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REVIEW

Feeding dendritic cells with tumor antigens: self-service buffet or à la carte?

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Adoptive transfer of autologous dendritic cells (DC) presenting tumor-associated antigens initiate and sustain an immune response which eradicate murine malignancies. Based on these observations, several clinical trials are in progress testing safety and efficacy with encouraging preliminary reports. In these approaches, ex vivo incubation of DC with a source of tumor antigens is required to load the relevant antigenic epitopes on the adequate antigen presenting molecules. Recent data show that in some instances exogenous DC artificially injected into malignant tissue or endogenous DC attracted to the tumor nodule by means of gene transfer of GM-CSF and CD40L into malignant cells result in efficacious antitumor immunity. In the case of intratumoral injection of DC the procedure is curative only if DC had been genetically engineered to produce IL-12, IL-6 or to express CD40L. Evidence has been obtained showing that intratumoral DC can capture and process tumor antigens to be presented to T-lymphocytes. Although the exact mechanisms of tumor antigen acquisition by DC are still unclear, available data suggest a role for heat shock proteins released from dying malignant cells and for the internalization of tumor-derived apoptotic bodies. Roles for tumor necrosis versus apoptosis are discussed in light of the 'danger theory'. Gene Therapy (2000) **7,** 1167–1170.

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Intratumoral release of DC for immunotherapy

The use of *ex vivo* differentiated dendritic cells (DC) to induce or amplify antitumor immune responses therapeutically is finding its way into the clinic with encouraging results in pilot studies.[1] DC have unique capabilities to induce T cell-dependent immunity due to their outstanding array of membrane MHC antigen presenting molecules, cytokines, costimulatory factors and ability to traffick into lymphoid organs.[2–4] In murine models, *ex vivo* gene transfer strategies have been used to provide DC with the genes coding for cytokines to enhance their immune adjuvant activity further[5–9] or with genes coding for tumor rejection antigens in order to present them to relevant T cells.[1,10]

Different sorts of genes and vectors have been used to transduce DC with tumor antigens and notable success has been achieved with recombinant adenovirus[11–13] and naked or liposome formulated RNA.[14,15] Alternatively, tumor antigens can be loaded on to DC MHC antigen presenting molecules by pulsing the cells with synthetic peptides,[16–18] purified proteins,[19] tumor lysates,[18,20] or crude eluted peptides from malignant cells.[21] Coculture of DC with tumor cell lines can also result in sufficient antigen uptake by DC to stimulate CTL-mediated immune responses able to generate antitumor protective immunity.[22] Injection of immature cultured DC with irradiated tumor cell lines also induced protective, but low levels of therapeutic immunity.[23] Moreover, intratumoral injection of DC was not therapeutically efficacious (unless the tumor was surgically removed), when specific antitumor immunity developed.

Although intratumoral injection of DC by itself has very modest therapeutic results,[8] two recent reports have shown that if DC are engineered to secrete IL-12 with recombinant retrovirus[7] or adenovirus,[8] they induce very intense therapeutic antitumor immunity against the treated malignant nodule and coexisting noninjected tumor nodules. The same has been observed when intratumorally injected DC have been modified with an adenovirus coding for CD40L.[24] The antitumor effector cells in either case were primarily $CD8^+$ cytotoxic T cells,[8] presumably primed by the adoptively transferred DC, which had actively migrated into T cell areas of draining lymph nodes after picking up the tumor antigens from malignant cells.[7,8] Intratumoral injection of artificially cultured DC is not the only possibility, since endogenous DC can be attracted to the tumor tissue with the help of specific chemotactic factors. For instance, tumors transfected with GM-CSF become infiltrated by DC and, if the tumor is cotransfected with CD40L to activate and mature these infiltrating DC, very powerful antitumor immune responses are unleashed.[25]

IL-12 is a cytokine which induces IFNγ production from many cell types such as T cells, NK cells[26] and dendritic cells.[27] It has been shown to be important to differentiate responding T helper cells to a Th1 phenotype.

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Both the recombinant protein and its gene transduced into tumors have shown remarkable therapeutic activity due not only to enhancement of antitumor cellular immune responses but also to impairment of tumor angiogenesis.[28,29] It is clear that IL-12 secreted by intratumorally injected DC greatly up-regulates the antitumor properties of DC.[7,8] Several nonmutually exclusive mechanisms could account for this beneficial effect: (1) IL-12-secreting DC could prime a more intense CTL and Th1 response upon arrival at lymphoid organs; (2) IL-12 can stimulate DC in an autocrine fashion to acquire certain functions such as IFNγ secretion;[27,30,31] (3) IL-12 can act on the tumor stroma either directly or through secondary cytokines to promote inflammatory changes in endothelium to promote homing of effector tumor-killer T cells[32,33] or to impair tumor angiogenesis.[34]

