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Original Paper

Local Low-dose of Soluble GM-CSF Significantly Augments An Immune Response Against Tumour Antigens in Man

H. Mellstedt, J. Fagerberg and A. Österborg

Immune and Gene Therapy Laboratory, Department of Oncology, Karolinska Hospital, 17176 Stockholm, Sweden

INTRODUCTION

Granulocyte macrophage-colony stimulating factor (GM-CSF) is a pleiotropic cytokine augmenting several functions of importance for mounting an immune response against defined antigens. These functions comprise: recruitment of monocytes/macrophages and dendritic cells (DC) to inflammatory sites; growth support for DC; activation of antigen presenting cells including upregulation of MHC molecules, CD80/CD86 as well as adhesion molecules; promoting usage of the endogenous antigen presentation pathway of exogenous antigens; facilitating migration of DC to local lymph nodes; supporting growth of T cells probably through an indirect mechanism, etc.

Activation of these functions is of significant importance for the induction of a humoral and cellular immunity during vaccination. Systemic administration of GM-CSF to immuno-compromised patients restored their ability to mount an immune response against foreign microbial antigens (i.e. influenza, hepatitis B) [1]. This principle should also be of benefit in tumour vaccination.

There are many data from experimental systems as well as from human clinical trials indicating that cancer vaccines might be clinically useful. In man, colorectal carcinoma patients, stages B and C, have been immunised with autologous tumour cells mixed with Newcastle Disease Virus and shown to mount an immune response as well as to have survival benefits compared with historical controls [2]. These encouraging results have recently been further corroborated in a prospective randomised trial from The Netherlands comparing adjuvant vaccination in colorectal carcinoma patients Dukes' stages B_2 and C using autologous tumour cells mixed with BCG to no treatment. In this large clinical trial comprising about 250 patients a significant improved disease-free survival as well as overall survival were observed for vaccinated patients compared to the control group [3]. In non-Hodgkin's lymphoma, the tumour derived autologous idiotype coupled to KLH (key-hole limpet haemocyanin) has been used for vaccination. Tumour regression was noted in patients with a small tumour burden. In patients entering complete remission after chemotherapy adjuvant idiotype vaccination induced a significant prolongation in disease free survival of those patients who mounted an idiotype specific T cell response [4].

Tumour antigens

Having these promising clinical results in mind it seems urgently warranted to develop effective but also simple concepts for vaccination of cancer patients. There are a lot of candidate tumour antigens for immunisation. The availability of suitable antigens is not a limiting issue. The immunogens can be whole tumour cells as well as cell lysates. For vaccination, the antigen does not need to be defined; the whole repertoire of tumour antigens expressed on tumour cells can be utilised. From a practical and generic point of view, however, such an approach might turn out not to be generally applicable. Defined antigens are most likely to be used. These can be oncofetal antigens (e.g. CEA), tissue-specific antigens (e.g. tyrosinase, gp100), widely shared tumour antigens (e.g. MAGE-1, MAGE-3), mutated antigens (e.g. ras, p53, CDk4), oncogene products (e.g. HER-2/neu), viral proteins (e.g. HPV, EBV), mucins (e.g. MUC-1), idiotypic immunoglobulins, anti-idiotypic antibodies, etc. Thus, finding an antigen for immunisation is no longer a major issue.

A matter of debate is the most effective antigen formulation. It is likely that neither short peptides nor peptides loaded onto DC will ultimately prove to be effective, safe and versatile anticancer vaccines. Such vaccine formulations would be restricted with respect to HLA type and antigen specificities and/or too laborious with respect to application on a large scale. Rather, recent results indicate the use of DNA-, virus-, long peptide chains or protein-based vaccines that encode/harbour a multitude of epitopes.

The majority of tumour antigens considered for vaccination are 'normal' self antigens. Various measures have to be taken to break tolerance and make the patients able to respond to such tumour associated structures. There are several chemical compounds and products derived from microbial agents that can be used as adjuvants and have shown to be effective. However, the mechanisms of action of such agents are not very well understood. Various cytokines have also been considered for adjuvant use in tumour vacci-

Correspondence to H. Mellstedt, e-mail: hme@rah.ks.se

nation and among those GM-CSF might be the most promising for induction of a tumour specific immunity. Others, but not the top priority, are IL-2, IL-12 and γ-IFN.

