

ROUND TABLE

Tumor Destruction and *In Situ* Delivery of Antigen Presenting Cells Promote Anti-Neoplastic Immune Responses: Implications for the Immunotherapy of Pancreatic Cancer

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Summary

Antigen presenting cells (APCs) activate helper and cytotoxic T cells specific for antigens expressed by tissue cells, including neoplastic cells. This event occurs after the antigen transfer from tissue cells to APC, and is referred to as “cross-presentation”. The number and the state of activation of APC in the tumor control the outcome of cross-presentation, including the establishment of protective immune responses. Cell death favors cross-presentation. Cancer cells normally die, either spontaneously or as a consequence of targeted therapies. The transfer of tumor antigens from dying tumor cells to APCs *in vivo*, exploiting the cross-presentation pathway, has the potential of yielding novel immunotherapeutic strategies. Their success will depend on at least two factors: the induction of synchronized cell death in the tumor, and the recruitment of activated dendritic cells in the tumor. Under normal conditions, pancreatic cancer represents a privileged environment; its profound chemoresistance reflects limited apoptosis after chemotherapy. Moreover, it usually contains only a few cells endowed with APC function. Endoscopic ultrasonography offers attractive possibilities of circumventing this privilege, including the delivery of ultrasound, radiofrequency or radiation in order to destroy the tumor and the

delivery *in situ* of autologous APC or appropriate chemotactic signals. In general, loco-regional approaches offer the possibility of using the tumor of each patient as a complex antigen source, thus limiting the risk of tumor escape and reducing the need for extensive *ex vivo* handling of the neoplasm and of the patient APCs.

Tumor Immunogenicity and Antigen Presenting Cells

The diagnosis of pancreatic cancer is often established in patients who have advanced diseases, with approximately half of them having metastases and the majority of the rest having locally diffused, unresectable disease. Moreover, this tumor is highly resistant to chemotherapy [1], with quite an unsatisfactory systemic mode of treatment and an extremely low survival rate. Novel therapies are sorely needed [1, 2, 3]. In recent years we have developed a better understanding of the interaction between the tumor and the host. Neoplastic cells express specific antigens which can be recognized by immune cells, leading to regression of the neoplasm and patient cure [4, 5]. Immune-mediated regression implies that tumor-specific T cells have been properly activated, a task accomplished *in vivo* by specialized

immune cells, referred to as antigen presenting cells (APCs).

Dendritic cells (DCs) are the most potent APCs. They originate from CD34+ bone marrow progenitors and reach peripheral organs *via* the blood. At the periphery, they develop to immature DCs, specialized in sampling tissue antigens *via* macropinocytosis, phagocytosis or receptor-mediated endocytosis. Primary pro-inflammatory signals switch the main function of DCs from antigen-uptake to presentation. Maturing DCs rearrange the actin-based cytoskeleton, acquire the sensitivity of attracting signals generated in the lymph nodes, where they migrate *via* afferent lymphatics. DCs complete the processing of the antigenic material on the way to the lymph node and up-regulate the expression of the molecules involved in the antigen presentation to the T cells [6, 7].

DCs infiltrate several tumors, including melanomas and carcinomas of the lung, stomach, prostate thyroid and breast. Tumor cells, by release of factors such as IL-6, IL-10, and vascular endothelial growth factor, influence DCs, inhibiting their maturation and their ability to migrate to secondary lymphoid organs and activate T cells [8]. Tumor - associated DCs are indeed often endowed with a low allostimulatory capacity, particularly if isolated from the progressing metastatic lesions. Moreover, at least in breast carcinomas, DCs are compartmentalized, with relatively large numbers of immature cells "trapped" in the lesion while mature DCs are preferentially found within the peritumoral areas [9]. A better survival rate after lymphadenectomy with a favorable clinical outcome and even the spontaneous regression of metastatic melanomas are associated with the infiltration of the tumor parenchyma by mature DCs, establishing close interactions *in situ* with tumor specific cytotoxic T cells [10].

