Commentary

Dendritic Cells as Adjuvants for Class I Major Histocompatibility Complex–restricted Antitumor Immunity

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The demonstration of autologous CD8^{+} CTL with specificity for class I MHC–restricted tumor antigens is a cornerstone of the field of tumor immunology (1, 2). Immunotherapy of malignant diseases is therefore an attractive possibility, but the frequency of immunoreactive T cells is extremely low and variable in the tumor-bearing host (3, 4). The critical challenge now is to augment the frequency of active, tumor-specific lymphocytes, thus translating a trace repertoire into effective immune responses in vivo. Three papers in this issue have begun to address this challenge by using dendritic cells to elicit antigen–specific, tumor-resistant immune responses in mice (5–7).

Approaches to Active Cell-mediated Immunotherapy of Malignant Disease

Tumor Cells Transduced with Cytokines. With the advent of molecular approaches to alter cell function, investigators have evaluated the therapeutic use of tumor cells transduced with cytokine genes to enhance in vivo immunity. The rationale is that cytokine production by the tumor would increase protective local and systemic responses, including the expansion of CTLs, the recruitment of helper T cells and NK cells, and the enhancement of host APC function, while minimizing systemic toxicity (8, 9). A number of cytokines have exhibited activity in vivo in rodents, including resistance to subsequent challenges by native, nontransduced tumors. Dranoff et al. separately evaluated eight cytokines, each encoded by a retroviral construct and expressed in the B16-F10 melanoma cell line (10). GM-CSF–transduced tumor cell vaccines proved to be the most effective in eliciting potent, durable, tumor-specific immunity, and successful vaccination required both CD4^{+} and CD8^{+} T cells. It was hypothesized that GM-CSF expressed by the tumor vaccine might locally recruit and enhance the activity of host APCs, like dendritic cells. Further studies using parental → F$_1$ bone marrow chimeras in fact demonstrated that priming of an antitumoral T cell response can occur in the context of MHC products on host APCs, rather than the MHC molecules expressed by the immunizing tumor (11).

Enhancing Costimulatory Signals Delivered by Tumors. Among the many plausible mechanisms by which tumors may escape immune rejection is their failure to exert the necessary and sufficient costimulatory signals for stimulating resting or unprimed T cells. The CD28/CTLA-4/CD80/CD86 family of molecules represents attractive candidates to enhance immune responsiveness against tumors, given their capacity to enhance cytokine production and prevent anergy. Some murine tumor models have in fact demonstrated that transduction of B7-1/CD80 genes for costimulation with tumor antigen can elicit a host response against a tumor in an antigen-specific fashion (12–15). This has resulted in actual tumor rejection, as well as antigen-specific immunologic memory manifested by resistance to rechallenge by nontransduced tumor cells.

Dendritic Cells as an Approach to Enhance the Host Response to Tumor Antigens. Dendritic cells are bone marrow–derived leukocytes whose properties for antigen presentation and initiation of T cell–dependent immune responses are more developed and far more potent than those of other antigen-presenting cells (16). Although a trace leukocyte population, dendritic cells are widely distributed in vivo at portals of entry for efficient antigen capture. They can also retain and present antigen in immunogenic form for several days (17, 18). Dendritic cells' migratory properties via blood and afferent lymph facilitate their movement between sites of origin and antigen capture to the T cell–enriched areas of lymphoid tissue. There they are positioned to sample large numbers of circulating T cells and select infrequent clones with specific reactivity for the presented antigen. This in turn should foster the egress of activated, antigen-specific T lymphocytes into the periphery as effectors.

In addition to the formation of peptide–MHC ligands for clonotypic TCRs, dendritic cells express high levels of critical accessory signals, both costimulatory and adhesive, including B7-1/CD80, B7-2/CD86, ICAM-1/CD54, ICAM-3/CD50, and others (19–22). This is pertinent to the murine models cited above, where costimulation with tumor antigen has proven critical to tumor immunogenicity. This potent accessory phenotype is also presumed to

support the capacity of dendritic cells to initiate CD8$^+$ CTL responses, independent of CD4$^+$ help (23–26).

Several reports have documented the ability of antigen-pulsed dendritic cells to prime class II MHC–restricted T cells in vivo (17, 27). By using parental dendritic cells to immunize F$_1$ hybrids, one of the critical experimental findings was that priming and immune responsiveness occurred only in the context of the shared parental MHC haplotype. No reactivity was demonstrated in the context of the un-shared haplotype, proving that antigen was not just shed from the immunizing dendritic cells and presented by host APCs.

It has also been shown that murine dendritic cells loaded with peptide can prime antigen-specific CD8$^+$ CTLs in vivo (28, 29). By using as few as 0.5–1 $\times$ 10^5 GM-CSF–generated, murine bone marrow–derived dendritic cells pulsed with OVA peptide, Porgador and Gilboa (29) have generated potent, antigen-specific CTL activity. Immunization with OVA-pulsed dendritic cells induced CTL that lysed not only targets pulsed with the OVA peptide, but also target cells expressing OVA peptide after transduction with OVA protein. Alternative adjuvants to dendritic cells could not prime these antigen-specific CD8$^+$ CTL.

Investigators in this and other contexts are now applying methods for generating large numbers of dendritic cells from precursor populations. No longer are investigators dependent upon the small numbers of terminally differentiated dendritic cells that can be obtained from most tissues like blood. In both murine and human systems, GM-CSF has proven to be an essential cytokine in dendritic cell growth and differentiation (30–39), with additional contributions by TNF–α or CD40L/gp39 (32–35, 40–42), IL-4 (35, 43), and c-*kit* ligand (41, 42).

