

# Optimizing Dendritic Cell Function by Genetic Modification

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The potential for effective immune therapy for cancer has been met with considerable enthusiasm because of a number of fundamental advances in our understanding of the immune responses to cancer. First, previous questions about the existence of tumor antigens have largely been answered, since tumor-associated and tumor-specific antigens clearly exist and, when they are recognized by antigen-specific T cells, can lead to cell lysis. Second, the requirements for initiating T-cell-mediated immune responses have been elucidated and include a first signal (the presentation of antigen within the groove of MHC [i.e., major histocompatibility complex] molecules) and a second signal (the interaction of costimulatory molecules with T cells). The central role of antigen-presenting cells, particularly dendritic cells, in inducing the appropriate immune responses is becoming better understood (1). Since the induction of primary immune responses is thought to be a seminal element in inducing immune responses against tumor-associated or tumor-specific antigens, dendritic cells have become a focus of investigations directed toward developing tumor-specific immunotherapy (2,3).

Dendritic cells are thought to have all of the known (and possibly as yet unknown) elements required for inducing primary antigen-specific T-cell responses. Indeed, *in vitro*, dendritic cells have been used to stimulate antigen-specific T-cell responses from naive peripheral blood mononuclear cells, a strategy that for many years was not possible without first priming cells *in vivo* by immunization. In this issue of the Journal, Hodge et al. (4) report on the use of murine dendritic cells modified with recombinant poxvirus vectors expressing a triad of costimulatory molecules (TRICOM) (i.e., B7-1, ICAM-1 [intercellular adhesion molecule-1], and LFA-3 [leukocyte function-associated antigen-3]) to enhance their performance in activating T cells. They report that these TRICOM-modified dendritic cells produce superior stimulation of naive and peptide-specific T cells compared with unmodified dendritic cells. An obvious question is raised: If dendritic cells already express B7-1, ICAM-1, and LFA-3 and are inherently effective in priming immune responses, how can genetic modification of dendritic cells to express these molecules improve their function?

This question exists because of the complexity of the dendritic cell system and the heterogeneity of dendritic cells. The heterogeneity in the dendritic cell system exists at multiple levels such as 1) precursor population—at least two subsets of dendritic cell precursors circulate in the blood (1), 2) anatomic location—dendritic cells exist in virtually all sites in the body, including the epidermis and dermis of the skin, spleen, lymph nodes, thymus, blood, and all organs (1), 3) function—subsets appear to have different functions (1), and 4) outcome of immune response—notably the induction of tolerance or immunity (1). Variables in dendritic cell biology also exist in the current view of dendritic cell ontogeny, inasmuch as dendritic cell progenitors in the bone marrow give rise to circulating precursors that home to tissues, where they reside as immature cells with high phagocytic capacity. After a signal such as tissue damage,

immature dendritic cells capture antigen and then migrate to the lymphoid organs, where they select antigen-specific T cells, thereby initiating immune responses.

Although many signals for dendritic cell maturation and activation are known, the optimal signals and the sequence to deliver these signals to generate optimal T-cell immunity *in vivo* are not known. Contemporary clinical trials of cancer immunotherapy use dendritic cells from a variety of sources, such as isolated directly from unmobilized or Flt3 ligand-mobilized peripheral blood or generated *in vitro* from CD34<sup>+</sup> progenitors, CD14<sup>+</sup> monocytes, or from adherent peripheral blood mononuclear cells cultured in the presence of granulocyte-monocyte colony-stimulating factor, interleukin 4, and other cytokines. These dendritic cell populations are generally heterologous and often immature, and, although they are relatively efficient in antigen uptake and processing, they actually express low levels of B7-1, ICAM-1, and LFA-3. Indeed, Hodge et al. (4) observed that the mean fluorescence intensity for these molecules, an indicator of the level of expression, was increased threefold to fivefold after infection with the TRICOM-containing vectors. These immature dendritic cells often are loaded with antigen(s) in a variety of forms, such as peptides, proteins, tumor lysates, apoptotic bodies, or tumor cell fusions. In addition, antigen loading and processing facilitated by gene transfer in the form of DNA or messenger RNA encoding that antigen have been the subject of numerous investigations (5). Antigen-loaded dendritic cells are then administered directly, or they may be processed further by maturation with a variety of second signals, such as tumor necrosis factor- $\alpha$ , CD40 ligand, or monocyte-conditioned media (6). Immature or mature dendritic cells are then administered to patients through a variety of routes, such as intravenous, intradermal, subcutaneous, and even direct intralymphatic or intranodal injection (2). The use of exogenous cytokines or cocktails to trigger dendritic cell activation has merit, and a variety of strategies are being investigated to determine the optimal agent or combination of agents to mature dendritic cell and the sequence that they should be administered (8).

It is the mature dendritic cells, present within secondary lymphoid organs, that express relatively high levels of costimulatory molecules, such as B7-1, and other molecules, such as ICAM-1 and LFA-3, permitting antigen presentation. Four issues, however, remain pertinent to the role of gene modification of dendritic cells. First, are all dendritic cells appropriately matured, so that the population of cells uniformly express high levels of these molecules? Hodge et al. (4) achieved 90%–98% efficiency of dendritic cell infection, as demonstrated by a reporter molecule. Second, does expression of these molecules persist? Al-

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though not addressed by Hodge et al., it is not clear that prolonged expression of these molecules is necessary to trigger T-cell activation. Third, can expression of these molecules at even higher levels enhance the function of the mature dendritic cells either by directly facilitating interactions with T cells or by facilitating interactions with T cells that can provide other signals, such as CD40 ligand, RANK/TRANCE, 4-1BB, and OX40 ligand? Hodge et al. demonstrated that dendritic cells, matured by either tumor necrosis factor- $\alpha$  or by a CD40-specific monoclonal antibody, possessed even greater T-cell-stimulatory activity when infected with the TRICOM-containing vector. Fourth, does genetic modification affect dendritic cell function in other ways? Hodge et al. noted that dendritic cells infected with a vaccinia vector had diminished antigen-presenting cell function, but this diminished function was more than compensated for by the use of a vector containing TRICOM. Therefore, the article by Hodge et al. demonstrates that, from the standpoint of what can be done to optimize delivery and processing of an antigen as well as to optimize expression of costimulatory molecules known to be important in activating cellular immunity, one approach is to ensure the expression of the critical molecules by use of an exogenous expression system, such as viral vectors engineered to express the apparently important molecules.

Hodge et al. (4) demonstrate in a series of experiments that their strategy for modifying dendritic cell is effective and improves the performance of dendritic cells generated *in vitro*. However, it is not clear that this will remain the optimal strategy. Should other molecules be expressed, should other molecules be repressed, and is the use of the viral vector system that may inhibit some of the cellular functions of the dendritic cells sub-optimal? In addition, it is not clear if dendritic cells will migrate with great efficiency when matured and then administered (7), if

dendritic cells that are fully matured (either by cytokine combinations *ex vivo* or by genetic modification) will function optimally when administered to patients, or whether immature dendritic cells should be administered and then matured *in vivo* in the appropriate microenvironment. Nonetheless, the series of experiments reported by Hodge et al. (4) demonstrate, in a direct fashion, that dendritic cell function can be manipulated by genetic modification and set the stage for testing these cells for their biologic activity and their role in eliciting authentic anti-tumor responses in patients.

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