Capitalizing on the Immunogenicity of Dying Tumor Cells
Catia Fonseca and Glenn Dranoff

Abstract
Cancer cell death occurs continually during tumor development and progression, whereas the selective killing of surviving cancer cells remains the primary objective of antineoplastic treatments. Recent insights into the immunologic consequences of cancer cell death have begun to elucidate the ways in which host antitumor immunity is shaped during cancer pathogenesis and then modulated by therapeutic intervention. Dying tumor cells evoke a range of host responses, dependent in part upon the mode of cell death, which may either impede or foster additional immune-mediated cancer destruction. Within the tumor microenvironment, the capture of apoptotic tumor cells by macrophages and dendritic cells may trigger tolerance networks that contribute to immune suppression, whereas the uptake of necrotic cancer cells may engender inflammatory pathways that fuel antitumor cytotoxicity. Milk fat globule epidermal growth factor 8, a phosphatidylserine-binding protein, and MHC class I chain–related protein A, an NKG2D ligand, play key roles in these competing outcomes. A deeper understanding of the mechanisms underlying the immunogenicity of dying cells informs the crafting of strategies that exploit endogenous or treatment-induced cancer cell death as the basis for stimulating sustained host antitumor cytotoxic reactions.

Background
Cancer cells devise a myriad of strategies to antagonize cell death, but these countermeasures prove to be imperfect (1). Although the economies of cell proliferation and accumulation dominate cell demise during disease progression, pathologic examination of established cancers typically reveals evidence for both single cell and zonal areas of tumor death. Mixtures of apoptosis, necrosis, and autophagy frequently may be discerned histologically. These varying forms of cellular destruction reflect the failure of tumors to cope adequately with the challenging burdens of cellular stress (2). Such ongoing insults include DNA damage and genomic instability; perturbed signal transduction and transcriptional networks; the unfolded protein response, hypoxia, nutrient deprivation, immunologic attack; and likely many others (3). Notwithstanding the intense effort directed toward unraveling the mechanisms underlying cancer cell death, much less attention have been devoted to understanding the host response to tumor cell demise.

A spectrum of host responses to cell death empowers the immune system with the dual abilities to monitor the integrity of healthy tissues and to respond rapidly to tissue injury (4). Under steady-state conditions, the capture of apoptotic cells serves as an immunoregulatory event that helps maintain tolerance, whereas the detection of necrotic cells evokes an inflammatory cascade that mobilizes effector responses. However, the development and progression of cancer typically involves both apoptotic and necrotic forms of cell death. What are the implications of this mixed pattern of cellular demise for the induction of antitumor immunity?

The crafting of genetic and biochemical strategies to characterize cancer antigens has yielded the insight that most patients mount tumor-specific T cell and antibody reactions (5). Because cancer cells typically fail to express the repertoire of costimulatory molecules required for stimulating T cells directly, adaptive immune recognition is likely accomplished through an indirect mechanism that exploits the capture of dying tumor cells by host mononuclear phagocytes (6). The concurrent acquisition of apoptotic and necrotic cells by dendritic cells and macrophages in the tumor microenvironment, however, might result in a set of conflicting signals that drive tolerance and effector responses in parallel. Under these conditions, a form of immunologic “gridlock” may ensue, in which neither the regulatory nor activation pathways achieve full control.

Histopathologic analysis typically reveals the presence of various immune cells within or circumscribing tumors, indicative of a nascent host response, whereas specific morphologic patterns of reactivity have been linked with varying clinical outcomes. Although dense intratumor T-cell infiltrates are rare, they are tightly associated with a decreased incidence of recurrent disease and reduced mortality in multiple cancer types, including malignant melanoma, follicular lymphoma, and carcinomas of the colon, ovary, liver, and kidney (7–13). The combination of brisk intratumoral CD8+ T cells and rare FoxP3+ Tregs connotes a particularly favorable
prognostic index, suggesting that the balance of effector and regulatory cells might modulate disease course in some patients.

In murine models of methylycholanthrene-induced carcinogenesis, endogenous host responses contribute to tumor dormancy (14). In this state of immune equilibrium, the coordinated activities of CD4+ and CD8+ T cells, IFN-γ, and interleukin (IL)-12 mediate disease control. The attainment of immune equilibrium in humans is highlighted through multiple case reports of the inadvertent transplantation of metastatic melanoma to recipients of renal allografts who were maintained on immunosuppression; tumors arose in these patients notwithstanding intervals as long as 16 years from the putative surgical cures of early stage melanomas in the donors (15).

This dynamic of host reactivity raises the possibility that antitumor immunity might contribute to the therapeutic efficacy of some conventional cancer treatments. Consistent with this idea, the durability of clinical responses to oncologic therapy is closely linked to the presence of brisk intratumoral T-cell infiltrates in diverse cancers (6). Perhaps antineoplastic treatments modulate the mixture of apoptotic and necrotic cell death within tumors, thereby altering the balance of effector and regulatory T cells in favor of more intense and sustained protection. How might this be achieved?