Dendritic Cells, Cross-Presentation and Tumor Cell Death

The transfer of antigens expressed in neoplastic tissues to DCs for the productive

activation of T cells is a key event for the initiation of immune responses against the tumor. The transfer of intracellular antigens among living cells is a rare event. In contrast, cell death associates with the loss of the compartmentalization of intracellular components which are released in the microenvironment, where they elicit inflammatory responses [11, 12]. Moreover, antigen presenting phagocytes efficiently internalize apoptotic tumor cells, process them in dedicated intracellular vesicles and cross-present their antigens *in vivo* and *in vitro* [13, 14, 15, 16]. Tumor-associated DCs are apparently equally proficient at internalizing dying tumor cells, processing them and cross-priming tumor specific T lymphocytes [17].

Tumor cell death is important for generating a proper phagocytic meal for antigen presenting DCs by feeding them with tumor antigens [18]. Tissue damage *per se* activates immune responses [19, 20, 21] with acute but generally transient immune responses against intracellular antigens [22, 23, 24]. The preferential recognition of antigens contained in dying cells is facilitated by the release of soluble factors ("immune endogenous adjuvants") which amplify and sustain the function of APCs and the establishment of T cell dependent immune responses, *in situ* and at a distance [25, 26]. Therefore, the massive death of tumor cells, overwhelming the phagocytic ability of local scavenger cells, generates a pro-immune environment. Immunostimulatory factors include uric acid [27] and heat shock proteins, whose expression is often deregulated in neoplastic tissues [28]. Other intracellular factors released by necrotic cells with similar functions still remain to be identified.

Both antigen release and the delivery of immunostimulatory signals to APCs possibly contribute to the results described using radiofrequency ablation in an experimental mouse system [29]. Radiofrequency ablation is minimally invasive, widely used in different clinical settings and results in the generation of large burden of tumor cell debris [30]. Radiofrequency ablation alone

induced a specific immune response, dependent of the cellular arm of the immune system, capable of protecting a fraction of mice against a second challenge with a lethal dose of living tumor cells [29]. This result suggests that even procedures commonly used in clinical practice which result in the synchronized death of tumor cells, have the potential of initiating a specific immune response. It is, of course, quite clear that, in the absence of interventions aimed at amplifying and sustaining the elicited response, the treatment *per se* is unlikely to show any clinical benefit at a distance. Even in the model described in the study by Den Brok *et al.*, only were about the 20% of the treated animals actually protected [29].

Several factors contribute to limiting the efficacy of the response. They include the lack of activated APCs in the tumor in the correct time-frame for internalizing dying tumor cells and cross-presenting their antigens. Moreover, ablative treatments induce the *in vivo* release of pro-inflammatory factors and the production of acute phase proteins, such as the short pentraxin C-reactive protein, which is produced in the liver under the control of IL-6 [31]. Pentraxins bind to dying cells and control their immunogenicity, regulating their interaction with antigen presenting cells and controlling their interaction with key factors of the innate immune response, like the complement system [32, 33, 34, 35]. It is tempting to speculate that elevated levels of acute phase pentraxins, which possibly physiologically prevent the induction of autoimmunity after massive cell death [34], also contribute to quenching the immunogenicity of dying tumors and limiting protective immunity after ablation of human cancers.

Taken together, these findings imply that several interacting factors are involved in the initiation of antineoplastic immunity, including the availability of APCs at the tumor site, their activation state, the extent of cell death taking place in the neoplasm, the possible recruitment of immunosuppressive circuits at the site of the tumor cell death.

