Review

Immunological Therapy of Human Tumors by Gene-Modified Cellular Vaccines

(cancer / gene therapy / vaccines)

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Pre-clinical studies and rationale

The need to improve the immunogenicity of cancer cells to provide a better and clinically more efficient stimulation of tumor-bearing individuals emerged in the late 80s. In fact, it was previously found that tumor-resected mice or mice given nonreplicating (irradiated) neoplastic cells often developed resistance to challenge with cells of the same tumor used to immunize, whereas tumor-bearing animals appeared refractory to reject their neoplasms upon a similar immunization procedure. It was concluded that, at least during the late phase of tumor growth, the host's immune system undergoes an antigen-specific immunosuppression (tolerance), which weakens or prevents an efficient, therapeutically significant immune response to take place. In addition, results of cancer patients, particularly with melanoma, treated with different cellular and subcellular-based vaccines were rather disappointing, indirectly confirming a lack or weak immunogenicity of the vaccine preparations used in these clinical trials. Based on his previous studies with interleukin-2 (IL-2) given locally (Bubeník et al., 1986), Jan Bubeník was one of the first to propose that such a tolerant state could be overcome by conferring upon tumor cells the ability to release an immunostimulatory cytokine (e.g. IL-2) (Bubeník et al., 1988). In seminal papers, Jan Bubeník was able to demonstrate that local release of IL-2 by tumor cells transduced with cDNA encoding the IL-2 gene resulted in growth inhibition not only of the transduced neoplasms but also of the wild-type parental cells concomitantly growing in the same animal (Bubeník et al., 1988; Bubeník et al., 1991).

This new approach was then the focus of several groups of investigators with the purpose of understanding the mechanism by which local release of cytokines causes a better, systemic immune response by the host against tumor antigens. In a series of elegant experiments, Colombo and Forni in Italy, Thomas Blankestein in Germany and the group of the John Hopkins University (D. Pardoll, G. Dranoff and H. Levinsky) in the States, uncovered the details of the so-called cross-talk between tumor cells, inflammatory (particularly granulocytes) and immune cells, which is driven by local secretion of the cytokine and entails at least two different steps, one nonspecific and the second more specific due to the involvement of T lymphocytes (Colombo et al., 1992). To better assess **the role of each cytokine** release in this phenomenon, Forni and coworkers have transduced the same weakly immunogenic, murine mammary adenocarcinoma (TS/A) with genes coding for different cytokines (IL-2, IL-4, IL-7, IL-10, interferon-α (IFN-α), IFN-γ, tumor necrosis factor-α (TNF-α)). These authors then compared mice immunized by these cytokine gene-transduced tumors for the features of the induced reaction and its impact on the growth of a subsequent challenge of the parental, mock-transduced tumor. Tumor cells transduced with IL-10 or IFN-γ were the less effective in preventing the growth of a subsequent challenge of TS/A cells while IL-2- and IL-7-gene modified cells were the most effective ones (see Musiani et al., 1997). Moreover, the role of each cell subpopulation infiltrating the rejected tumors varied with the secreted cytokine, though granulocytes and $CD8^+$ lymphocytes appeared to have a prominent role (Musiani et al., 1997). Several attempts to increase the efficacy of gene-modified tumor cells were then carried out by using combinations of cytokine genes which can augment both T and NK antitumor activities (e.g. IL-12, IL-18, IL-1) (Oshikawa et al., 1999) or of cytokine and co-stimulatory molecule genes that improve the stimulation of naive T cells (Gaken et al., 1997). However, not all gene combinations resulted in a higher immunogenicity; in fact, while IL-4 and B7.1 do synergize (Cayeux et al., 1996), the simultaneous expression of IL-2 and B7.1 in the same tumor does not (Cayeux et al., 1997). It should also be considered that two or more of such genes can be inserted into a recombinant vaccinia

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Abbreviations: IFN – interferon, IL – interleukin, GM-CSF – granulocyty/macrophage-colony-stimulating factor.

virus by a "cassette" system to be efficiently used to treat tumor-bearing mice (Carroll et al., 1998).

