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Original Paper

Combination of GM-CSF with Antitumour Vaccine Strategies

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CANCER VACCINE DEVELOPMENT

THE DEVELOPMENT of clinically efficient cancer vaccines depends on five critical steps:

(1) Characterisation of relevant cancer antigens, which will constitute the immunogenic component of the vaccine. The strategy in this area is focused on a balance between the degree of tumour specificity and characterisation of antigens that can be of use for defined groups of cancer patients (shared tumour antigens) rather than for individual cancer patients only (unique cancer antigens).

(2) Methods of vaccinating cancer patients, that will reproducibly give rise to strong immune responses in a majority of patients. Since patients with a large tumour burden and different levels of tumour or treatment induced immunosupression are likely to be included in vaccine trials, this is a central issue in cancer vaccine development. Research in this area is thus concentrated on the development of adjuvants that will result in the relevant T cell responses, mainly cytotoxic T lymphocyte (CTL) and TH1 responses. Other important issues are antigen dosage; route of injection, number of injections and intervals between injections, i.e. timing of restimulation or booster vaccinations and duration of vaccination to yield prolonged protection.

(3) Immune response monitoring. In the development phase, the main way to obtain information relevant to the efficacy of the vaccination procedure is through immunological monitoring of the immune responses in the patients. Thus, immunological rather than clinical endpoints is the focus of early stage (phase I/II) cancer vaccine trials. Until now, the methods used to follow immune responses against single epitope antigens, such as synthetic peptides in patients with concomitant disease, have not been sensitive enough. Recent technical advances, such as the use of synthetic human leucocyte antigen (HLA)-peptide complexes (tetramer technology) and the ELISPOT technique, may have solved some of these initial problems. Also, there seems to be a growing consensus that monitoring immune responses *in situ*, i.e. in the tumour itself, in draining lymph nodes or even at the site of vaccination or a delayed type hypersensitivity (DTH) test is going to be far more informative than measurements made in peripheral blood samples.

(4) Development of treatment resistance. Already at this step in the development of cancer vaccines, evidence for tumour escape due to antigen or HLA-loss variants is accumulating. This problem may be approached by two different strategies, one involving the use of vaccine cocktails to minimise the chance of antigen escape, the other focusing on vaccinating patients at an early stage with minimal tumour burden, such as patients with minimal residual disease.

(5) Determination of clinical effects. The final step is the development of clinical trials that will allow the determination of a clinical efficacy of the vaccines. Such trials are likely to focus on two different patient groups and will have different objectives. In patients with end stage disease, it is hoped that cancer vaccines, which so far have demonstrated no serious side-effects, may prolong survival and increase the quality of life of the patients. In the adjuvant situation, the hope is that vaccination may consolidate what has been obtained by the previous treatment, such as surgery, by eliminating residual cancer cells and thus reducing the fraction of patients that relapse.

During the last 5 years, significant advances have been made in these fields. With the advent of molecularly defined cancer antigens that can be made synthetically, the potential to develop cancer vaccines has become a clinical reality. As the majority of these newly defined tumour specific/associated antigens are recognised by T cells, vaccine trials focus on ways to orchestrate the immune system so as to produce the desired type of immune response. Since much of the activity in this field is centered around the generation of CTL responses against small peptide epitopes, and since the most common adjuvant used in animal studies, i.e. complete Freund's adjuvant (CFA), is not suitable for human vaccinations, little was to be learnt from previous vaccine experience in humans or animal models.

THE FUNCTION OF ADJUVANTS IN VACCINATION

Basically, an adjuvant has two functions. Firstly, it will provide a vehicle for delivery of the vaccine (antigen) and in many cases this will also result in a depot effect due to slow release of the active antigen from the site of vaccination.

Secondly, components of the adjuvant, such as mycobacteria, will cause a general inflammatory reaction at the site of vaccination. This is now understood as a complex cellular reaction and the accompanying release of active cytokines and chemokines. Detailed knowledge of these inflammatory processes and the cloning of the various factors involved has allowed us to start to orchestrate immune responses *in vivo* by employing recombinant cytokines and chemokines in combination with molecularly defined vaccines.

GM-CSF AS AN ADJUVANT IN HUMAN CANCER VACCINES

Animal experiments testing the immunogenicity of a large number of cytokine-transfected cancer cell lines, pinpointed GM-CSF as an important immune activator resulting in tumour protection in experimental models [1]. Local production of granulocyte macrophage-colony stimulating factor (GM-CSF) by the transduced tumour cells or GM-CSF delivered locally by injection at the site of established tumours resulted in an induction of antitumour reactive draining lymph node (LN) cells. These sensitised LN cells were capable of tumour rejection in adoptive transfer experiments (data not shown). The potency of GM-CSF as an adjuvant for cancer vaccines was confirmed in multiple laboratories and subsequently formed the basis for clinical trials using GM-CSF transduced cancer cells as vaccines [2]. A large number of experiments as well as data from clinical trials have now firmly established the potency of GM-CSF as an adjuvant both for antibody responses and T cell mediated responses (data not shown). An updated summary of clinical cancer vaccine protocols using GM-CSF as adjuvant registered in the NIH database for clinical trials is given in Table 1. A similar database for Europe is currently not available.

