Commentary

Dendritic Cells as Adjuvants for Immune-mediated Resistance to Tumors

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The Problem: Tumor Immunity Is Not Effectively Induced in Tumor-bearing Hosts. Animal experiments as well as clinical experience, notably in melanoma, indicate that the immune system can recognize and kill tumor cells. In particular, CTLs recognize MHC class I–peptide complexes on the tumor cell surface, and the peptides are derived from nonmutated or mutated genes that can be primarily expressed in the tumors (1–3). Why then does the immune system fail to eradicate most antigenic cancers? One clue is the observation that CTLs for melanoma and other human tumors are not known to be expanded. For example, CTL precursors are not increased in melanoma patients, whereas humans who are infected with a virus like influenza frequently show expansions in virus-specific CTLp. Therefore tumors have antigens for T cells, but these do not appear to be immunogenic in vivo.

The Hypothesis: Lack of Tumor Antigen Presentation by Dendritic Cells In Vivo Is a Major Problem that Can Be Bypassed by Delivery of Tumor Antigens on Autologous Dendritic Cells. One possible reason for a lack of tumor immunity is that the antigens are not being presented by dendritic cells (DCs). DCs are antigen-presenting cells that are specialized to prime helper and killer T cells in vivo (recently reviewed in references 4 and 5). To do so, DCs perform a series of coordinated tasks. Immature DCs develop from hematopoietic progenitors and are strategically located at body surfaces and in interstitial spaces of most tissues. There, DCs are equipped to capture antigens and to produce large numbers of immunogenic MHC–peptide complexes. In the presence of maturation-inducing stimuli such as inflammatory cytokines or stimulation via CD40 (6), DCs upregulate adhesion and costimulatory molecules to become more potent, terminally differentiated, stimulators of T cell immunity. At the same time, numerous intracellular MHC II compartments seem to discharge MHC II–peptide complexes to the cell surface where they can be unusually long lived (7, 8). DCs also migrate to secondary lymphoid organs to select and stimulate rare antigen-specific T cells (9, 10).

Nonetheless, there is no evidence that DCs capture and process antigens from malignant cells in vivo. Tumors, for example, can make products like IL-10 (11, 12) and vascular endothelial growth factor (13) that could decrease DCs development and function. Is a lack of tumor antigen presentation by DCs a major problem in generating T cell–mediated resistance to tumors in vivo? If so, could DCs be used as adjuvants to induce strong tumor-specific immunity?

Some Evidence: Administration of DCs that Are Presenting Tumor Antigens Induces Therapeutic Immunity in Tumor-bearing Mice. The ability to explore DCs as adjuvants for immunization against tumors emerged with better knowledge on their growth and development. By applying appropriate cytokines, such as GM-CSF, one can generate large numbers of DCs ex vivo from mice, rats, and humans (14–23, and Talmor, M., A. Mirza, S. Turley, I. Mellman, L.A. Hoffman, and R.M. Steinman, manuscript submitted for publication). Therefore, autologous DCs from tumor-bearing patients can be expanded ex vivo, charged with tumor antigens, and reinfused to induce tumor-specific T cells including CTLs. This new approach would bypass the proposed obstacle, i.e., that tumor antigens do not access DCs in vivo. Prior work using bone marrow–derived murine DCs have shown induction of CTL-mediated tumor immunity and resistance in vivo (see review in reference 24).

Two new papers in this issue of the journal (25, 26) emphasize the treatment of established tumors with DCs and the use of gene transfection to charge DCs with a model antigen. Both Specht et al. and Song et al. used the murine tumor CT26.CL25, a subclone of the CT26.WT BALB/c undifferentiated colon adenocarcinoma that is stably transduced with *Escherichia coli* β-galactosidase as a model antigen. After intravenous inoculation of CT26.CL25 tumor cells and formation of lung metastases, no β-galactosidase–specific CTLs were noted. However, after a single intravenous or subcutaneous injection of $4–5 \times 10^5$ antigen-bearing DCs, (*a*) a specific CTL response was induced, (*b*) the number of lung metastases decreased, and (*c*) survival was improved. The DCs were generated from marrow progenitors (Song et al. also used a murine epidermis-derived DC line) and transduced with the β-galactosidase gene via retroviral (25) or adenoviral (26) vectors. These findings illustrate the lack of tumor antigen presentation in vivo and its reversal with tumor antigen-charged DCs. It is now necessary to study authentic tumor antigens, rather than models like β-galactosidase. Interesting other new strategies for the induction of tumor immunity, such as naked DNA encoding tumor antigens and cytokine-transfected tumor cells, are also dependent on the presenting function of host DCs (27).