However, animal models from which the conclusions that cytokine gene-transduced tumor cells are more immunogenic and can be used as effective anti-tumor vaccines suffer from two important drawbacks, owing to the use of a) vaccines consisting of non-irradiated, growing tumor cells, and b) tumor-free, healthy rather than tumor-bearing individuals.

As for the first point, it has been clearly shown that irradiation drastically reduces the immunogenicity of gene-modified tumor cells (Cayeux et al., 1996). The second point is also crucial and often underestimated, because patients have been often vaccinated without knowledge of their immune status (primed? unprimed? actively tolerant?) against antigens of their own tumors. The presence of the tumor mass is known to functionally downregulate the immune system and, therefore, it may considerably weaken the patients' reaction which follows administration of gene-modified vaccines. However, studies of the frequency of melanoma antigen-specific cytotoxic T lymphocytes (CTL) in metastatic patients have revealed that a sizeable fraction of them possess melanoma-specific (e.g. MelanA/MART1-specific) T cells, as evaluated by limiting dilution analysis (LDA) and/or tetramer technology (Romero et al., 1998; Anichini et al., 1999). Such a different immunological state of cancer patients may impact on the outcome of vaccination with gene-modified tumor cells.

Clinical studies

Based on the results of animal studies, several phase I/II clinical trials have been initiated, though only a limited number of these have been then concluded and published, mainly in melanoma patients. A limited clinical response rate was found in these studies, which ranged from 2 to 10% (including complete and partial responses) (Parmiani et al., 2000a). But even the T-cell response to the vaccine was scanty, with 10–30% of patients generating such a response as evaluated by cytokine release or cytotoxicity (Arienti et al., 1996). Both autologous and allogeneic tumor lines transduced with cytokine genes have been used, though no major differences in terms of clinical and/or tumor-specific immune responses have been reported (see Parmiani et al., 2000b). In addition, of the many cytokine genes transduced into human tumor cells (e.g. IL-2, IL-4, IL-7, IL-12, granulocyte/macrophage-colony-stimulating factor (GM-CSF), IFN-γ) then used as vaccines, none appeared to be reproducibly more immunogenic as compared to the other ones. Actually, a direct comparison between the same tumor line transduced with two different cytokine genes and given to two clinically similar groups of patients is lacking. However, tumor cells transduced with GM-CSF are those which have been shown to generate the strongest local immunoinflammatory reactions (Soiffer et al., 1998; Simons et al., 1999).

Concluding remarks

The reasons of the weak immune response and low clinical response rate in patients vaccinated with cytokine gene-transduced tumor cells have been recently discussed (Parmiani et al., 2000b) and can be summarized as follows. A) Cancer cells have been often used (particularly in the early studies) without characterization of their antigenic profile, thus precluding a straightforward interpretation of the presence or absence of the immune response to tumor antigens; B) tumor cells were not checked for expression of co-stimulatory molecules which, in case of patients already primed against their own tumor antigens (Anichini et al., 1999), would help in increasing the antigen presentation by tumor cells themselves in addition to cross-priming; C) the amount of the cytokine released locally after injection of irradiated gene-transduced tumor cells has been rarely assessed and may impact on the immunostimulatory properties of the vaccine; D) lack of systemic activation of the immune response generated locally. These and other factors may have contributed to the insufficient immune stimulation and clinical response observed in these studies.

However, I believe that there is room for improvement in the outcome of this vaccination approach, both at the immunological and clinical levels, taking into consideration the many advances of basic immunology and vaccinology and the information that has been collected from the clinical studies as well. Thus, I propose that improvements can be made in the construction of gene-modified vaccines by a) using tumor cells known to express molecularly defined antigens; b) introducing, in addition to genes encoding cytokines, genes encoding T-cell co-stimulatory molecules; c) increasing the amount of cytokines released locally by irradiated cells, and d) co-administrating adjuvant cytokines (e.g. IL-2, IL-12, IFN-α) systemically in order to expand the T-cell pool activated by vaccines and help in upregulating the major histocompatibility complex (MHC)/peptide complexes by target tumor cells. Therefore, it is still entirely possible that the hypothesis put forward by Jan Bubeník more than ten years ago and which has attracted the work of many investigators will pay off in the next few years of preclinical and clinical work.

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