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Conference on cancer vaccines

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Introduction

The Istituto Superiore di Sanità and the Italian Association of Immunology organized an international conference on cancer vaccines, held in Rome on 15–16 November 1999, to discuss the state of the art and future prospects in this field. No less important was the occasion it offered to define strategies for implementing and coordinating research in Italy. Cancer prevention and therapy deserve special attention from the authorities responsible for the promotion of public health. Although vaccination performed in patients with advanced neoplastic disease rarely results in objective responses, it is generally expected that such treatment will be beneficial for patients with minimal disease or for patients free from disease but at high risk of recurrence. Anticancer vaccination can be effectively directed against certain infectious agents involved in the pathogenesis of certain human tumours. Moreover, a new class of patient, or even potential patients who are at genetic or environmental risk of developing cancer, can be detected through genetic screening or by more sophisticated, diagnostic tools.

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Dendritic cells overcome the obstacle of immune tolerance

In the opening lecture, Drew Pardoll (Baltimore, USA) reviewed the manner in which dendritic cells (DC) deliver tolerogenic signals in response to tumour antigens in the absence of costimulation. In order to be effective, cancer vaccines must break down tolerance and activate a "cryptic" T cell population that avoid the induction of tolerance by low-affinity binding. Whether tolerance or T cell activation occurs depends on the ability to activate the relevant antigen-presenting cell (APC) since DC may deliver tolerogenic signals in response to tumour antigens in the absence of costimulation. "Danger signals" or a CD40 trigger activates DC to mature to a stage characterized by elevated expression of costimulatory molecules on the cell surface.

This point was well illustrated by Kees Melief (Leiden, The Netherlands), who showed that an E1a peptide derivative from adenovirus can induce immune suppression when given in complete Freund's adjuvant whereas the same peptide is immunogenic if loaded on DC. A better response can be obtained if an elongated peptide is used that requires DC processing for presentation. DC activation and maturation are critical steps for proper T cell priming. Reviewing the role of interferon α (IFNα) in cancer immunotherapy, Filippo Belardelli (Rome, Italy) underlined its importance in linking innate and adaptive immunity by acting as an adjuvant for maturation and functional activation of human DC from peripheral blood monocytes (in the presence of granulocyte/macrophage-colony-stimulating factor, GM-CSF) in vitro and in humanized scid mice.

Differentiation of leukemic $CD34^+$ cells into DC would favour the correct presentation of known and unknown leukaemia antigen(s) to T cells. This and other immunological strategies to treat human leukaemia were reviewed by Robin Foà (Rome, Italy).

In vivo targeting is a matter of choice: DC or tumour cells?

Without any ex vivo manipulation, DC can be activated to perform cross-priming after allovaccination. Tumour cells engineered to secrete cytokines when injected in vivo are, for some time, a depot for both tumour antigens and cytokines, which, like GM-CSF, may affect DC function (D. Pardoll). IFNα can favour tumour cell apoptosis expecially following gene transfer into tumour cells (Belardelli). Thus cytokines capable of simultaneously inducing tumour cell apoptosis and differentiation/activation of both DC and T cells may be of value for a more efficient presentation of tumour antigens and initiation of an immune response.

Co-transduction of both GM-CSF and CD40L enables the direct interaction of tumour cells with DC, which isolated from the transduced tumours, show uptake and presentation of endogenous tumour antigens (Mario P. Colombo, Milan, Italy). DNA vaccine can be delivered orally by using *Salmonella* as the carrier of a plasmid containing tumour antigen cDNA. A surrogate tumour antigen, like β-galactosidase (M. P. Colombo) or human carcinoembryonic antigen (CEA), (Ralph A Reisfeld, La Jolla, USA), delivered to APC in the gut is presented such that it can effectively induce protection against a challenge with tumour cells transduced with β-galactosidase or CEA respectively. Recombinant antibody-cytokine fusion proteins were presented as an alternative mode of delivering a high cytokine concentration specifically to the tumour site. Using an antibody to the Ep-CAM and human interleukin-2 (IL-2) linked to the C_H3 portion of the Fc tail of the mAb to treat CT-26 liver and lung micrometastases, R. A. Reisfeld had obtained a strong therapeutic effect.