Pancreatic Cancer as a Privileged Tissue

Extensive tumor cell death takes place in human cancer, either spontaneously or as a consequence of targeted therapies. The case of pancreatic cancer is peculiar; its poor prognosis is indeed associated with resistance to cytotoxic drugs, a phenomenon known as chemoresistance [1]. Of importance, pancreatic adenocarcinomas comprise an important mesenchymal stromal component. Continuous tumor-stroma interactions have been proposed to lead to the generation of anti-apoptotic soluble and gaseous messengers [36]. Chemoresistance could therefore associate to apoptosis resistance, resulting in the lack of cell death induction at the tumor site. A second factor possibly restricting the immunogenic potential of pancreatic cancer is the lack of DCs as compared to other neoplasms [37]. Both lack of cell death and lack of interaction between tumor and host APCs might contribute to poor T cell priming and failure of tumor rejection [5]. The reasons for the selective paucity of DCs in pancreatic cancer (and not, for example, in laryngeal, colorectal, lymphoma, uterine, gastric, papillary thyroid, or lung cancers, in which the presence of tumor-associated DCs correlates with improved survival [37]) are poorly understood. Theoretically, DC apoptosis could be induced by the tumor [38]. Indeed, the exposure of DCs propagated *in vitro* to the anti-apoptotic gaseous messenger, nitric oxide, both protects them from apoptosis *in vivo* and enhances their function in the tumor [39]. However, some evidence suggests that a defective homing of DCs to the tumor, more than the active induction of cell suicide of infiltrating DCs, is involved. Schmidt *et al.* developed an experimental model of pancreatic cancer based on the implantation of ductal pancreatic adenocarcinoma cells in healthy mice; this model nicely reproduces several features of the human disease, including the lack of infiltrating DCs [40]. These authors therefore relied on ultrasound-guided orthotopic inoculation to inject DCs into the growing tumors. DC survival was apparently

unaffected by the tumor environment *in vivo* while, provided that they had been previously loaded with tumor antigens, DCs generated a potent antineoplastic immune response, quite superior to the response elicited by DC vaccination s.c. or i.v.. These results suggest that, once transferred into the pancreatic cancer, DCs not only survive but have a conserved antigen presenting function.

APCs and Loco-Regional Strategies

Direct injection of DCs into the tumor lesion has been proposed as a reliable approach in experimental systems. This procedure minimizes the need of *in vitro* loading with tumor antigens, circumvents the problem of efficient homing systemically delivered DCs to the tumor, and can be efficiently combined with other approaches, including chemotherapy and radiotherapy [41, 42, 43]. However, alternative approaches exist to enrich the tumor with activated APCs, which may prove valuable in the clinical setting. For example, Furumoto *et al.* [44] directed circulating DCs to experimental tumors inducing the expression of the CCL20/MIP-3alpha chemokine by gene transduction or by directly injecting the recombinant protein into the tumor. Depending on the model used, the simple attraction of DCs *in situ* was sufficient to elicit a specific anti-tumor response, dependent on CD4 and CD8 T cells. However, the activation state of DCs is a limiting factor (see above) and the receptor for CCL20/MIP-3alpha is down-regulated during DC maturation. In more stringent models, the simultaneous injection of unmethylated cytosine-guanine rich sequences and immunostimulatory oligonucleotide motifs to activate DCs was necessary in order to obtain clinically relevant protection [44]. Several other factors contribute, including the immunosuppression generated by the tumor or by conditioned infiltrating leukocytes, such as IL-10 [45, 46], which are known to inhibit DC maturation and function. Not surprisingly, in other studies the reversal of these inhibitory

influences, for example by an IL-10 blockade, was also necessary in order to establish a protective antineoplastic immune response [47].

Conclusions

Most of the findings described until now were obtained in experimental models. In the future, verifying whether immunotherapeutic strategies based on these insights will provide an advantage in clinical settings will be a major challenge. Pancreatic carcinoma is a natural candidate, given the unsatisfactory results of the therapeutic tools currently available. Moreover, the lack of infiltrating DC seems to quite clearly lay a possible roadmap for future study. The poor induction of cell death in the context of the tumor by systemic therapy could possibly be circumvented by ablative approaches, which result in efficient destruction of the targeted tissues (e.g. Chan *et al.* [48]). Endoscopic ultrasonography (EUS) is, at present, a powerful tool for imaging and substantiating diagnosis. However, its possibilities for anti-tumor therapy include the local destruction of tumor cells by ultrasound, radiofrequency or radiation [49]. The same approach may be exploited to locally deliver cells (including APC) or pharmacological agents (including drugs and chemokines). A creative use of the possibilities of EUS [49] will prove valuable for immunotherapy, increasing the possibility of a successful outcome of novel vaccination procedures.

Keywords Apoptosis; Dendritic Cells; Endosonography; Immunity, Natural; Necrosis

Abbreviations APC: antigen presenting cell; DC: dendritic cell

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