The principles governing the host reaction to the turnover of normal cells provide a useful framework for considering the consequences of cancer cell destruction (4). The programmed death of normal cells (apoptosis) involves a well-orchestrated sequence of events that culminates in the formation of surface blebs and the sequestration of intracellular components with the potential to effectuate lysis (16). Apoptosis is a critical feature of tissue homeostasis, and the orderly detection and clearance of cellular corpses is essential for proper tissue remodeling and renewal. Defects in the management of early apoptotic cells may allow the resurrection of some cells that otherwise were committed to die, thereby increasing the risk of transformation (17, 18). Various parenchymal cells seem capable of ingesting their dying neighbors, but the host immune system is especially well-adapted for scavenging cellular remnants and debris (19).

The principal immune components charged with this task are macrophages and dendritic cells, the key mononuclear phagocytes. These antigen-presenting cells use a multiplicity of receptor/ligand pairs to recognize and engulf apoptotic cells (20). Whereas scavenger receptors, complement components, and collectins all play important roles in clearance, a primary "eat me" signal seems to be phosphatidylserine. The centrality of this detection scheme is underscored by its conservation throughout metazoans, including the paradigmatic model system, Caenorhabditis elegans (21). Phosphatidylserine is normally retained within the inner leaflet of the surface membrane through the tonic activities of a transporter/flippase, but upon execution of programmed death, this lipid is translocated to the exterior envelope of the cell (22).

Mononuclear phagocytes accomplish phosphatidylserine-based uptake of apoptotic cells through several secreted and surface proteins (Fig. 1). Milk fat globule epidermal growth factor 8 (MFG-E8) and the closely related molecule Del-1 are released into the tissue microenvironment, wherein their discoidin domains bind phosphatidylserine on cellular corpses. These are then internalized through the binding of RGD sequences, encoded in the epidermal growth factor domains of MFG-E8 and Del-1, to the αvβ3 and αvβ5 integrins expressed on the phagocyte surface (23, 24). A second set of opsonins that similarly bind phosphatidylserine are growth arrest–specific gene 6 and protein S, although these secreted proteins promote phagocyte engulfment through interactions with the tyro family of receptor tyrosine kinases (Mer, Axl, and Tyro-3; refs. 25, 26). The membrane proteins TIM-1 and TIM-4 additionally detect phosphatidylserine and contribute to the uptake of apoptotic cells by phagocytes (27). The essential role of these pathways in tissue homeostasis is underscored by the development of persistent inflammation in mice harboring mutations in these gene products (28).

Indeed, the efficient clearance of cellular corpses by mononuclear phagocytes results in the elaboration of immunosuppressive mediators, particularly transforming growth factor β and IL-10, which empower dendritic cells and macrophages to stimulate FoxP3+ expressing and other regulatory T-cell subsets that support the maintenance of immune tolerance (29, 30).

In contrast to the orderly clearance of apoptotic cells, necrotic cell death triggers a vigorous inflammatory response that is aimed at restraining the cause of tissue damage (31). Phagocytes that encounter necrotic debris produce a broad range of proinflammatory cytokines such as IL-1β, IL-6, IL-12, and IL-23. These in turn engage a network of soluble and membrane factors that orchestrate the recruitment and activation of multiple innate and adaptive immune elements that control invading pathogens and noxious agents (Fig. 1). Concurrently, the elaboration of regulatory molecules such as transforming growth factor β and IL-10 is attenuated, thereby temporarily dampening the immunosuppressive circuits, which facilitates the mobilization and evolution of the effector cascade.

The triggering of this inflammatory response by mononuclear phagocytes requires the MyD88 adapter protein, which functions in both IL-1 and Toll-like receptor signaling (32). The latter pattern-recognition molecules detect not only microbial signatures but also the biochemical sequelae of necrotic cell death, which include the spillage of cytoplasmic heat shock proteins and the release of nuclear HMGB-1, a normal component of chromatin (33). The phagocyte ingestion of damaged nucleic acids may further provoke a cytosolic recognition system for DNA that engenders the production of inflammatory cytokines, particularly type I IFNs (34). The engulfment of uric acid generated in foci of necrosis may also engage the inflammasome, a cytoplasmic protein complex that stimulates IL-1β secretion, which in turn triggers the nuclear factor-κB– and Jun kinase–signaling cascades to elicit the transcription of multiple inflammatory cytokine genes (35). The surface exposure of calreticulin, a protein usually resident in the endoplasmic reticulum, may additionally serve as a key signal that activates macrophages and dendritic cells to propagate the effector response (36).