New approaches for discovering tumour antigens

To broaden the CTL response to melanoma and to overcome critical hurdles such as epitope loss, down-regulation of HLA and T cell tolerance, new antigens restricted to more stable HLA are needed. Chiara Castelli (Milan, Italy) searched for epitopes recognized in the context of HLA-B or -C using autologous mixed lymphocyte/tumour cell cultures to avoid A-locus down-regulation, which commonly occurs in melanoma. She identified two Cw8-restricted peptides derived from gp100 and TRP-2. She also described the use of DC pulsed with heat-shock proteins isolated from melanoma cells as a way to stimulate both CD4 and CD8 T lymphocytes, with the hope of using such lymphocytes to clone the antigen(s) presented.

β2-Microglobulin-knockout mice, carrying the HLA-A2 transgene as a unique MHC molecule, were used by Lea Eisembach (Rehovot, Israel) to identify novel peptide sequences after immunization with tumour-derived peptide fractions loaded on DC. Overlapping uroplakin peptides were identified from both transgenic mice and patients with bladder transitional carcinoma, thus validating the murine model approach. Novel peptide sequences from prostate antigens PSA, PSMA and PAP and from breast cancer antigens MUC-1 and BA46-1 had been identified using the HLA-A2/β-2 mice.

Carbohydrate antigens

The formulation of a saccharide-based vaccine has been discussed by S. Canevari (Milan, Italy), aberrant glycosylation being one of the most constant features of tumour cells. The globo-H hexasaccharide, as detected by mAb CaMBr1, is highly over-expressed in breast, ovary, lung and prostate carcinomas on the entire cell surface, whereas the normal counterparts have only a moderate and polarised expression pattern. Antigen expression is associated with poorer clinical prognosis in small-cell lung and breast cancers. The minimal antigenic structure of globo-H has been identified and the synthetic Fuc(1-2) Gal(1-3) GalNAc(1-3) Gal mimics the cellular antigen. The synthetic tetrasaccharide was fused with an adjuvant, either keyhole limpet haemocyanin (KLH) or CRM197, and tested in animal models before a clinical study was proposed.

G. Ragupathi (New York, USA) presented results of clinical trials with glycoconjugate vaccines. On the basis of the observation that naturally occurring antibodies against GM2 and gangliosides in melanoma patients may have a protective effect, the goal was to develop a ganglioside vaccine that is reproducibly capable of inducing IgG antibody responses upon immunization. Since differential expression of gangliosides is characteristic for certain tumour entities, specific natural or synthetic gangliosides might be beneficial vaccines. GM2 covalently linked to KLH plus the immunological adjuvant QS21 is optimal for inducing an IgG antibody response in vaccinated patients. The same approach has been applied to several other carbohydrate antigens, resulting in induction of antibodies against GD2, GD3, GD3-lactone, fucosyl-GM1, globoH, syalyl-Tn, Tn, TF and MUC1 in most patients. However, whether clinical remission correlates with such a response is still debatable. Numerous phase I–III clinical trials are in progress in multiple tumours including breast and prostate cancer. The use of polyvalent vaccine is planned.

Non-specific immunity and prophylactic vaccination

The tumorigenic processes leading to mammary carcinoma in BALB/c mice carrying the activated *HER2/neu* oncogene under the control of the MMTV promoter are accelerated by the knocking out of the IFNγ gene. Reporting this observation, Guido Forni (Torino, Italy) showed that the same tumorigenic process is greatly impaired by treating transgenic mice with IL-12. IL-12-activated tumour inhibition was associated with

mammary infiltration by reactive cells, production of cytokines and inducible nitric oxide synthetase (iNOS), reduction in microvessel number and a high degree of haemorrhagic necrosis. Interestingly, IL-12 treatment is more efficient in preventing the early stage of transformation and is optimal for treating initial hyperplastic nodules. The results underscore the importance of non-specific immunity and iNOS induction. This correlation was also reported by D. Pardoll for mice vaccinated with GM-CSF-transduced tumour cells. Biopsies of the DTH reaction site of patients receiving GM-CSF-transduced cell vaccine also revealed infiltration of eosinophils and iNOS induction.