Pre-clinical studies of GM-CSF in tumour vaccination

A classical experiment on the importance of GM-CSF as an adjuvant in tumour vaccination was published in 1993 by Mulligan and coworkers in a mouse melanoma system. GM-CSF transduced tumour cells were shown to be the most immunogenic compared to tumour cells transfected with a variety of other cytokines to induce a protective as well as therapeutic immunity [5]. These results have repeatedly been confirmed in many preclinical models. The effect is probably mediated by a sustained release of low concentration of GM-CSF, which activates neighbouring antigen presenting cells, which in turn take up, process and present tumour antigens from damaged tumour cells. The importance of prolonged exposure of GM-CSF was obvious by the findings in the mouse B16 melanoma system showing that immunisation with tumour cells together with soluble GM-CSF in saline was not sufficient to induce an effective immunity while immunisation with tumour cells together with GM-CSF encapsulated in microspheres (slow-release formulation) induced an immunity comparable to transduced tumour cells [6]. Similar results have been reported from a mouse lymphoma model. Mice were immunised intraperitoneally with lymphoma cells together with a low-dose of mouse GM-CSF for 1 day and 4 days, respectively. Only when GM-CSF was administered for 4 days was an effective immunity induced. Moreover, a low-dose of GM-CSF seemed to be more effective than a high-dose [7]. Similarly, tumour cells transduced with GM-CSF were more effective in mounting an immunity if they were low-producers as compared to high-producers [8]. Rhesus monkeys have been vaccinated with an anti-idiotypic antibody mimicking the Lewis Y antigen expressed on adenocarcinoma cells. Two systemic adjuvant schedules of GM-CSF were compared. The antigen was given together with 10 μg/kg b.w. of GM-CSF for 1 day and the same dose for 4 consecutive days. Only those monkeys that received the 4 days GM-CSF schedule developed antibodies against the idiotype, i.e. antibodies recognising the nominal tumour associated antigen Lewis Y [9].

Collectively these preclinical data strongly support the use of GM-CSF as an adjuvant for vaccines. It appears that GM-CSF should be given for a few consecutive days. Based on the present experience, 4 days seem to be recommendable. Moreover, a low-dose of GM-CSF seems to be sufficient.

CEA vaccination with and without soluble GM-CSF in man

Mice immunised with recombinant CEA expressed in vaccinia virus were protected against challenge with murine colon carcinoma cells expressing the human CEA and regression was noted of established tumours [10]. Most colorectal carcinoma cells in the vast majority of patients express CEA. Patients immunised with vaccinia CEA mounted a CTL response against tumour cells loaded with cytotoxic CEA peptides and against tumour cells exhibiting endogenously produced CEA peptides.

Protein antigens are an option to use for the development of a vaccine strategy in cancer patients. The full-length external domain of human CEA was produced in a baculovirus expression system. Compared with native (n) CEA baculovirus produced recombinant (r) CEA is less glycosylated while the amino acid sequence is exactly the same. This rCEA preparation was used to immunise colorectal carcinoma patients Dukes' stages B and C. Half of the patients received rCEA absorbed onto alum alone while the other half also received soluble GM-CSF. rCEA was given subcutaneously (s.c.) day 1 and GM-CSF was administered at a dose of 80 μg/day s.c. locally at the same site as rCEA for 4 consecutive days (days 1–4). The patients were immunised seven times during 1 year. At each immunisation, the same procedure was repeated. GM-CSF induced only a local redness and swelling with slight tenderness. A report on the results of the immunity induction during the first 9 months has recently been published [11]. All patients in the GM-CSF group developed a strong IgG response against rCEA while only one third of the non-GM-CSF patients mounted a weak antibody response. All patients in the GM-CSF group developed a strong rCEA specific proliferative T cell response as well as type I T cells (IFN-γ secreting T cells). In 45% of these patients also a weak type II T cell response (IL-4 secretion) was evoked. Both MHC class I and class II restricted rCEA specific T cells were noted. A specific cellular response (proliferation and/or cytokine secretion) against nCEA could be found in 90% of the patients in the GM-CSF group although at a significantly lower level than against rCEA. In the non-GM-CSF group a very weak rCEA specific T cell response was noted in the majority of patients and in this group a type II T cell response seemed to dominate. No significant auto-immune reactions were noted.

The significantly lower T cell response against nCEA as compared with rCEA might be explained by difference in glycosylation. Immunogenic epitopes may be exposed on rCEA which are hidden by carbohydrate moieties on the nCEA molecule.

Since that report all patients have been followed for at least 30 months from start of vaccination, i.e. at least 18 months from the last immunisation. In the GM-CSF group the T cell response against rCEA remained at the same high level as at the time of the last rCEA immunisation. However, in the non-GM-CSF group the specific T cell response had disappeared (H. Mellstedt and colleagues, Division of Oncologic Biotherapy, Karolinska Institute, Sweden).

GA 733 plus GM-CSF vaccination in colorectal carcinoma patients

Practically all colorectal carcinoma patients express on the vast majority of their tumour cells the tumour antigen GA73-3 [12]. This differentiation antigen is also termed CO17-1A, KSA or EpCAM. This particular antigen can induce a spontaneous immune response in cancer patients. About 15% of colorectal carcinoma patients have IgG antibodies against this structure and autoreactive T cells can also be found at diagnosis [13]. Moreover, the therapeutic mouse monoclonal antibody (MAb) 17-1A (Panorex®) is directed against this antigen and promising clinical results have been presented with this antibody when colorectal carcinoma patients Dukes' stage C were treated in the adjuvant situation [14]. These data might indicate that GA73-3 may be a suitable antigen for immunisation.

