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Editorial

Immunological approach to gene therapy of human cancer: improvements through the understanding of mechanism(s)

Immunological gene therapy of human cancer can be divided into two main approaches: (1) genetic manipulation of neoplastic or normal cells to construct vaccines; and (2) insertion of genes into lymphocytes to be used in the adoptive immunotherapy.

Based on previous mouse studies, Gansbacher *et al*[1] proposed in 1992 to vaccinate cancer patients with tumour cells releasing cytokines upon transfection *in vitro* with genes carried by retroviral vectors.

Since then, several clinical studies have been initiated following the demonstration in animal models that cytokine (IL-2, IL-3, IL-4, IL-6, IL-7, IFN-γ, GM-CSF and, more recently, IL-10 and IL-12) gene-transduced tumour cells can generate a systemic antitumour immunity and that vaccines constructed with such cells can even cause regression of established neoplasms.[2] These clinical trials were carried out using either autologous or allogeneic gene-transduced tumour cells. However, only a few papers describing the results of these studies in melanoma and renal cancer have been published.[3,4]

The major problem in the interpretation of data from these clinical protocols, in terms of both immune and clinical responses, is the uncertainty in the mechanism of immunisation that is activated by cytokine gene-modified cellular vaccines. In fact, when this approach was started, the assumption and the main rationale were that tumour cells expressing HLA class I could function as tumour antigen presenting cells (APC) for HLA class I-restricted T cells that can recognise antigens thanks to the help of the locally released cytokine. This notion, however, has been challenged in the past few years by experiments showing that antigen is presented to naive T cells in the draining lymph nodes by bone marrow-derived, autologous APC (cross-priming).[5]

While those studies were ongoing, the discovery of molecularly defined tumour antigens recognised by T cells, particularly in melanoma,[6] has offered a potential alternative in peptide-based vaccines. Even in this case, however, the mechanism of immunisation remains to be elucidated. We need, therefore, to answer the question of whether a cellular approach to cancer vaccines with gene-modified tumour cells still deserves to be pursued or should be abandoned in face of the availability of well-defined peptide antigens.

This decision should be based first of all on the efficacy of these approaches which in turn will depend on the knowledge of the mechanism of T cell activation (if any) by locally injected vaccine.

In fact, if cross-priming is the predominant mechanism in vaccination with tumour cells, then expression of HLA and/or costimulatory molecules by such cells could be useless, these requirements being provided by the host's APC. Recent studies in mice appear to conflict with this conclusion by showing that B7.1 expression by IL-2-transduced, irradiated tumour cells confers a higher therapeutic activity against established tumours.[7] This could be explained by the ability of the gene-modified neoplastic cells to function as APC, though recruitment and activation of NK cells by B7.1 that could ultimately favour a cross-priming cannot be excluded.

Animal models from which several of the above conclusions were drawn, however, suffer from two important limitations, namely the frequent use of non-irradiated replicating tumour cells as vaccines and of tumour-free, rather than tumour-bearing, animals. The latter point is crucial because patients have been vaccinated without knowing whether they were primed to at least some of the tumour antigens present in the cellular vaccine. Therefore, since direct antigen presentation by tumour cells requires intact cells expressing enough numbers of HLA/antigen complexes and considering that, in patients, irradiated and sometimes even allogeneic cells are injected, one should conclude that such a mechanism could only be operative for a few days, before tumour cells are destroyed or have died off after irradiation. However, this may occur only in already primed patients in whom memory T cells can traffic to subcutaneous tissues, but not in naive individuals. A further limitation of this process lies in the frequency of antigen-specific CTL precursors which varies greatly in different patients despite their similar clinical stage. Therefore, it is likely that in the majority of cases and at the later stage of vaccine administration, cross-priming becomes the predominant mechanism of antigen presentation. In this case, tumour cell debris is processed by the host's APC and tumour antigens can be presented through both HLA classes I and II pathways, thus allowing even $CD4^+$ Th cells to be activated. Since the local release of cytokines has been shown to favour this process and human tumour cells can provide a wide spectrum of antigens, it appears that vaccination with cytokine gene-transduced tumour cells deserves to be explored further. A detailed discussion of these mechanisms has been published recently.[8] A recommendation is that in the construction of these vaccines, one should select tumour lines known to express a wide spectrum of T cell-defined antigens in an immunogenic form. This will allow stimulation of T

