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OPINION

Will modern cancer vaccines reach clinical practice?

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Introduction

In recent years several ways of achieving successful immunotherapy (IT) of cancer have been suggested. In contrast to the clinical concepts of the late 1980s and early 1990s clinicians and tumour immunologists have concentrated on developing active specific IT protocols, ie modern cancer vaccines.

Cancer cells are poor immunogens[1] and scientists have described the mechanisms in tumour cells to escape immunosurveillance. Immunologists have discussed bases for the optimal induction of specific cytotoxic T lymphocytes (CTL), in order to elicit a specific antitumour immune response that will effectively eradicate growing tumours.[2] Based on better knowledge and understanding of the biochemistry of major histocompatibility peptide complexes, of antigen (Ag) processing presentation and of the co-stimulatory requirement for effector and memory functions of T cells, experimental strategies have been developed to access novel vaccination approaches such as DNA, recombinant viruses, CTL-defined peptide epitopes, proteic Ag, gene-modified tumour cells or dendritic cell (DC)-based vaccines.

Tumour cells express tumour associated Ag or rejection Ag (TAA) that might be discernible targets for effector T cells provided that a TAA-specific T cell repertoire is available and that T cells can be efficiently triggered and can gain access to the distant metastatic sites.[3] The TAA is the immune regulator: the place, dose and time of availability of the TAA in secondary lymphoid organs determines whether an immune response is induced and for how long it lasts.[4]

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Gene-modified tumour vaccines

Most if not all tumours, including non-immunogenic tumours, encode tumour-rejection antigens which may be used for the induction of protective immunity. Furthermore, the $CD8^+$ cytotoxic T cells (CTL) are best equipped to recognise tumour cells as foreign and lead to their eradication. This basic knowledge has shifted the emphasis in vaccine development from induction of humoral responses to vaccines inducing cellular responses. There is now growing evidence that somatic cells, tumour cells, or cells infected by pathogens do not as a rule present antigen to naive $CD8^+$ CTL. Rather, the ability to activate naive $CD8^+$ T cells is the exclusive province of professional antigen-presenting cells (APC), whereby antigen is transferred from cells expressing the antigen to the APC, a process which has been referred to as cross-priming, re-presentation or indirect presentation and popularised as the 'danger theory'.[5] A number of observations, old and recent, argue that indirect presentation is an important, if not a major pathway for induction of CTL responses *in vivo*. The use of genetically modified autologous tumour cell based vaccines (GMTV), has received much attention.[6,7] 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. This was based on an older notion which prevailed some time ago; in addition to antigen presented by the tumour cells, full maturation of the tumour-specific CTL required cytokines secreted by activated $CD4^+$ T

cells. The current view is that the role of cytokines or costimulatory molecules in GMTV is to enhance the transfer of tumour antigens to professional APC for activation of naive CTL precursors, as may be the case with interleukin-2 (IL-2) or granulocyte/macrophage colony stimulating factor (GM-CSF) secreting GMTV, or to enhance the expansion of activated or memory CTL, as may be the case with B7-I (CD80) expressing GMTV. Evidence suggest that the main form of professional APC is the dendritic cell.

Dendritic cell based vaccines

The rationale behind the use of dendritic cells in tumour vaccination is based on the fact that the limiting factor in order to achieve tumour CTL induction *in vivo* is the transfer of antigen from the tumour cell to the dendritic cell. Direct loading of dendritic cells with the relevant antigen is an effective method of accomplishing it.[7]

Vaccination against tumours with the help of dendritic cells is thus a logical approach. One has to consider that, regardless of the method of immunisation, class I restricted antigens have to be introduced into the dendritic cell system to activate the CTL arm of the immune response. This is the essence of DC-based vaccination: loading *ex vivo* cultured DC with antigen which are then re-infused into the patient. By contrast, the mechanism of action of most forms of GMTV is directly to activate the DC system *in situ* and thereby enhance the transfer of tumour antigen to the DC system. Thus the primary goals of DC vaccination and GMTV are the same: channelling antigen to the DC system.