GM-CSF AS AN ADJUVANT FOR PEPTIDE BASED CANCER VACCINES

The mechanism for the adjuvant effect of GM-CSF is believed to be through its effect on antigen presenting cells. Early experiments in humans [3] showed that leprosy patients that had been given 7.5–45 μg/d of recombinant human GM-CSF by the intradermal route demonstrated local skin reactions that appeared within 24–48 h and persisted for more than 6 days. Re-injection of sites led to enhanced areas of epidermal reaction. Microscopy of the sites demonstrated that a major change in the dermis was a progressive accumulation of Langerhans cells (LC), which peaked on day 4. The recruitment of LC was found to be selective. In comparison no increase in dermal LC was seen when GM-CSF was injected subcutaneously. One explanation for the recruitment of LC at the site of GM-CSF injection may be that GM-CSF is a member of the heparin-binding factor family and can bind to heparan sulfate [4]. It may thus be sequestered in the proteoglycan component of the extracellular matrix following intradermal injection. The accompanying gradient that builds up around the site may function as a chemokine gradient for immature dendritic cells precursors entering the skin from the blood and subsequently result in accumulation and maturation of LC at the site. Interestingly experiments performed *in vitro* indicate that picomolar concentrations of GM-CSF function as a chemokinetic mediator for LC [5]. GM-CSF may, therefore, also be one of the critical factors triggering the egress of LC from their epidermal environment. It can be concluded from these experiments that GM-CSF is a key regulatory cytokine determining the fine control in LC trafficking in the skin and thus of antigen presentation following injection of antigen by the dermal route. GM-CSF is, therefore, a very attractive biological response modifier (or adjuvant) for weakly immunogenic vaccines such as peptides given intradermally.

Table 1. *GM-CSF in clinical cancer vaccine trials*

- GM-CSF expressing tumour cells: 1/23
- Recombinant GM-CSF vaccinia virus: 1/23
- GM-CSF with tumour cells: 4/23
- GM-CSF with rec. virus vaccines: 4/23
- GM-CSF with synthetic peptides: 9/23
- GM-CSF with idiotype vaccine: 4/23

Source: PDQ Clinical Trial Search (http://cancernet.nci.nih.gov/cgi-bin/cancerform).

Experiments in an animal model tested this possibility [6]. These authors observed that intradermal inoculation of GM-CSF was more efficient than subcutaneous inoculation in increasing the number of epidermal class II positive cells. Furthermore, intradermal immunisation was more effective than other routes in eliciting tetanus toxoid specific humoral and cellular immunity. In addition, intradermal GM-CSF administered as a single dose with antigen compared favourably with complete Freund's adjuvant and alum in generating an immune response. Finally, they were able to show that rat neu peptides inoculated with GM-CSF could elicit a strong DTH response, whereas peptides alone were non immunogenic. Based on these observations, a phase I study of Her-2/neu peptides with GM-CSF as an adjuvant in patients with stage II or VI Her-2/neu expressing cancers has been initiated (NCI PDQ Clinical Trial overview).

These animal experiments were also confirmed in humans by results obtained in a pilot clinical trial [7] assessing the immune responses to synthetic melanoma-associated peptides injected intradermally. After 3 cycles of immunisation with peptides alone, followed by systemic GM-CSF during the fourth cycle, enhanced DTH responses and $CD8^+$ CTL responses were observed. Objective tumour regressions were documented in all the responding patients, indicating that the use of GM-CSF supports CTL-mediated tumour rejection *in vivo*. Other studies have been less convincing, reporting only minor effects of using GM-CSF in combination with cancer peptide vaccines. The discrepancy in this case is probably related to the choice of a less efficient route of injection of the cytokine.

In cancer patients with tumours harbouring a mutation in one of the genes of the RAS family of oncogenes, mutant ras peptides corresponding to mutations in the tumour tissue may be used as a tumour specific cancer vaccine [8]. T cells that were specific for a 12 Gly→Val mutation could be isolated from a pancreatic cancer patient following ras peptide vaccinations. After cloning *in vitro*, T cells of both the $CD4^+$ and the $CD8^+$ subset were shown to kill tumour cells in a mutant ras specific way. These T cells recognised nested epitopes within the vaccine peptide and containing the 12Gly→Val amino acid substitution [9]. Several recent phase I/phase II clinical trials in patients with colorectal carcinomas, pancreatic carcinomas and melanomas using intradermal injection of mutant ras peptides and GM-CSF have confirmed the experimental animal data of Disis and colleagues

[6]. From these data, the use of GM-CSF clearly results in strong immune responses against mutant ras in a majority of patients even with terminal disease. On the basis of these combined results it is fair to conclude that GM-CSF has a great potential as a biological response modifier or adjuvant in cancer vaccine strategies.

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