cells directed against as many antigens as possible, thereby increasing the possibility of overcoming the main escape mechanism, namely the loss of HLA class I and/or antigen(s) by tumour cells growing in the body and exposed to selection by the host's immune system.

To avoid the requirement of transfecting the cytokine gene (eg IL-2, IL-4 or IL-12) into autotologous tumour cells of each patient, an alternative method has been proposed, namely to transfect autologous or even allogeneic fibroblasts and admix them with fresh or short-term cultured autologous tumour cells before injection into the patient. However, this approach has not been particularly successful in generating an immune response or a clinical response in melanoma patients and has been abandoned. The use of purified autologous dendritic cells transfected with genes encoding tumour antigens by viral vectors appears more promising.[9] *In vitro* studies show that such transfected cells can stimulate patients' lymphocytes and, therefore, lend themselves as novel vaccines for cancer patients. Recently, mouse models have shown that dendritic cells can even be loaded with nucleic acids, both DNA and RNA, that may allow amplification of genes encoding the appropriate antigens even from a limited number of tumour cells.[10] Clinical trials have been initiated to test such a dendritic cell-based vaccine in melanoma patients.

In the past, a few attempts have been made to insert genes coding for tumour cytotoxic cytokines (eg TNF-α) into T or LAK cells. These experiments have met with limited success due to the inefficiency of gene transfection and *in vivo* tumour targeting.[11] Genetic manipulation of T-lymphocytes has been recently used to prepare reagents for an adoptive transfer in patients who developed EBV lymphomas after allogeneic bone marrow transplantation. In these studies, donor T cells were sensitised *in vitro* to the appropriate EBV antigen and transfected both with a marker gene, that allowed their identification *in vivo*, and with a suicide gene (eg HSV thymidine kinase). The latter will permit the destruction of transfected lymphocytes upon administration of the pro-drug ganciclovirin in case GVH disease occurs in patients receiving the cells.[12]

However, the use of genetically manipulated lymphocytes in clinical settings is still limited due to the difficulty of generating large numbers of tumour-specific T cells at the single patient level without using high amounts of cytokines which may cause apoptosis or expansion of nonspecific T cells.

To bypass such a problem, chimeric T cell receptors have been constructed using chimeric receptor genes consisting of antibody variable regions (the antibody being directed to known tumour antigens) combined with T cell activating molecular domains. Once targeted on to tumour cells by the antibody scFv, the chimeric receptor triggers T cell killing machinery resulting in tumour cell destruction. Such reagents are being used in two main clinical settings, namely with renal and ovarian cancer patients. It has been recently shown that nude mice i.p. xenotransplanted with ovarian cancer cells can be cured by injection of autologous T cells bearing a chimeric receptor with a scFv of an antifolate receptor highly expressed by ovarian cancer cells.[13]

The testing of such an approach in the clinical setting is eagerly awaited to assess its effective antitumour activity. However, it still remains to be determined how easy the preparation of T body lines for each patient, the pattern of circulation of such cells throughout the body, their lifespan, etc is.

In conclusion, genetic manipulation of tumour cells and their use as vaccines has provided several insights into the mechanism of immunisation. Such information can now be exploited to improve the ability of gene-modified cells further to generate a potent antitumour response. Even the gene modification of immune effector cells to confer upon them a more specific targeting of tumour cells is opening new opportunities for novel therapeutic approaches in cancer. The years ahead will ultimately tell us how successful such genetic approaches to cancer therapy will be.

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