Review

Tumor Cell Death and ATP Release Prime Dendritic Cells and Efficient Anticancer Immunity

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Abstract

By destroying tumor cells, conventional anticancer therapies may stimulate the host immune system to eliminate residual disease. Anthracyclines, oxaliplatin, and ionizing irradiation activate a type of tumor cell death that elicits efficient anticancer immune responses depending on interferon γ (IFNγ) and the IFNγ receptor. Thus, dying tumor cells emit danger signals that are perceived by dendritic cells (DC), which link innate and cognate immune responses. Recently, we observed that ATP was released by tumor cells succumbing to chemotherapy. ATP activates purinergic P2RX7 receptors on DC, thus activating the NLRP3/ASC/caspase-1 inflammasome and driving the secretion of interleukin-1β (IL-1β). IL-1β then is required for the adequate polarization of IFNγ-producing CD8+ T cells. These results imply a novel danger signal, ATP, and a novel receptor, P2RX7, in the chemotherapylelicted anticancer immune response. Cancer Res; 70(3); 855–8. ©2010 AACR.

Setting the Stage

Some regimens of chemotherapy mediate direct cytotoxic effects on the tumor and, in addition, elicit indirect antitumor effects resulting from the immunogenicity of cell death (1, 2). Anthracyclines, oxaliplatin, and ionizing irradiation can trigger immunogenic cell death, whereas many other anticancer agents fail to do so. We delineated the molecular mechanisms underlying the recognition of dying tumor cells by dendritic cells (DC) and found two major checkpoints that dictate the immunogenicity of cell death. First, optimal phagocytosis of chemotherapy- or radiotherapy-treated tumor cells by DC requires the translocation of endoplasmic reticulum (ER)-resident calreticulin and disulfide isomerase ERP57 to the plasma membrane of dying tumor cells (3–5). Second, the chromatin-binding high mobility group box 1 protein (HMGB1) must be released by dying tumor cells and must bind to its receptor toll-like receptor 4 (TLR4) on DC to facilitate antigen processing of the phagocytic cargo (6). However, additional signals emanating from dying tumor cells are required for the full-blown maturation and differentiation of DC and T cells, respectively. Therefore, we addressed the contribution of damage-associated molecular patterns (DAMP) to the efficiency of chemotherapy in mouse tumor models (7).

Results

Components of the immune system required for therapeutic effects. Immunosurveillance of tumors involves lymphocytes and requires an intact interferon γ (IFNγ)/IFNγR pathway (8–10). To investigate the contribution of these immune effectors in the efficacy of chemotherapy, we compared chemotherapy-induced tumor growth retardation in immunocompetent mice and in mice bearing various immunodefects affecting B and/or T cells [Rag1−/−, nu/nu, or wild-type (WT) mice treated with a depleting anti-CD8 mAb] or the IFNγ/IFNγR system [Ifng−/−, IfngR−/−, or WT treated with neutralizing anti-IFNγ antibodies]. The depletion of CD8+ T cells or the removal of the IFNγ/IFNγR pathway reduced the therapeutic effects of oxaliplatin against CT26 colon cancer, EL4 thymoma, and abolished the antitumor effects of anthracyclines (doxorubicin or mitoxanthrone) against CT26 cells or MCA-induced sarcomas. In contrast, other molecules such as IL-12Rβ2, perforin, and TRAIL were dispensable for the efficacy of these cancer chemotherapies in the same tumor models. Chemotherapy promoted a tumor-specific T-cell response that was detectable in the tumor-draining lymph nodes (LN), 7 to 10 days after the systemic administration of oxaliplatin. After ex vivo restimulation with tumor antigens, oxaliplatin-induced CD4+ T cells mainly produced interleukin-2 (IL-2), whereas chemotherapy-induced CD8+ T cells produced high levels of IFNγ. These chemotherapy-induced T-cell responses apparently resulted from local cancer cell death because they were detectable in draining LN (but not distant LN) and because dying (but not living) tumor cells inoculated in the footpad could mimic these immune effects (7).

