NEWS AND VIEWS

Problem solving for tumor immunotherapy

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Tumor cells express defined antigens that can be recognized by tumor-destroying ($CD8^+$) cytotoxic T lymphocytes (CTLs). As most cancer patients obviously do not mount efficient T-cell responses against their tumors, the task is clear: immunotherapies must induce cancer-destroying T cells in patients. Although this goal appears straightforward, effective immunotherapy has remained elusive because of three major problems: first, for many tumors, no or not enough suitable antigens are known; second, no consensus exists for the best antigen formulation or the route of immunization; and third, tumors under immune attack tend to be selected for antigen loss variants. These three problems lie at the heart of two studies[1,2] published in this issue and one published recently in *Nature Medicine*[3].

Currently, problem number one—that of identifying specific tumor antigens—is closest to solution. An increasing number of antigens are being found by screening for gene products differentially expressed in tumors as opposed to normal tissues and by testing for antigenicity. In addition, vaccination trials are underway with preparations containing multiple unidentified tumor antigens, such as heat shock proteins isolated from autologous tumors or allogeneic dendritic antigen-presenting cells (APCs) fused with autologous tumor cells, the latter with surprising success[3].

Problem number two—optimizing antigen presentation and delivery—is more complex, as illustrated by the high number of antigen formulation and immunization modes and routes currently employed in clinical trials. Antigen formulations can take the form of peptides, proteins, DNA, RNA, viral vectors, modified cells either alone or together with adjuvants, cytokines, or in vitro-derived dendritic cells. The approach used by Cho et al.[1] is a new combination of a protein antigen coupled to adjuvant-like immunostimulatory DNA.

Problem number three—loss of antigen—can be potentially avoided if immunization is carried out at an early stage in the disease and with multiple antigens. If this fails, it may be possible to restore antigen expression, as suggested by Alimonti et al.[2].

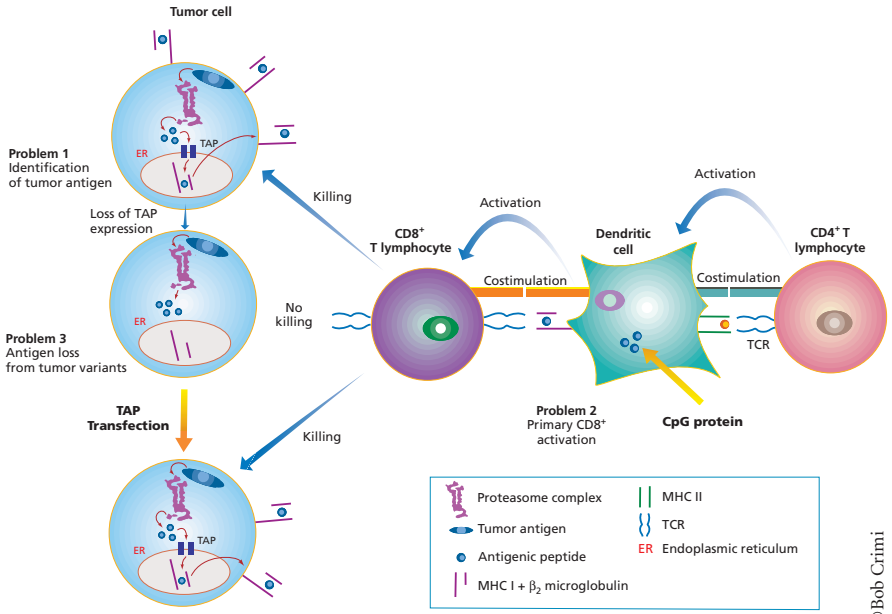

Figure 1. Three main problems in inducing $CD8^+$T lymphocytes to kill tumor cells in vivo: Efficient activation of the CTL needs interaction with an activated dendritic cell (problem number 2). Identification of new tumor antigens and dealing with antigen loss variants are also critical points for improving immunotherapy protocols in cancer patients (problems number 1 and 3). Red arrows show two examples of strategies that may be used to boost CTL respopnses against tumors, as described by Cho et al. and Alimonti et al. in this issue.