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If antitumor immunity can be induced with DCs in vivo, it should become possible to explore potential obstacles to successful immunotherapy of cancers. For example, once CTLs are induced, it will then be easier to determine if the CTLs migrate to the tumor and are activated there, and if there is a loss of tumor antigen presentation. If one cannot induce CTLs via DCs, has tolerance to the tumor antigen been induced?

Clinical trials are now addressing the safety and efficacy of tumor antigen–charged DCs. Melanoma is being emphasized because this is a prevalent tumor for which many antigens have been shown to be recognized by CTLs. Several variables have to be addressed to identify optimal strategies, and we would like to consider some of these.

Several Methods to Generate Human DCs for Clinical Use. Recent studies have uncovered complexity in the DC lineage with several subsets, functions, and maturation stages (22, 23, 28, 29). Besides classical immunostimulatory "myeloid" DCs, there may be regulatory fas-ligand bearing "lymphoid" DCs (30). With respect to the stage that will prove optimal for immunization, the evidence indicates that the more potent mature DCs should be prioritized. The many groups that have demonstrated the efficacy of DCs in mice have primarily used mature, marrow-derived cells.

Immunostimulatory human DCs are generated from proliferating $CD34^+$ and from nonproliferating $CD14^+$ progenitors. The $CD34^+$ method uses GM-CSF and TNF-α as cytokines, and either bone marrow, cord blood, or adult blood as a source of progenitors (16–18). Adult blood is the most accessible, but requires G-CSF pretreatment of the patient to increase the otherwise minimal percentage of $CD34^+$ cells. The $CD14^+$ method uses GM-CSF and IL-4 as key cytokines (22, 23), and the abundant monocyte fraction as the starting population. One variable among labs is the type of serum that is being used, FCS versus human serum or plasma. FCS batches vary considerably in efficacy, and batches that are LPS-free do not appear to work well (Schuler, G., unpublished data). For therapy, FCS also might be infectious and immunogenic, and it therefore should be avoided, especially since it is feasible to generate potent DCs in the absence of FCS (21–23). Hsu et al. (31) also have used preformed DCs from blood, vaccinating B cell lymphoma patients with the Ig idiotype from the tumor.

A set of criteria has developed to document the maturation of DCs. MLR stimulatory activity should be strong, since mature human DCs induce a strong MLR at DC/T cell ratios of 1:1,000 with stimulation indices of 20 or more. Mature DCs have large veils or sheet-like processes that extend in several directions from the cell body. The phenotype includes expression of CD83 and CD25, and high levels of CD86 and HLA-DR. When cytokines are removed, mature DCs are stable, whereas if cells are not matured, they can revert to adherent macrophages. With maturation, the DCs also can produce large amounts of IL-12 and become resistant to the suppressive effects of IL-10 on antigen-presenting function.

The Frequency and Route of DC Injections. In mice, mature DCs induce immunity to tumors after injection by the subcutaneous or intravenous routes. The optimal injection site for human immunization needs to be determined. It may be that upon intracutaneous injection, DCs will activate in the draining lymph nodes those T cells that preferentially home back to skin and are therefore most effective at this site. In mice, recent data indicate that upon intravenous injection, DCs home not only to spleen but also to liver-draining lymph nodes (32). Direct intranodal injection is another possibility (33), but should not be necessary given the homing properties of DCs.

The development of strong memory would reduce the frequency of DC injections, but to date, there has been little work on T cell memory after DC priming in situ. Strong memory might be facilitated by the fact that MHC II–peptide complexes can be retained for long periods, providing the DCs themselves with a type of "memory" (7, 8). Human studies will indicate how long memory persists after DC priming, and how often the DCs will have to be given to achieve optimal immunity.