In conclusion, tumor regression promoted by oxaliplatin or anthracyclines elicits a tumor-specific IFNγ-polarized CD8+ T-cell immune response, which is critical for the efficacy of chemotherapy.
The inflammasome NLRP3 is mandatory for the efficacy of chemotherapy. Inoculation of anthracycline or oxaliplatin-treated tumor cells in the footpad induced a potent IFN-γ-polarized CD8+ T-cell response relying on TLR4 signaling in DC (6). We investigated the possible contribution of other receptors for DAMPs that could account for the priming of IFN-γ-producing T cells during anticancer chemotherapy. In addition to TLR, NOD-like receptors (NLIR) recognize DAMPs and link inflammation to innate immunity (11, 12). In macrophages, the so-called "inflammasome" serves as a central sensor for DAMPs (13). The NLRP3 (CIAS1/CRYOPYRIN)-inflammasome is a multimeric protein complex that activates the protease caspase-1, which mediates the processing and secretion of pro-inflammatory cytokines (IL-1β, IL-18, IL-33). The NLRP3 inflammasome can be activated by endogenous danger signals (DAMPs) or pathogen-associated molecular patterns (PAMPs), which all act in concert to induce K+ efflux (14–17). Gain-of-function mutations in the human NLRP3 gene cause autoinflammatory disease that can be suppressed by the systemic administration of IL-1R antagonists (18, 19). To investigate the putative role of the NLRP3 inflammasome in anticancer chemotherapy, we compared tumor growth kinetics of EL4 sarcomas treated with oxaliplatin in WT mice versus mice deficient in each component of the inflammasome [NLRP3, its adaptor molecule apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) or caspase-1] or its final product IL-1β. Each individual player of this complex was required for the success of chemotherapy. The key contribution of IL-1β in the antitumor effects mediated by oxaliplatin and/or oxaliplatin was also shown in CT26 colon cancer and more importantly in spontaneous tumors induced by MCA (7).

To analyze the role of the NLRP3 inflammasome in the antitumor immune response elicited by chemotherapy, we inoculated live versus dying tumor cells exposed to chemotherapy ex vivo and determined the capacity of draining LN T cells to produce IFN-γ in response to tumor antigens. In these circumstances, T cells from mice deficient in individual components of the inflammasome/IL-1β axis (NLRP3, Casp-1, IL-1R1) failed to mount optimal IFN-γ-polarized CD8+ T-cell responses. Moreover, caspase-1-deficiency resulted in deficient IFN-γ production by tumor antigen-specific CD8+ T cells, whereas normal mice mounted such T-cell responses in the draining LN of established tumors treated by systemic oxaliplatin injections (7). Hence, we showed that the NLRP3 inflammasome is critical for the immunogenicity of cell death triggered by anthracyclines, oxaliplatin, or x-rays.

Key contribution of ATP and purinergic receptors. Multiple distinct bacterial products or endogenous damage signals (such as toxins, ATP, uric acid crystals, alum, silica) stimulate the NLRP3 inflammasome resulting in the proteolytic auto-activation of caspase-1 (15, 20, 21). One of the most pleiotropic activators of the NLRP3 inflammasome is extracellular ATP, which is released from stressed cells and acts on purinergic receptors, mostly of the P2x subclass (22). To our surprise, multiple distinct cell death inducers (cadmium, etoposide, mitomycin C, oxaliplatin, cis-platin, staurosporine, thapsigargin, mitoxanthrone, doxorubicin) induced the release of ATP in vitro from dying tumor cells, by 8 to 20 hours postexposure (7, 23). This ATP release became undetectable when cells were incubated with the ATP-degrading enzyme apyrase or with inhibitors of ATP synthesis such as antimycin A plus deoxyglucose (A/D) or the oxidative phosphorylation uncoupler 2,4-dinitrophenol (DNP). When oxaliplatin-treated EG7 or anthracycline-treated CT26 cells were admixed with ATP scavengers (A/D, apyrase, DNP) or nonselective P2x receptor antagonists (iso-pyridoxalphosphate-6-azophenyl-2,5′-disulfonate or oxidized ATP), they lost their capacity to elicit protective antitumor immune responses upon subcutaneous inoculation into normal mice. ATP depletion or P2x receptor blockade also abolished the capacity of oxaliplatintreated EG7 cells [which express the model antigen ovalbumin (OVA)] to prime OVA-specific cells for IFN-γ production (7). Altogether, we generalized the finding that dying tumor cells release ATP, which is indispensable for their immunogenicity. In contrast, the immunizing capacity of candidate antigen proteins admixed in TLR adjuvants is not blocked by P2x receptor antagonists and hence is likewise independent of endogenous ATP.

