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Activation of dendritic cells by tumor cell death

Mara Cirone,* Livia Di Renzo, Lavinia V. Lotti, Valeria Conte, Pankaj Trivedi, Roberta Santarelli, Roberta Gonnella, Luigi Frati and Alberto Faggioni

Department of Experimental Medicine; Istituto Pasteur-Fondazione Cenci Bolognetti; Sapienza University; Rome, Italy

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A growing number of studies indicate that cell death can be either immunogenic or not, depending on its modalities, the type and the activation state of the cells, and finally, the environment where it happens. Increased understanding of the immunogenicity of cancer cell death will significantly improve the outcome of chemotherapeutic treatments.

Cell death can be induced in cancer cells by physical or chemical treatments. Chemotherapeutic drugs kill tumor cells often by inducing apoptosis that can be accompanied by autophagy or, in a later stage, by cell necrosis. However, in order to completely eliminate cancer cells and avoid cancer recurrence, it is of fundamental importance the activation of the immune system.[1] Dendritic cells (DC) are powerful antigen-presenting cells (APCs) that play a pivotal role initiating a specific immune response and in the eradication of apoptotic cancer cells by mediating the cross-presentation of tumor antigens to the cytotoxic T cells. Thus, one of the fascinating issues in oncoimmunology is inducing DC activation by tumor cell death to increase the effect of cancer therapy. Immunogenicity is dictated by dying cells' exposure or release of several molecules able to activate the diverse components of the immune system, such as macrophages, natural killer cells (NK) and DC.[2,3] The most characterized molecules involved in the immunogenicity of cell death are: calreticulin (CRT), heat shock protein (HSP) 70 and 90, ATP and high mobility group box 1 (HMGB1).[4] HSPs and CRT are located in the cytoplasm or into the endoplasmic reticulum (ER) and their exposure or release seems to be linked to ER stress, although the underlying mechanisms have not been fully elucidated yet. More recently, it has been reported that autophagy can play a role in the immunogenic cell death, because during the autophagic process, that may precedes the cell death, molecules such as ATP are released to recruit and activate DC.[5] HMGB1, another immunogenic molecule, is released when there is a severe damage of the plasmamembrane such as that induced by cell necrosis or necro-apoptosis, that represents the final event of an apoptotic cell death.

We have recently shown that anticancer drugs Bortezomib (Velcade), a proteasome inhibitor and Tyrphostin AG 490, a Janus Activated Kinase 2/signal trasducer and activator of transcription-3 (JAK2/STAT3) inhibitor, induce mmunogenic cell death in primary effusion lymphoma (PEL).[6] PEL is a non-Hodgkin's lymphoma characterized by poor response to conventional chemotherapy and by an extremely aggressive clinical course. By coculturing Bortezomib- and AG 490-killed PEL cells with DC we found that they were able to activate DC, as ndicated by the upregulation of CD83 and CD86 markers. However, nonetheless cell died by apoptosis, seen as Annexin V staining and higher percentage of sub G_0/G_1 DNA content, the use of broad-spectrum caspase-inhibitor zVAD-fmk only slightly reduced DC activation in the cocoltures with killed-PEL cells. These results suggest that apoptosis itself in PEL is not responsible for the immunogenicity of both Bortezomib and AG 490. Rather, important for DC activation was PEL cell surface exposure of CRT, HSP90 and HSP70. Thus, reduction in DC maturation was achieved by a cocktail of antibodies directed against CRT, HSP90 and HSP70. Since caspase-inhibitor treatment only slightly reduced CRT, HSP90 and HSP70 expression, these findings indicate that additional mechanisms other than apoptosis are responsible for their exposure. Studies are in progress to elucidate how it occurs, exploring if and which pathways of ER stress response are induced in PEL cells by Bortezomib and AG 490 and if other cell death modalities are involved in the immunogenicity occurred with these drugs. Since PEL is a virus-associated lymphoma, it will be also interesing to explore if during the Bortezomib-induced cell death, viral antigens, hidden in live cells, become exposed on cell surface, possibly in association with HSPs.