The advantage of a non-specific antitumour response is that it can be directly applied to a broad range of individuals, irrespective of the presence or specificity of any tumour-associated antigen they may express. Preneoplastic lesions may be new target to look at. They do not yet display genetic instability, and should also be more vulnerable to immune attack, since their cells do not markedly remodel the extracellular matrix or produce suppressive factors.

Clinical applications

The third session, devoted to clinical application, was opened by Dirck Schadendorf (Heidelberg, Germany) presenting an update of clinical results in which autologous DC were used for vaccination in more than 50 patients with metastatic melanoma. Results of the evaluation of 32 patients – all in a metastatic stage – were presented. He reported that adverse effects are mild and treatment is easily performed on an outpatient basis. Immunological responses were detectable in 90% of patients against the helper protein KLH, and 14/21 of patients demonstrated a positive DTH reactivity against the peptides used for immunisation, loaded onto dendritic cells. Major clinical responses were seen in 25% of patients, who had all previously failed conventional treatments. Analysis of non-responding patients revealed several immune escape mechanisms operating with the loss of individual HLA alleles, loss of tumour antigens and protein transporters. A prospective randomised clinical trial to compare a standard chemotherapy to vaccination with peptide-pulsed DC is currently underway.

Giorgio Parmiani (Milan, Italy) summarised his experience of using cytokine-modified tumour cell vaccines for the management of melanoma patients. An allogeneic melanoma cell line was transduced with the IL-2 or the IL-4 gene, in order to evaluate the safety and toxicity of such cellular therapy and also the induction of cytotoxic T lymphocyte (CTL) responses and potential antitumour effects. Samples of 5×10^7 or 15×10^7 gene-modified tumour cells were injected at 2-week intervals and immunological tests were performed before and after three vaccinations. Immunisation was found to be feasible and there was no toxicity; however, the clinical efficacy was limited with no major response. Enhanced CTL activity towards autologous melanoma cells or/and melanoma-associated peptides was found in only 1 out of 3 patients analysed. An improved immunogenicity is expected from co-transduction of co-stimulatory molecules like B7.1.

Vaccination protocols targeting MAGE proteins were discussed by N. van Baren (Brussels, Belgium) providing an overview of the current vaccination protocols initiated at the Ludwig Institutes for Cancer Research. Testicular cancer antigens are primarily expressed in tumours exhibiting demethylation, such as melanoma and head and neck, lung and bladder carcinomas, and are mostly located on the X chromosome. More than 200 patients have currently been treated with MAGE peptides in various protocols with only minor adverse effects. Only 68 patients completed the vaccination scheme, with a total of 11 responders (7/25 responding to MAGE-3/A1; 2/17 responding to MAGE-3/A2 peptides). A common observation is the slow onset of a clinical response (up to 1 year) and often slow clinical regression. No CTL could be detected in these patients.