Tumor Antigens and Loading Methods. For assessing variables that lead to optimal immunogenicity, peptide-pulsed DCs should be suitable. One can then test if the peptide-specific CTLs also kill the tumor in question and not just experimental, peptide-pulsed targets. The value of immunizing MHC class II–restricted, $CD4^+$, helper and killer T cells needs to be explored. Investigators also are studying many approaches to bypass the MHC restriction that is imposed by the use of peptides. Genetic transduction is one such approach, as carried out by Song et al. (26) and Specht et al. (25), and will allow the DCs to tailor the peptides from a tumor antigen so that the peptides can be presented by any given patient's HLA products. Genetically transduced DC might also express MHC-peptide complexes for very long periods, which may be vital given recent data on the relatively short half life of MHC I–peptide complexes on DCs (8).

A single dose of DCs modified with adenoviral and retroviral vectors can be effective for immunizing tumor-bearing mice, as shown by the two papers in the current issue. For repeated vaccinations, induction of antiviral or antivector immunity may pose problems. Nonviral or modified viral vectors therefore need exploration.

If exogenous proteins can target MHC I products of human DCs, as described for mouse marrow–derived DCs (34), recombinant tumor antigens will need to be tested. An exciting goal is to arm DCs with the full antigenic spectrum of tumor cells. Some reported approaches are to transduce DCs with tumor-derived RNA (35), to fuse DCs with whole tumor cells (36), and to induce DCs to phagocytose and present whole tumor cells (Albert, M.L., B. Sauter, and N. Bhardwaj, manuscript submitted for publication).

Another recent development is the production of monoclonal antibodies that recognize MHC–peptide complexes (37, 38). This could soon permit researchers to monitor and optimize the many possible antigen-loading procedures for DCs.

Monitoring the Efficacy of Active Immunization with DCs. Pilot experiments (31, 39, 40) suggest that DC injections are safe, and larger trials are underway. It will be important now to demonstrate immune efficacy, and use these responses as a guide to optimizing this approach. Proving that immunization has occurred, particularly for CTLs, is not straightforward. Classical measurements for effector CTLs in blood (^{51}Cr–release assays) may be insensitive, whereas standard precursor frequency measurements are laborious, especially for kinetic studies. Restimulation in vitro to activate memory CTLs is far from routine with human cells, but may now be feasible after DC enrichment (41). Novel quantification methods for $CD8^+$ T cells such as semiautomated ELISPOT analysis (42) and binding of tetrameric MHC–peptide complexes (43) should be tested.

Additional Strategies to Avoid Tumor Evasion of the DC-induced Immune Response. There are animal models in which it is difficult to reject established tumors with immune activated T cells (44). After DC-based immunization procedures are established, it will be possible to investigate potential obstacles to the efficacy of tumor-specific CTLs. Ancillary strategies may be required, for example, to (*a*) mobilize and activate the immune T cells in the tumor, (*b*) obviate tumor-mediated resistance as by expression of fas ligand (45) and inhibitory cytokines (12, 13), and (*c*) reverse the downregulation of antigen presentation that can occur (46, 47). Supplemental anti tumor approaches such as inhibitors of proteases (48) and angiogenesis (49) may be necessary.

Some Future Issues in DC-based Immune Therapy and Vaccination. The new animal experiments in this issue indicate that DC therapy for established tumors can be a significant strategy. Clinical studies of DC therapy for existing tumors and DC vaccination at the time that a primary tumor is removed should both be considered. Current methods for DC-based immunization are within the realm of other cell therapies in terms of feasibility.

It is also possible that ex vivo generation of DCs will be superseded by methods to effectively load DCs with tumor antigens in situ, and to activate their stimulatory and migratory functions. Antitumor immunity may develop if DCs (or other cells) are substantially expanded in vivo by, e.g., flt3 ligand (50). A recent report even reveals that DCs themselves can have an NK-like, cytolytic activity on certain targets (51).

We have emphasized human studies here, but the careful animal experiments of Specht et al. and Song et al. in this issue illustrate that a lack of tumor antigen presentation by DCs can be an important cause for the absence of T cell–mediated resistance in tumor-bearing hosts. Additionally, the ex vivo and genetic transduction approaches in these two papers are of wide interest for human studies. This is an intriguing time for using DCs to actively manipulate the immune response to tumors and other clinically important antigens.

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