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 population 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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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, grow-
ing 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; Anichi-
ni et al., 1999). Such a different immunological state of
cancer patients may impact on the outcome of vaccina-
tion 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 cli-
nical 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 gener-
ating such a response as evaluated by cytokine release or
cytotoxicity (Arienti et al., 1996). Both autologous and
alloimmune 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 trans-
duced 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 immu-
oinflammatory 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 dis-
cussed (Parmiani et al., 2000b) and can be summarized
as follows. A) Cancer cells have been often used (par-
cularly in the early studies) without characterization of their
antigenic profile, thus precluding a straightforward inter-
pretation of the presence or absence of the immune re-
sponse 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-trans-
duced tumor cells has been rarely assessed and may
impact on the immunostimulatory properties of the vac-
cine; D) lack of systemic activation of the immune re-
sponse 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 consider-
ation the many advances of basic immunology and vac-
cinology and the information that has been collected from
the clinical studies as well. Thus, I propose that impro-
vements can be made in the construction of gene-modified
vaccines by a) using tumor cells known to express mole-
cularly defined antigens; b) introducing, in addition to
genes encoding cytokines, genes encoding T-cell co-
stimulatory molecules; c) increasing the amount of cyto-
kines released locally by irradiated cells, and d) co-ad-
ministrating 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 histocom-
patibility complex (MHC)/peptide complexes by target
tumor cells. Therefore, it is still entirely possible that the
hypothesis put forward by Jan Bubenik more than ten
years ago and which has attracted the work of many
investigators will pay off in the next few years of pre-
clinical and clinical work.

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