunosuppressive activity and are effective antigen-presenting cells. Ocul. Immunol. Inflamm. *8*, 177–187.

Straus, S.E., Sneller, M., Lenardo, M.J., Puck, J.M., and Strober, W. (1999). An inherited disorder of lymphocyte apoptosis: the autoimmune lymphoproliferative syndrome. Ann. Intern. Med. *130*, 591–601.

Sun, J.C., and Bevan, M.J. (2003). Defective CD8 T cell memory following acute infection without CD4 T cell help. Science *300*, 339–342.

Takahashi, T., Kuniyasu, Y., Toda, M., Sakaguchi, N., Itoh, M., Iwata, M., Shimizu, J., and Sakaguchi, S. (1998). Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. Int. Immunol. *10*, 1969–1980.

Thompson, C.B. (1995). Apoptosis in the pathogenesis and treatment of disease. Science *267*, 1456–1462.

Torchinsky, M.B., Garaude, J., Martin, A.P., and Blander, J.M. (2009). Innate immune recognition of infected apoptotic cells directs T(H)17 cell differentiation. Nature *458*, 78–82.

Ullrich, E., Bonmort, M., Mignot, G., Kroemer, G., and Zitvogel, L. (2008). Tumor stress, cell death and the ensuing immune response. Cell Death Differ. *15*, 21–28.

Unsinger, J., Kazama, H., McDonough, J.S., Griffith, T.S., Hotchkiss, R.S., and Ferguson, T.A. (2010). Sepsis-induced apoptosis leads to active suppression of delayed-type hypersensitivity by CD8+ regulatory T cells through a TRAIL-dependent mechanism. J. Immunol. *184*, 6766–6772.

Winau, F., Weber, S., Sad, S., de Diego, J., Hoops, S.L., Breiden, B., Sandhoff, K., Brinkmann, V., Kaufmann, S.H., and Schaible, U.E. (2006). Apoptotic vesicles crossprime CD8 T cells and protect against tuberculosis. Immunity *24*, 105–117.

Xie, Y., Bai, O., Yuan, J., Chibbar, R., Slattery, K., Wei, Y., Deng, Y., and Xiang, J. (2009). Tumor apoptotic bodies inhibit CTL responses and antitumor immunity via membrane-bound transforming growth factor-beta1 inducing CD8+ T-cell anergy and CD4+ Tr1 cell responses. Cancer Res. *69*, 7756–7766.

Zitvogel, L., Casares, N., Péquignot, M.O., Chaput, N., Albert, M.L., and Kroemer, G. (2004). Immune response against dying tumor cells. Adv. Immunol. *84*, 131–179.