Because the high affinity receptor for ATP is the purinergic receptor P2RX7, we assessed the ability of p2rx7−/− mice to mount a chemotherapy-induced IFN-γ-polarized CD8+ T-cell response and to control the growth of established tumors after chemotherapy. Importantly, oxaliplatin failed to control tumor progression in p2rx7−/− mice, and, similarly, oxaliplatin-treated OVA-expressing EG7 could not elicit OVA-specific T-cell responses in LN harvested from p2rx7−/− mice. Next, we showed that host DC were the cells harboring functional P2RX7 receptors during chemotherapy. In transgenic mice expressing the diphtheria toxin receptor on their DC (under the control of the CD11c promoter), diphtheria toxin depletes LN DC, and this maneuver readily abolishes the CD8+ T-cell response elicited by dying EG7 cells. The adoptive transfer of bone marrow-derived WT (but not in p2rx7−/−) DC loaded with dying EG7 restored the OVA-specific CD8+ T-cell response in p2rx7−/− mice. Accordingly, WT DC loaded with oxaliplatin-treated EG7 cells in vitro produced IL-1β in a NLRP3-, ASC-, and casp-1-dependent fashion, in conditions in which IL-12p40 secretion was independent of the NLRP3 inflammasome. As discussed above, ATP released by dying tumor cells engages P2RX7 receptors on DC, thereby triggering the NLRP3 inflammasome that culminates in IL-1β production and DC-mediated IFN-γ-polarized CD8+ T-cell response. How then could IL-1β contribute to the immunogenicity of cell death? We hypothesized that IL-1β may control and/or switch the quality of the priming of naïve CD8+ T cells during the...
treatment of a tumor by chemotherapy (7). We produced three lines of evidence supporting this contention.

First, DC loaded with dying EG7 cells induced the polarization of naive OT-1 cells (which express a transgenic T-cell receptor that recognizes an OVA-derived peptide, SIINFEKL) into IFNγ producing lymphocytes in a caspase-1- and IL-1β-dependent manner \textit{in vitro} and \textit{in vivo}. IL-1β receptor antagonist or neutralizing anti-IL-1β antibodies suppressed IFNγ polarization in this \textit{in vitro} priming system. Similarly, OT-1 cells were activated (CD69 expression) upon their adoptive transfer into WT mice, but not into casp-1−/− mice, following immunization with dying EG7 cells. IL-1RA also prevented the expansion of SIINFEKL/Kb tetramer-specific T cells in LN draining the immunization with dying EG7. Second, activation of naive CD3+ T lymphocytes by a cocktail of anti-CD3/anti-CD28 mAb in the presence of IL-1β (but not IL-6 or TNFα) resulted in a IFNγ polarizing effect that was similar to the one described for IL-12 (7). Third, the failure of p2rx7−/− and casp-1−/− mice to mount OVA-specific IFNγ-polarized CD8+ T-cell response after immunization with oxaliplatin-treated EG7 could be overcome by recombinant IL-1β coinjected with the vaccine (7).

In conclusion, IL-1β contributes to the full differentiation of IFNγ-polarized CD8+ T cells during the priming of anti-tumor immune responses that are elicited as a byproduct of anticancer chemotherapy.

**Discussion**

Our results identify tumor-derived ATP as a new DAMP, which is required for cancer cell death to be immunogenic. Our data are compatible with a scenario (Fig. 1) in which ATP activates P2RX7 receptors on DC, thereby stimulating the aggregation and/or activation of the NLRP3/ASC/Casp-1 inflammasome, the proteolytic maturation of caspase-1, pro-IL-1β cleavage, and consequent IL-1β release. IL-1β then is required for the priming of IFNγ-producing tumor antigen-specific CD8+ T cells (7). In accord with previous studies (24), IFNγ, rather than perforin or TRAIL-dependent cytotoxic activities, mediates the anticancer activity of T lymphocytes that have been primed in a P2RX7/NLRP3/ASC/Casp-1/IL-1β-dependent fashion.

New links between inflammasomes and cognate immunity are being progressively unraveled. Eisenbarth and colleagues

![Image](https://cancerres.aacrjournals.org/content/70/3/857/F1.large.jpg)
(25) showed that the NLRP3 inflammasome pathway stimulated by aluminum-based adjuvant (alum) directs a humoral adaptive immune response. Mice deficient in Nlrp3, ASC, or caspase-1 failed to mount an antibody response to antigen administered with alum, yet generated normal responses against antigens emulsified with complete Freund’s adjuvant. The Nlrp3 inflammasome was required for the production of IL-1β and IL-18 by macrophages in response to alum (25). It is noteworthy that in response to dying tumor cells, IL-18 was not produced by P2RX7/NLRP3-activated DC and IL-18 was not required for the immunogenicity of cell death in vivo (7). During lung infection with influenza A virus, ASC and caspase-1 (but not NLRP3) were required for CD4+ and CD8+ protective T-cell immunity, mucosal IgA production, and systemic IgG antibody responses (26), suggesting that each immune response relies on a specific mode of inflammasome activation and perhaps a specific pattern of inflammasome-dependent cytokines.

Importantly, we showed that a loss-of-function allele of P2RX7 that reduces the affinity of P2RX7 receptor for ATP compromises the efficacy of anthracycline-based chemotherapy in breast cancer patients, implying that the pathway we elucidated has clinical relevance and will presumably be clinically implemented for a better management of “personalized chemotherapy.”

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**

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