Editorial

Immunotherapy of cancer with dendritic cell-based vaccines

The field of cancer immunotherapy has been reinvigorated through the appreciation that most tumors, including those that are nonimmunogenic, encode tumor rejection antigens that enable them to induce protective immunity.¹ This appreciation, and the recognition that the cellular arm of the immune response is best equipped to recognize tumor cells as foreign and lead to their eradication, has shifted the emphasis in vaccine development from vaccines which favor the induction of humoral responses to vaccines which favor the induction of cellular responses, in particular the CD8⁺ cytotoxic T cell (CTL) arm.

One such approach, the use of genetically modified autologous tumor cell-based vaccines (GMTV), has received much attention.² The original working hypothesis of the GMTV approach was to provide cytokines to the CTL precursors as a means to circumvent the dependence on CD4⁺ T helper cells.² This was based on the notion prevailing at that time that in addition to antigen presented by the tumor cells, full maturation of the tumor-specific CTL required cytokines secreted by activated CD4⁺ T cells. There is now growing evidence that somatic cells, whether tumor cells or cells infected by pathogens, are not capable of priming naive CD8⁺ CTL. Rather, the ability to activate naive CD8⁺ T cells is an exclusive property of a specialized type of cell called professional antigen presenting cells (APC). Accordingly, antigen is transferred from cells expressing the antigen to the APC, a process that is triggered by inflammatory reactions which cause the degradation and release of antigen from the dying cells. This process has been referred to in the literature as 'cross priming', 're-presentation' or 'indirect presentation' and popularized as the 'danger theory'.³ A number of observations, old and recent, argue that indirect presentation is an important if not the major pathway for induction of CTL responses *in vivo*. The current view is, therefore, that the role of cytokines or costimulatory molecules in GMTV is to enhance the transfer of tumor antigens to professional APC for activation of naive CTL precursors. Evidence suggests that the main type of professional APC is the dendritic cell (DC).⁴ Hence, the working hypothesis underlying the use of DC in tumor vaccination is that the limiting factor in tumor-specific CTL induction *in vivo* is the transfer of antigen from the tumor cell to the DC, and that direct loading of DC with the relevant antigen is an effective method to bypass this limiting step.

Three issues are considered here: (1) tumor vaccination

strategies: GMTV *versus* DC-based vaccines; (2) the pros and cons of using defined tumor antigens *versus* unfractionated tumor-derived material; and (3) the form of antigen to load on to DC: polypeptide, polynucleotide, DNA or RNA.

GMTV and DC vaccines are forms of cellular therapies which require *ex vivo* manipulation of the patients' cells. The complexities associated with cellular therapies notwithstanding, they must be considered if they represent the only potential treatment to a terminal disease as is the case for most forms of cancer. While GMTV were highly effective in animal studies, translation to clinical settings turned out to be quite difficult. This is primarily due to the relative inefficiency of gene transfer techniques when applied to primary human tumor cells, the difficulty of obtaining a sufficient number of tumor cells from the patient, and the overall complexity of the procedure. Compared with GMTV, preparation of DC vaccines is a more 'user friendly' and clinically manageable process. At present, DC can be generated from cancer patients by culturing adherent PBMC from the patients for 5–7 days in the presence of cytokines.⁵ This process is relatively simple in contrast to the isolation or culture of sufficient tumor cells from patients. While the source of tumor antigen may be a contentious issue (see below), loading of DC with antigen is easy compared with transduction of freshly isolated or cultured human tumor cells.

A number of tumor antigens recognized by CD8⁺ CTL have been identified and molecularly cloned.⁶ The disadvantage of using defined tumor antigens to load DC is that one is currently limited to a small number of cancers in which candidates for tumor rejection antigens have been identified.⁶ The ideal formulation of antigen would consist of a mixture of commonly expressed tumor rejection antigens, that is, antigens that are capable of inducing protective immunity, as opposed to simply inducing antigen-specific CTL. It is not known which of the currently known tumor-associated antigens will serve as effective antigens in a vaccine formulation and it is impossible to predict when such antigens will be identified. Vaccination with total tumor-derived material as the source of antigen may therefore be preferable to using a single defined tumor antigen. Vaccination with unfractionated tumor-derived antigens could induce autoimmune responses directed against 'self' antigens. In view of the recognition that passive tolerance (ignorance) may represent a major pathway for averting autoimmunity against peripherally expressed antigens,⁷ this possibility has to be considered. With one exception, however, the majority of animal studies has shown that effective tumor immunity can be established in the absence of any

visible signs of autoimmunity. Interestingly, the effector arm of the T cell response was capable of distinguishing between tumor cells and normal cells expressing the cognate antigen.⁸ While all of this is encouraging, clinical studies using total tumor-derived antigens must be carefully monitored for autoimmune manifestations.

The major limitation of using protein or peptide-encoded antigens derived from tumor cells is that the amount of tumor tissue available from patients is often limited or heavily 'contaminated' with normal tissue. The use of nucleic acid-encoded antigens offers a means of generating sufficient antigenic material from very few tumor cells, hence expanding the ability to treat patients with tumor-derived antigen preparations to practically everyone. At present we have shown that RNA transfection is an effective means of delivering antigen to DC.^{9,10} While there is no reason to think that similarly potent strategies cannot be developed with DNA-encoded antigens, the use of RNA offers several potentially important advantages over DNA which include increased safety and technical ease.

Efficiency of transfection aside, what are the possible advantages and disadvantages of using tumor antigen in the form of DNA or RNA in vaccine formulations? DNA is of course more stable than RNA and hence simpler to handle, an important consideration, especially in clinical settings. DNA-encoded antigens are also likely to persist and be presented longer than RNA-encoded antigens in the transfected DC. A potentially significant advantage of using RNA-encoded antigens is safety. The half-life of stable mRNA species in the mammalian cell is less than 24 h, while unintegrated DNA can persist and function in nondividing cells for extended periods of time measured in months. Considerations of safety come into play when tumor antigens which are mechanistically implicated in the neoplastic process, such as E6 or E7 genes of human papilloma viruses, are considered. Of greater concern would be vaccination with total tumor-derived antigens in the form of DNA.

There is also an important technical advantage of using RNA- compared with DNA-encoded antigens. Generation of a cDNA expression library from the tumor cell will require the cloning of the amplified cDNA product into an expression plasmid to place an appropriate promoter 5' to the cDNA and a polyadenylation signal 3' to the cDNA. The cloning step is labor intensive and requires considerable skills to generate sufficiently large

representative libraries comprising $>10^6$ members. By contrast, no cloning is needed to generate a mRNA-based library because a 28 nt long T7 promoter can be easily accommodated in the primers used for amplification. Thus, generation of mRNA-based expression libraries consists of simple test tube reactions which are completed overnight and generate large representative libraries.

In summary, from an immunological view there are compelling reasons to vaccinate with DC loaded with the total repertoire of antigens from a given tumor. For the technical and safety issues cited above a reasonable approach would be to use tumor-derived RNA for this purpose.

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