The AG 490-induced cell death and its immunogenicity are less explored. AG 490 acts by inhibiting STAT3 phopsphorylation and inducing apoptosis in cancer cells which display a constitutive activation of this molecule, such as PEL. STAT3 is also phosphorylated in the DC in the tumor environment and its activation correlates with their immunosuppression.[7] We found that AG 490 is non-toxic for DC cocoltured with PEL and several studies have shown that it can be also utilized to restore DC function in the tumor environment. Because of its action on both immune and tumor cells, the use of AG 490 as an inducer of immunogenic cell death could be very promising. Moreover, as STAT3 has been inversely correlated with autophagy, which has been reported

*Correspondence to: Mara Cirone; Email: Mara.Cirone@uniroma1.it
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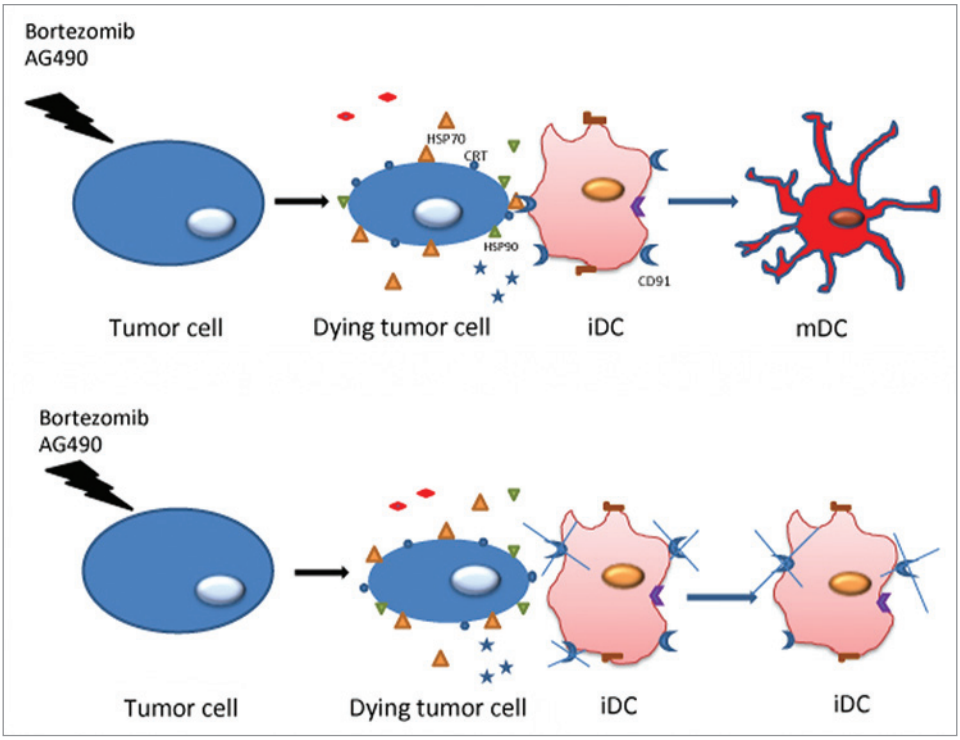

Figure 1. Schematic representation of Bortezomib- and AG 490-induced expression of Calreticulin, HSP 70 and 90 on the cell surface of dying tumor cells. CD91 neutralization by monoclonal antibody or RNA interference prevented the DC activation.

to be essential for monocyte differentiation into DC,[8] it will be interesting to evaluate whether STAT3 inhibition could promote DC autophagy and subsequently restore DC differentiation.

To further analyze the molecular mechanisms responsible of DC activation, we found complete inhibition of DC maturation by using a neutralizing antibody directed against CD91 (**Fig. 1**), also known as LRP1, a polyfunctional molecule that has been reported to be the common receptor for CRT, HSP70 and HSP90.[9] Similarly, strong reduction of DC activation was obtained following CD91 knockdown by small interference RNA. CD91 is one of the receptors for damage associated molecular patterns (DAMPs) able to mediate the phagocytosis and subsequent cross-presentation of foreign antigens. It can also bind the human defensins that, with an autocrine regulatory loop, upregulate its expression and activate DC. Its importance in the immune response is also underlined by the finding that it is the only molecule upregulated on the DC surface of long-term, non-progressor HIV-infected patients.[10]

Our results demonstrate a pivotal role of CD91 in the DC activation mediated by Bortezomib- and AG-490-killed PEL. It will be interesting to unveil the molecular pathways activated in DC after CD91 engagement and whether CD91 upregulation, for example by using defensins, might be exploited to enhance the immune response and increase the efficacy of the immunogenic chemotherapies.

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