Several papers have shown the ability of dendritic cells to activate naive T cells, $CD4^+$ T-helper cells as well as $CD8^+$ CTL. Animal studies have also shown that DC loaded with tumour antigens induce potent antitumour immunity. Whilst animal studies on cancer vaccines do not tell us what will work in the human patient, they serve as an important screening tool to compare and analyse possible clinical vaccination strategies.

GMTV and DCs: practical aspects

Both GMTV and DC vaccines require *ex vivo* manipulation of the patients' cells. Whilst GMTV were highly effective in animals studies, the practical problems related to the translation to clinical settings turned out to be a limiting factor. This is primarily due to the relative inefficiency of the transfer techniques when applied to primary human tumour cells but also to the difficulty of obtaining a sufficient number of tumour cells from the patient, as well as the overall complexity of the procedure. Compared with GMTV, preparation of DC vaccines is a clinically manageable process. The major problem is the production of purified TAA for loading on to DCs. Once completed, DCs themselves can be generated from cancer patients in relatively simple protocols by culturing adherent peripheral blood mononuclear cells from the patients for 5–7 days in the presence of cytokines followed by antigenic loading. Further simplifications of the DC vaccine preparation may soon be achieved based on the observation that Flt3-ligand mobilisation significantly augments resident DC in the blood of healthy volunteers, which may hopefully eliminate the need to culture PBMC *ex vivo* for DC generation in future cancer vaccine protocols.[3]

Does it matter, in the clinical approach, whether a DC and peptide-based strategy is used?

The most advanced approaches in the field of immunotherapy have been achieved by treating patients with metastatic malignant melanoma. This tumour has long defied conventional cancer therapies and has earned its reputation as one of the deadliest of human cancers. Yet despite its characteristic resistance to chemotherapy and radiation, melanoma is one of the few human cancers to which host immune responses can be reproducibly demonstrated. In the early 1990s, the isolation of tumour-reactive $CD8^+$ cytotoxic T lymphocytes (CTL) from the peripheral blood of melanoma patients, or tumour-infiltrating lymphocytes (TIL) from melanoma tissue allowed the functional cloning of numerous genes encoding melanoma antigens recognised by T cells.

Recently two studies have been published describing different approaches to peptide-based therapeutic melanoma vaccination in humans, which depend on facilitating the 'presentation' of peptide antigens to T cells. In one study DCs were adopted as the Ag presenting system[8] and in the other vaccination was based on plain peptide together with incomplete Freund's adjuvant and with help of IL-2 injections at low doses to increase the CTL response.[9] Each group has reported tumour responses of relevance in a substantial proportion of

patients. One might wonder, reading about the two trials whether the way to alert DCs to function has any relevance at all, or is the knack just to have them pulsed anyhow by the antigen without regard to a direct or indirect process.

Nestle and colleagues[8] describe the immunisation of 16 patients with advanced melanoma using peptide-loaded DC. Dendritic cells propagated from patients' blood were pulsed with a mixture of melanoma peptides chosen for their ability to bind to the individual patient's class-I HLA molecules required for recognition by CTLs. A crude tumour lysate was substituted for the peptide combination in the treatment of some patients. The highly immunogenic helper protein keyhole limpet hemocyanin (KLH) was also included to recruit $CD4^+$ helper T cells and support the maturation of a memory CTL response. The pulsed dendritic cells were then administered by a novel route of direct injection into lymph nodes under ultrasound guidance to support an efficient entry into the lymphatic system. Toxicity was minimal and limited to mild local reactions. Tumour regressions were seen in five out of 16 patients, with complete responses lasting for more than a year in two patients. Clinical responses were accompanied in all cases by antigen-specific skin test (DTH) reactivity. Remarkably, two of the five responding patients received tumour lysate-pulsed dendritic cells in which the identity of the relevant tumour antigens was not known.[8] Therefore this approach may be immediately applicable to other human cancers lacking well characterised tumour antigens.