To trigger the immune system properly, DC should be activated. Indeed, DC derived from cultures of monocytes or bone marrow precursors with GM-CSF and IL-4 display a so-called immature phenotype. In this stage DC avidly acquire antigens, but are poor at stimulating specific T cells.[3,4] Certain stimuli are known to reverse these properties in a process named maturation which encompasses the orchestrated regulation of multiple genes. Many factors promoting DC maturation have been identified and can be classified into three groups: (1) bacterial or viral components (ie bacterial DNA,[35] Lypopolysaccharide,[36] dRNA,[37] etc); (2) endogenous proinflammatory factors (ie IL-1, TNFα,[38] IL-12,[30] or possibly released heat shock proteins[39]); (3) DC interaction with activated T helper cells providing stimulation through surface proteins such as CD40 and/or MHC-class II.[40,41] An immature phenotype of DC to be injected in the tumors was chosen to allow DC to take up and present antigen from tumor cells and then migrate into lymph nodes, but a formal proof for this concept has not yet been published. Stimulation by gene transferred IL-12 and CD40L is probably critical in such settings to enhance certain DC functions which are necessary for therapeutic efficacy. It has been published recently that intratumoral injection of DC engineered with recombinant adenovirus to secrete IL-7 also displayed potent antitumor properties.[9] In this case the main effect of the IL-7 transgene is likely the expansion of antitumor T cell clones, although relevant direct or indirect effects of IL-7 on DC cannot be excluded.

Mechanisms of antigen capture

A key issue is the mechanism of antigen uptake from tumor cells. Antigen capture inside the tumor tissue has been proved by recovering DC from the malignant nodule and by testing their ability to activate *in vitro* T cells specific for tumor antigens and to immunize naive mice for CTL induction.[25] In this setting DC were found to internalize TUNEL+ apoptotic material, but although this mechanism has been proposed to be important for antigen transfer in *in vitro*,[25] the finding has not been stressed since there was no proof that apoptotic bodies were from tumor cells. Other studies suggest that cells dying through necrosis rather than by apoptosis are the most immunogenic, since they leave their remains in a fashion prone to pass their antigens into DC.[42] In fact, release of proteins such as hsp-70 by dying cells has been shown to be very efficient means of antigen transfer to the antigen presenting pathways of DC.[43,44] The mechanisms of action of hsp70 are still elusive, although one possibility is the proposed ability of hsp to chaperone immunogenic peptides into APC.[45] In addition, factors released by dying cells can activate DC maturation, especially when not cleared by scavenger macrophages.[46,47]

In most tissues natural cell death occurs via apoptosis. Such a process is likely ignored by the immune system or may be involved in the induction of tolerance to normal self components.[48] In contrast, we propose the concept of a stressful cell death, in which cells, regardless of whether they are dying by apoptosis or necrosis, release these endogenous DC activators, which are known to be overexpressed in cells under stress.[49] Accordingly, cells dying in an environment containing microbial components or proinflammatory cytokines will also end up with their antigens being presented in an immunogenic fashion. This is consistent with the overall concepts of the danger theory proposed by Matzinger,[50,51] according to which naive T cell precursors only become activated if they see their antigen on an activated (or mature) dendritic cell. Injection of DC in a tumor mass can trigger such stressful cell death events by mechanical damage caused by the needle and the injected fluid. In addition, DC can execute direct cytotoxicity against certain tumor targets[52] and/or they can locally activate NK cells.[53] Interestingly, IL-12 gene transfer can increase these NK cell activating properties of DC. Therefore, strategies to enhance tissue necrosis in the tumor mass before artificial release of DC should be evaluated to enhance efficacy.

Tumor masses contain normal components in the stroma and malignant cells share most of their protein sequences with normal cells. This may raise concern about the risk of triggering autoimmunity against normal self tissue. Nonetheless, careful monitoring of mice successfully treated by intratumoral injection of gene modified DC have not shown signs of autoimmune disease.[8]

Intratumoral injection of DC engineered to produce IL-12 is now reaching the clinical arena due to its feasibility and preclinical results. The outcome of the trials will show if this promising approach is really safe and efficacious. In addition, repeated intratumoral injection of DC is feasible and, as long as there is enough injectable malignant tissue left, repetitive boost of antitumor immunity should be possible.

Conclusion

The explosion in the molecular identification of tumor antigens offers new hope for construction of successful vaccines for cancer. However, even now it seems clear that single antigens will not suffice for effective clearance of tumors consisting of polyclonal cells with a range of antigens expressed and lost. One trend has been the construction of poly-epitope vaccines delivered by DNA or viral vector-mediated vaccination approaches. However, even this à la carte construction of vaccine components leaves too much to chance and runs the risk of leaving out crucial antigenic components which may not even have been identified yet. Surely it is better to leave the dendritic cells to sample and display the antigens that may be most relevant to raising effective antitumor responses directly *in vivo*. Although our molecular skills at cloning tumor antigens are proceeding impressively fast, it seems more sensible to facilitate the entry of DC into tumors (either by gene modification of the tumor

cells or by direct intratumoral injection) and let them serve themselves from the feast of potential antigens than to assume that we can construct a better menu for them ourselves.

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