Antigen-pulsed Dendritic Cells Generate Protective Immunity to Tumors. All of these considerations provide a rationale for using dendritic cells as immunogens against malignantly transformed cells. Dendritic cells have been implicated in the generation of largely class II MHC–restricted host responses to tumors (44–46). The initiation by dendritic cells of class I MHC–restricted CD8$^+$ CTL immunity against tumors, however, has been experimentally tested in murine models for the first time in the papers under consideration here (5–7).

Celluzi et al. have used murine dendritic cells generated from bone marrow with the support of GM-CSF and IL-4 (6). Small numbers of these dendritic cells, pulsed with an OVA peptide that constitutes a strong CTL epitope [SIIN-FEKL], protected mice against a lethal challenge with an OVA-transduced tumor cell line. Protection was CD8$^+$ mediated and OVA specific. These mice survived a second challenge, however, by the more weakly immunogenic, untransduced, or parental tumor. These results complement earlier studies by Boon and colleagues using tum^{neg} variants (1), and they suggest the possibility that immunization by dendritic cells with a potent antigen, simultaneously expressed by a poorly immunogenic tumor, may effect subsequent resistance to the tumor itself. In reality, the great majority of tumor antigens are either unknown or indeterminate with regard to their immunogenic CTL epitopes. To counter this problem, Zitvogel et al. have used unfractionated, acid-eluted peptides from tumor cell lines of three different histologies and derived from two mouse strains to load syngeneic dendritic cells for vaccination (5, 47). This approach may have provided additional class II MHC–restricted epitopes for stimulation of helper lymphocytes and enabled the concomitant presentation of several tumor antigens. Dendritic cells were again generated from murine bone marrow under the influence of GM-CSF and IL-4, and they were again highly effective adjuvants in small numbers. Successful immunization by these antigen-pulsed dendritic cells was documented by the elimination or suppression of growth by small but established tumors, in contrast to rejection of a tumor challenge after vaccination. An important role for costimulatory signals provided by dendritic cells was demonstrated by the partial inhibition of their efficacy as immunogens when mice were systemically treated with CTLA4-Ig. Other accessory ligand–receptor interactions could be expected to account for the remaining activity. Important contributions by cytokines (e.g., IL-12, TNF-α, and IFN-γ) to tumor resistance were also identified.

Paglia et al. evaluated the generation of tumor-specific CTL and in vivo tumor protection against a β-galactosidase (β-gal)–transduced murine tumor cell line (7). Two experimental findings merit highlight. The first is that in vivo priming of CTL and protection against a subsequent tumor challenge required only small numbers of antigen-pulsed dendritic cells. Standard microbial adjuvants proved inactive, as did unpulsed dendritic cells or antigen alone. The second notable finding is that GM-CSF–generated, bone-marrow–derived dendritic cells were able to process intact soluble β-gal protein for peptide presentation via class I MHC to CD8$^+$ CTL.

Caveats and Future Directions. Despite the substantial interest in these reports, several precautions are warranted. First, these are all murine tumor models. It has yet to be proven that human dendritic cells present tumor antigens and elicit specific T cell immune responses, even in vitro. Next, significant helper responses are most likely being generated in vivo because of the exposure of the dendritic cells to FCS during in vitro expansion and differentiation. The substitution of FCS-free cultures for human trials may require the addition of class II MHC helper epitopes to class I MHC antigen–pulsed dendritic cell immunization strategies.

The longevity of the response has also not yet been rigorously tested. Zitvogel et al. (5) did use three to four biweekly immunizations with peptide-pulsed dendritic cells, but any advantage conferred by booster immunizations on the long-term durability of positive responses remains unanswered. Also untested is the ability of dendritic cell immunizations to overcome tumor-specific suppressor populations. Early studies in the field of tumor immunology by North and colleagues have in fact demonstrated that CD4$^+$ lymphocytes can actively suppress protective cell-mediated immunity under conditions of high tumor burden (48).

Where, then, does one proceed? Implicit in all of the papers considered here is the usefulness of methods to generate large numbers of dendritic cells, rather than relying on the isolation of scarce, terminally differentiated populations from accessible sites like human blood or mouse spleen. This field is moving forward because of the definition of cytokines that enhance dendritic cell growth and development from proliferating and nonproliferating precursors in both mice and humans. As noted above, GM-CSF plays a pivotal role, additionally supported by TNF-α, CD40L/gp39, IL-4, c-kit ligand, and likely additional cytokines that have not yet been identified (32–38, 40–43).

Among the remaining challenges will be how to load dendritic cells with the correct antigens for stimulation of antitumoral immunity. An obvious strategy in addition to the methods presented in this issue is the transduction of genes encoding relevant proteins into dendritic cells as they divide from cycling precursors and intermediates. In all of these cases, dendritic cells could tailor peptides to their own MHC molecules, obviating the need to synthesize tumor-specific peptides, most of which have stringent MHC restrictions.

It remains unclear, however, whether dendritic cell immunizations would induce immunity against established disease in hosts with high tumor burdens. An objective more likely to succeed would be to test dendritic cells as adjuvant therapy for minimal residual disease after definitive treatment of primary tumors. Melanoma offers an obvious starting point, in view of the extent to which tumor antigens and lymphocyte reactivity are already defined for this malignancy (2). Many of the issues still confronting the use of dendritic cells for cancer immunotherapy, however, will require carefully planned human trials.

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