Rosenberg and co-workers[9] took a clear-cut different approach in developing their melanoma vaccine. The specificity and affinity of immunogenic peptides for class-I HLA molecules depends on specific 'anchor' residues at either end of the peptide. An immunodominant peptide of gp100 with a single anchor residue and an intermediate affinity for HLA-A2 was modified to endow it with dual anchor residues and enhanced CTL-generating activity *in vitro*. Subcutaneous administration of the unmodified peptide mixed with incomplete Freund's adjuvant (IFA) failed in most cases to elicit cytokine-secreting, peptide specific T cells in the blood of melanoma patients. In contrast, vaccination with the modified peptide induced T cell responses in 91% of cases. Curiously, however, administration of high dose interleukin (IL)-2 following modified peptide vaccination reduced the frequency of T cell responses to only 16%. However, in these patients a noticeable clinical response rate of 42% was observed, with no responses seen in the patients receiving modified peptide alone.[9] Tumour regressions were seen not only in skin, lymph nodes, lung and soft tissues, but also in liver and brain, sites typically more resistant to IL-2 therapy alone. The higher immunisation levels observed in the vaccinated patients without IL-2 treatment (91%) do not follow the clinical results of one complete response and the other partial responses (42% objective response) seen in the IL-2 treated group (16% immune response). This is a paradox since one would expect several clinical responses considering the higher immunisation rate in the first group. Several explanations are possible. It is likely that tumour cells do not contain the appropriate co-stimulatory or adhesion molecules required to activate the resting precursor cells that circulate in the peripheral blood as a result of immunisation. Peripheral anergy may thus result from contact with antigen in the absence of co-stimulation. IL-2 may be the cytokine required to eliminate this anergy but it seems that it can do so only partially. Furthermore, why does the specific immune response measured in peripheral blood decrease in the group responding well to the treatment?

A similar paradox is that observed by T Boon and collaborators. They have recently completed a multicentre study in which 39 metastatic melanoma patients received three subcutaneous injections of the MAGE-3.A1 peptide every 4 weeks without adjuvants.[10] Among 25 evaluable patients, seven (28%) displayed objective tumour regression, including three with complete response. Two of these had a disease-free response lasting for more than two years. In spite of objective clinical results they could not show any specific cytolytic T cell response. Again the question is: why is a clinical response to a specific cancer vaccination not followed by a measurable specific immune response in the periphery? Are basic immunologists missing something relevant by observation of humans compared with the evidence observed in animals? Is this discrepancy a major aspect in the design of specific immunotherapy trials? Is there a paradox, or not?

Conclusions

At this stage of development, immunotherapy of tumours has reached elegant animal models giving promising anti-tumoural results.

The effort of transposing these well designed models to humans has in a way confirmed the observations already acknowledged at the beginning of modern immunotherapy. The evidence obtained at laboratory

level is far from eligible for direct extrapolation and application to human beings, in expectation of similar clinical responses.

The way to induce effective specific DC activation may differ between the clinical approaches but it seems that it is impossible to reach similar levels of anti-tumour response.

It is conceivable that in the future the *ex vivo* DCs pulsing procedure can be avoided if the use of Flt3-ligand in clinical trials is effective in increasing specific immunisation and clinical response.

The *ex vivo* approach has advantages as well disadvantages. It seems that the *in vivo* approach with IFA may give clinical responses similar to those in the *ex vivo* DC model. However, one should be cautious and consider the possibility that vaccination with free peptide in IFA can, by altering the dosage, schedule, or route of peptide administration, result in tolerance rather than effective immunity. Finally, how should we consider the puzzling paradox of high rate tumour response *vs* low rate specific immune response in peripheral blood cells?

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