D. Pardoll discussed three clinical trials at the end of the opening lecture. In a phase I dose-escalating (up to 4×10^7 cells) study with 3 patients in each dosage group, patients with renal cell carcinoma were randomized into groups vaccinated with GM-CSF-transduced or non-transduced autologous tumour cells. Some mixed clinical responses were observed. T cell clones were obtained from immunized patients with the CD8 clone mainly recognizing the common tissue antigen of renal epithelium and CD4 clones preferentially recognising unique tumour antigens now being investigated with a view to gene cloning. Allogeneic tumour cell lines were then transduced with GM-CSF and are being used for the other two studies. In one, minimal residual disease is being treated after prostatectomy and the prostate-specific antigen (PSA) level has been chosen to monitor progress during the immediate follow-up. Of 13 patients treated so far, 7 have shown stabilization of their disease or a small reduction of PSA level and 2 a reduction of more than 50%. The third study is treating pancreatic cancer after surgery; vaccination is given before and after radiotherapy/chemotherapy (since the two together they prolong survival to 2 years in 10% of the patients). The assessment parameters here were the time to relapse and the DTH response to autologous tumour. Dose escalation up to 5×10^8 cells was divided into four levels. Patients treated at dose levels I and II relapsed within 18 months; however, 3/11 patients treated at levels III and IV remained disease-free for more than 2 years and showed a DTH response to autologous tumour cells.

Immunological follow-up

Elke Jaeger (Frankfurt, Germany) summarized data obtained following vaccination with GM-CSF and

peptide derived from melanosomal differentiation antigens. Out of 36 patients, 4 had a long-lasting (more than 1 year) remission with a slow response kinetic (over 4 years!) associated with increased CTL activity, DTH reactivity and vitiligo. Immunisation with MAGE peptides was only successful in 1 out of 11 patients and there was no DTH reactivity. For the rest, she underlined that an antigen-specific T cell response correlated with clinical response and that non-responders were characterised by antigen loss or loss of HLA alleles. Although MAGE-1 is much more frequently expressed (40%) than NY-ESO-1 (20%), antibodies can be detected in only 3% of patients carrying tumours positive for MAGE-1 but in almost 50% of those positive for NY-ESO-1. In most cases the antibody response is accompanied by a cellular T cell response. Antibody detection in sera of patients with NY-ESO-1-positive tumours was shown to correlate with the clinical course of disease.

Monitoring of antigen-specific cytolytic T lymphocyte responses by MHC/peptide tetramers was discussed by Pedro Romero (Lausanne, Switzerland) in the context of the analysis of the naturally occurring T cell response. Frequencies of CTL precursors (pCTL) detecting MelanA were found to be high in CD8 cells of PBL (around 0.07%) in healthy donors as well as in melanoma patients. In contrast, pCTL detecting tyrosinase were low or not detectable. Differentiation of naive, effector and memory T cells by co-expression of the appropriate cell-surface markers demonstrated that mostly naive or anergic T cells were stained by the tetramer complexes in PBL of donors or melanoma patients. However, MelanA-specific T cells could be found in metastatic lymph nodes (up to 3.8%) and were shown to express activation markers. Interestingly, PBL of patients immunised with MelanA-derived peptides undergo a dramatic change from a naive to an activated phenotype expressed on specific T cells upon vaccination.

The conference was closed by a round-table discussion focused on the present status and prospects of research on cancer vaccines in Italy. The participants agreed that this conference should be the basis for future meetings dedicated to critically evaluating the progress on cancer vaccines as well as stimulating coordinated research efforts at the international level.

Concluding remarks

It is clear that the definition of tumour-associated antigens – which began a decade ago – has elicited a plethora of novel therapeutic options including peptide vaccination, the use of APC or the utilisation of recombinant viruses or bacteria to expose the patient's immune system to tumour antigens. The role of CD4 cells and additional ways to activate the antitumour immune response fully, including CD40 activation, need more careful attention and will be of particular interest in the near future. Clinical phase I/II studies are still in their infancy but have shown some interesting clinical results, and the first phase III trials are being planned. There is still much room for improvement, including our understanding of how to induce and maintain a reliable and sufficiently strong immune response.

However, what really may change our perspective is the choice of patient to be treated. Patients apparently free from disease following conventional treatment and at risk of recurrences are beginning to receive vaccination and this will probably show the real potential of immunotherapy. New classes of patients or potential patients, i.e. individuals with a high genetic or environmental risk of developing cancer, will then increase the demand for a "soft" treatment like vaccination. We are all motivated and enthusiastic about this prospect; the new millennium calls for a prompt answer.