The complete external domain of the GA73-3 antigen has been produced in the above mentioned baculovirus system for vaccination. A controversial issue in tumour vaccination in man using self-antigens is for how long a period of time immunisation should continue. There are those who favour

repeated immunisations for a prolonged time to break tolerance against auto-antigens and to maintain an immune response, while others support the idea of a few immunisations not to induce immune suppression. In an ongoing trial of our own, patients receive only three immunisations with the recombinant GA73-3 antigen (weeks 0, 2 and 6) and at each immunisation soluble GM-CSF (75 μg/d for 4 consecutive days) is co-administered s.c. at the same site as the vaccine is delivered. This vaccination procedure has been carried out without other side-effects than a local reaction as described for CEA. An example of the T cell response in 1 patient is shown in Figure 1. As can be seen, a very strong T cell response was induced which was maintained for the whole observation period (10 months) only after 3 vaccinations. The specific T cell response could be inhibited by MHC class I as well as class II MAbs indicating that the reactivity was confined to both CD4 and CD8 T cells (data not shown).

Idiotype vaccination in combination with GM-CSF in multiple myeloma

The idiotypic immunoglobulin structures on the surface of myeloma cells can operationally be regarded as a tumour specific antigen. Myeloma patients have been shown to have natural humoral as well as cellular immunity against the autologous idiotype. A cellular immunity could be detected in most of the patients with a low stage while in advanced stage fewer patients exhibited an idiotype specific T cell immunity. In low stage, the idiotype reactive T cells were mainly of type I and MHC class I as well as class II restricted.

The idiotypic tumour-derived immunoglobulin can easily be isolated from serum of the patient. Such purified autologous idiotypic immunoglobulin from IgG myeloma patients has been used for vaccination of individual patients with an indolent disease. In the first series of patients, the idiotype adsorbed onto alum alone was used. A weak transient T cell response was noted in 60% of the patients. No side-effects were noted. No clinical effects were registered [15]. In a subsequent series, GM-CSF in the same dose-schedule as described above was added. All 5 patients included in the trial mounted an idiotype specific type I T cell response. Preferentially MHC class I T cells were induced. The patients were immunised six times during a 14 week period. The cellular response was sustained for a maximum of 1 year. One of the patients in this group has a major tumour response (>50% reduction of the M component conc.) [16]. Comparing the idiotype specific T cell responses between the group of patients immunised with idiotype alone or idiotype plus GM-CSF, the idiotype specific T cell reactivity was significantly enhanced in the GM-CSF group [17].

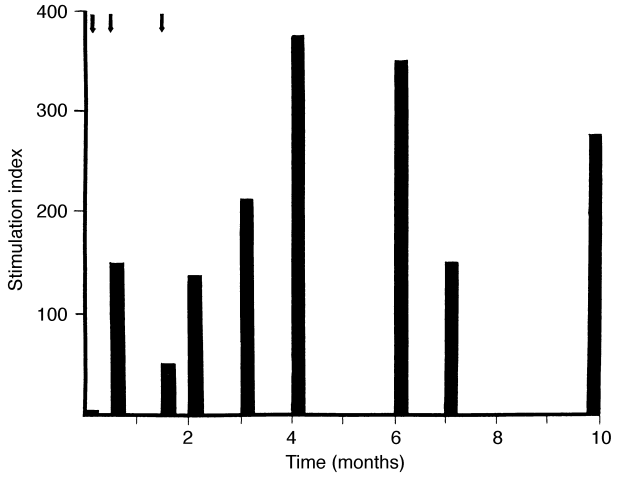

Figure 1. Specific GA73-3 proliferative response (stimulation index) in a patient with colorectal carcinoma stage C immunised with recombinant GA73-3 (400 μg) and local administration of GM-CSF (75 μg/day for 4 consecutive days). Arrows indicate time of immunisation.

CONCLUSION

From a theoretical point of view, GM-CSF should be one of the key-cytokines to combine with tumour vaccines (or other vaccines) to augment the initial phase of immunity induction. We here describe a straightforward, simple and cost-effective usage of soluble GM-CSF to significantly enhance the immune response without side-effects. This simple approach can be used for any kind of tumour vaccine preparation (e.g. whole tumour cells, peptides, proteins and virus expressed tumour antigens). An essential part of the concept is probably the prolonged administration of soluble GM-CSF. Besides GM-CSF other cytokines might also be considered to add. IL-12 is an interesting cytokine that promotes the T cell response towards a type I response, which is of importance for tumour rejection. Studies combining GM-CSF and IL-12 in tumour vaccination are in progress.

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