To understand the different strategies employed, it is necessary to outline briefly the way in which a CTL attacks a tumor cell. $CD8^+$ CTLs specifically recognize short peptide fragments presented by major histocompatibility complex (MHC) class I molecules at the cell surface of target cells. The process of protein degradation into peptides in the cytosol, their assembly with newly synthesized MHC proteins in the endoplasmic reticulum, and export to the cell surface is called antigen processing[4]. The peptides recognized by CTLs are derived either from viral proteins if the cells are infected, or from aberrantly expressed proteins in tumor cells. Thus, tumor-associated proteins that are solely expressed intracellularly are still visible for CTLs (but not antibodies) by virtue of MHC class I-associated peptide presentation.

However, cancer cells are poorly immunogenic themselves. In vivo CTL induction requires contact with a professional APC, such as the dendritic cell, which first takes up the tumor antigen and is activated itself by interactions with $CD4^+$ helper T cells (see Fig. 1)[5]. This leads to the increased expression of proteins from the B7 family and adhesion molecules, all of which are essential for the efficient co-stimulation and activation of T lymphocytes. If successfully activated, the $CD8^+$ CTLs kill the tumor cells as long as the antigen continues to be expressed.

Unfortunately, escape mutants of tumor cells also commonly arise. Apart from deletion of the antigen itself, one or several proteins of the antigen presentation pathway can become defective; this includes the peptide transporter TAP, subunits of the proteasome complex, or even class I components themselves (see Fig. 1)[6].

In this issue, Cho et al. report a new vaccine using DNA that, rather than encoding an antigen, acts as an adjuvant of a protein antigen. DNA-based vaccination of mice using a DNA sequence encoding the antigen and additional "immunostimulatory sequences"

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containing unmethylated cytosine/guanosine-rich motifs (CpG) have already been shown to induce antigen-specific CTLs[7]. Cho et al. have now coupled CpG sequences covalently to a well-known model protein, ovalbumin, and shown that this construct induces CTL activity against ovalbumin-expressing target cells upon injection into mice. Surprisingly, CTL induction by the CpG–protein construct is independent of $CD4^+$ help, as demonstrated by CTL activity in CD4 knockout mice. Vaccination with the construct protects against pre-established ovalbumin-expressing tumors and is mediated by $CD8^+$ effector cells.

There are two remarkable features in this new combination of DNA-adjuvant and protein antigen: First, the CpG sequences seem to direct the presentation of exogenous antigen to the class I pathway, although the mechanism implicated is unknown (see Fig. 1). Second, the construct bypasses $CD4^+$-mediated help, most likely by providing both a "danger" signal and differentiation signals directly to dendritic cells, as demonstrated recently for CpG–peptide mixtures in mice[8]. In addition, CTL induction with the CpG–protein construct is much more efficient than with other ovalbumin preparations.

Although this all sounds very straightforward, we do not know yet whether such constructs work in humans. Some immunostimulatory effects of CpG motifs have been described in human peripheral blood in vitro, most notably in dendritic cells[9], but immunization trials have not been reported. Thus, the efficacy of CpG–protein constructs for immunotherapy in patients remains to be tested.

Once efficient tumor-destroying T cells are induced, tumor cells may escape by antigen loss, as mentioned above. One way of achieving this is to downregulate TAP activity[6].

In a second paper, Alimonti et al. explore the possibility of restoring antigenicity by transferring one of the TAP genes into a TAP-deficient tumor cell via transfection or infection with a TAP1-containing vaccinia virus construct. The results suggest a beneficial effect, although it is unclear how only one of the two TAP chains can restore TAP activity (maybe TAP1 induces marginal intrinsic TAP2 expression?).

Overall, their data suggest that reintroduction of one deficient component of the antigen-processing machinery (i.e., TAP1) may be enough to restore the peptide-presenting capability and the in vivo antigenicity of certain cancer cells. Since virus-mediated gene delivery is not tumor-specific, potential induction of autoimmunity by increasing the activity of the antigen-processing machinery in normal cells must be considered.

To date, vaccinations with peptides, peptides loaded onto dendritic cells, recombinant or tumor-derived proteins, or modified cells have proved efficacious only in certain patients. Thus, a powerful and reliable method of immunizing patients is still sought. A decade's worth of clinical trials for antigen-specific cancer immunotherapy suggests that optimization of antitumoral effector cell stimulation, possibly combined with improvement of tumor antigenicity by gene transfer or cytokines, may provide the best option. As the papers in this issue illustrate, there may be many approaches to solving the same problem.

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