How do these insights into the host response to apoptotic and necrotic death inform our understanding of the immune consequences of oncologic treatments? Recent work indicates that some forms of cytotoxic cancer therapy, particularly...
radiation, anthracyclines, and platinum-based compounds, induce an immunogenic form of cell death characterized by the surface mobilization of calreticulin and the extracellular release of the nuclear protein HMGB1 (33, 36). Together, these modifications activate dendritic cells, through a Toll-like receptor–4 and MyD88-dependent pathway, to capture and cross-present efficiently tumor cell debris to evoke cytotoxic T-cells responses. Variants of Toll-like receptor–4 with high avidity for HMGB-1 are associated with superior clinical outcomes after anthracycline-based adjuvant chemotherapy in women with early stage breast carcinoma. Tumor cell immunogenicity may be further augmented through the induction of DNA damage that generates novel protein epitopes that provoke T-cell recognition (37). Cytotoxic therapies may additionally compromise regulatory T-cell circuits through a reduction in antigen load and the modification of lymphocyte subset dynamics during homeostatic expansion (38).

Oncologic treatments may also affect the expression of NKG2D ligands, which are induced on the surface of tumor cells in response to DNA damage (39). The human NKG2D ligands include MICA, a MHC class I–related molecule, the closely related MICB, and five UL16-binding proteins (40). These gene products manifest limited expression in healthy tissues but may be up-regulated in response to double-stranded DNA breaks through a pathway that involves ATM, ATR, CHK-1, and CHK-2 (39). Ligand-triggered NKG2D activation in natural killer cells, γδ-T cells, and CD8+ αβ-T cells culminates in potent cytotoxicity (41). In murine models, the engineered expression of NKG2D ligands on tumors provokes rejection in wild-type hosts through the coordinated activities of natural killer cells, CD8+ T lymphocytes, and perforin, whereas the administration of anti-NKG2D blocking antibodies increases susceptibility to chemical carcinogens (42–44). Notwithstanding the ability of NKG2D signaling to engender innate and adaptive antitumor responses, immune escape is frequently accomplished in patients through the shedding of MICA from tumor cells, which is mediated in part through the protein disulfide isomerase ERp5 (45). Shedding of MICA elicits the down-regulation of surface NKG2D, resulting in impaired cytotoxic function and tumor progression (46).
If host immunity participates in the response to conventional oncologic treatments, can induced tumor cell death be rendered more immunogenic? In this context, we found that some patients who achieved clinical responses to immunotherapy with granulocyte macrophage colony-stimulating factor–secreting tumor cell vaccines or blockade of CTL-associated antigen 4–generated high titer antibodies against MICA and ERp5 (ref. 47). These humoral reactions antagonized the immunosuppressive effects of shedding of MICA and restored innate and adaptive cytotoxicity. The antibodies also promoted the cross-presentation of ingested tumor antigens by dendritic cells and thereby augmented antitumor cellular immunity. Together, these findings raise the possibility that therapeutic monoclonal antibodies against MICA and ERp5 might be useful for cancer treatment, especially in combination with DNA-damaging agents that enhance NKG2D ligand expression (Fig. 2).

Will the concurrent uptake of apoptotic cells limit the ability of these strategies to enhance tumor cell immunogenicity? MFG-E8 and perhaps other phosphatidylserine-binding proteins are produced in the tumor microenvironment, where they may promote Treg expansion and local immunosuppression (30). Inhibiting the capture of dying cells through these

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**Clinical/Translational Advances**

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1 Fonseca et al, submitted.
pathways might therefore attenuate maintenance of the local tolerance networks (Fig. 2). In a therapeutic model of established melanoma in mice, the combination of granulocyte macrophage colony-stimulating factor—secreting tumor cell vaccines with a dominant-negative MFG-E8 mutant, which blocks phosphatidylserine-based phagocytosis (48), effectuated the complete regressions of established tumors in the absence of significant toxicities. The therapeutic immunity involved the inhibition of Tregs and the concurrent expansion of CD8\(^+\) cytotoxic T cells. Thus, systemic administration of anti–MFG-E8 antibodies or other approaches that mask phosphatidylserine might complement strategies that trigger immunogenic cell death. Furthermore, the gene products involved in the clearance of apoptotic cells play decisive roles in tumor progression through fostering angiogenesis, matrix metalloproteinase production, epithelial-to-mesenchymal transformation, and epithelial cell migration (49, 50). Thus, targeting these pathways might not only stimulate tumor immunity but also antagonize pathogenic tumor mechanisms.

Conclusions

Accumulating evidence indicates that the clinical activity of many cancer therapies involves the interplay of tumor cell autonomous effects and host immunity. An understanding of the host response to dying tumor cells provides compelling opportunities to render existing oncologic treatments more efficacious and to develop novel schemes to intensify endogenous antitumor reactivity. Analogous to the way in which an intact host response is required for antimicrobial agents to achieve pathogen control in infectious diseases, protective antitumor immunity will likely synergize with multiple forms of cancer therapy. Paradoxically, the critical role of genomic instability in driving tumor pathogenesis and the evolution of drug-resistant variants renders cancer cells highly amenable to immune recognition. Perhaps this vulnerability may now be actualized through capitalizing on the host response to death.

References

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*Clin Cancer Res* 2008;14:1603-1608.

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