Gut

Leading article

Towards immunotherapy of pancreatic cancer

A recent review of pancreatic carcinoma stated, "In spite of poor results, we must continue to search actively for more accurate methods of diagnosis and better methods of treatment"[1]; in light of this, further study of immunotherapy may be appropriate.

Gut readers will not be surprised to see this article as, every decade, immunotherapy becomes a "hot" topic and, as such, generates short lived enthusiasm. Thus, BCG, tumour lysates, and even interleukin (IL) 2 as a single agent, have recently been of interest to those involved in scientific research. Immunotherapy is currently at the fore as we can now, actively or passively, stimulate the immune system of patients with pancreatic cancer, creating an immunotherapeutic regimen which may be partially or completely effective in curing the disease. Why such optimism? Recently, developments in genetic engineering techniques have lead to breakthroughs identifying tumour antigens, the description of numerous cytokines (approximately 25, including IL 1–18, tumour necrosis factor (TNF) o/o, interferons (IFN) α, β, γ, and others), and more recently, chemokines, of which there are more than 30, including their receptors.

Using recombinant techniques, it is now possible to produce large amounts of antigens and cytokines for preclinical and clinical studies. Furthermore, these reagents, and the crystallisation of MHC class I and class II molecules, have shown us how antigens enter cells, are degraded into peptides, and presented by class I or class II molecules.[2 3] This process is particularly important because class I molecules present peptides to CD8 T cells. In tumour immunotherapy, this may give rise to killer T lymphocytes which function either by killing and/or inducing cytokine production, particularly IFN γ, TNF α, IL-2, and IL-12—that is, a Th1 response. The most effective therapy also requires CD4 mediated T cell help, with recognition occurring through longer peptides presented by MHC class II molecules.[4] It was previously thought that appropriate anchoring amino acids had to be present in the peptides to generate an efficient cytotoxic T lymphocytic response; however, we have recently shown that in experimental models, mucin 1 (MUC1) peptides (found in pancreatic cancer) bind with low affinity in an unusual way, while still generating effective cytotoxic T lymphocytes (CTL).[5 6]

As we now understand how class I and class II presentations work, consequently, we understand how antigens track through different pathways in the cell. It is difficult, however, to find the most appropriate carrier system, allowing the antigens to enter the chosen pathway and give the appropriate response. After passage through the cell membrane, peptides enter endosomes and subsequently the cytoplasm, prior to class I presentation. Certain bacterial derivatives—for example, listeriolysin-O, BCG, and oxidised mannan containing aldehydes, can trigger this process.[7 8] This sequence of events causes an internalised antigen to escape from the endosome, enter the cytoplasm and thus, via proteosomal processing and a transport associated protein dependent mechanism, is assembled in the endoplasmic network, with the MHC heavy and light chains, as class I molecules for presentation. The processing occurs in tumour cells which may be targets for CTLs (direct presentation) or shed antigens, which are processed by macrophages or dendritic cells (indirect presentation). The latter usually provide appropriate signals to cause sensitisation, as sensitised cells can act directly on the target cancers provided that they express MHC class I molecules—for example, HLA-A1 and A2.

Class II presentation requires passage through early and late endosomes into lysosomes, and association with various components (such as the invariant chain) for passage and presentation. At present, it is not likely that a single peptide binding to one HLA allele will suffice to induce appropriate CD4 help and a CTL response to eradicate tumours. Various strategies have been designed to couple helper epitopes to class I epitopes; multiple peptide epitopes are likely to be present in the cocktail (or string of beads) presented by different HLA alleles which generates appropriate T cell help.

The material provided by the identification of antigens, how they can be presented by class I or class II molecules to generate the Th1 response considered necessary to eradicate tumours, the addition of cytokines to amplify responses, and other such reagents, will be sufficient for many years of study. Other approaches are being made using carbohydrate antigens (such as Ca19-9 and Tag 72), in some vaccines. However, it is not yet clear how T cells can be manipulated to have anti-tumour effects against target carbohydrate antigens, although present studies are concentrating on CD1 antigens.[9] Finally, although we are not considering this area in this paper, monoclonal antibodies, particularly after humanisation, are now receiving more attention for passive immunotherapy.

The opinions given earlier can be applied to the immunotherapy of any solid tumour, where the aim is to generate killer T cells and also to increase their production and effectiveness with appropriate T cell help, possibly by using cytokines. How can this relate directly to pancreatic cancer? A number of tumour associated antigens, which are also found in other solid tumours, have been described

Abbreviations used in the paper: CTL, cytotoxic T lymphocyte; IFN, interferon; IL, interleukin; TNF, tumour necrosis factor; GM-CSF, granulocyte macrophage–colony stimulating factor.

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Table 1 Potential peptide targets in immunotherapy for pancreatic carcinoma

Antigen	*Approximate % incidence*	*Reference*
KAI1	89	23
MUC1	80	24
Her2/neu	50	25
PAP	20	26
NT-3	72	27
c-fos	75	28
Ki-ras	90	29
p53	67	30
CA 19-9	76	31
CA 17.1	90	31
TPA	85	32
TUM2-PK	71	33
CEA	39	33

PAP, pancreatic associated protein; NT-3, neurotrophin-3; TPA, tissue polypeptide antigen; CEA, carcinoembryonic antigen.

in pancreatic cancer—for example, MUC1 is found in virtually all pancreatic cancers, and is similar to that found in breast cancer. Indeed, it was cloned at the same time from both pancreatic and breast cancers.[10 11] Because of these similarities, many preclinical studies performed to study one particular cancer can be used in another; there are now many targets which could be used for immunotherapy of pancreatic cancer (table 1). Several different means have been described for the eradication of breast cancer tumours in mice by targeting the MUC1 antigen. One method is to use mannan MUC1, as we did in a previous study that was the first to demonstrate the generation of effective CTLs which could eradicate MUC1+ tumours in mice.[12] CTLs have also been induced by the administration of MUC1 with vaccinia virus or DNA–MUC1 constructs. These studies are of some interest, but the immunogenicity of human mucin in mice is of limited application to humans. More recently, MUC1 transgenic mice have been immunised to make an effective CTL response, particularly by fusing tumour cells with dendritic cells; these cells were able to prevent metastasis.[13] In vitro immunisation with dendritic cells has proved to be useful, particularly in overcoming the difficulty of immunising patients with cancer. The antigen is placed directly on the target antigen presenting cells (the dendritic cell) prior to transfer into the patient; adoptive transfer between relatives sharing the same HLA allele is also possible. Additionally, a cross reaction between pre-existing antibodies in patients and MUC1 tumour antigens diverts the immune response towards antibodies rather than cellular responses. In the absence of serum antibody, this can be overcome by in vitro treatment.[14] Thus, in vitro sensitisation of dendritic cells may be the optimal way to immunise patients.

The work on dendritic cells of interest as these are generated from patient's blood (possibly by pretreating the patient with granulocyte macrophage–colony stimulating factor (GM-CSF)). In the presence of IL-4 and GM-CSF, after a period of culture in vitro, mature dendritic cells are produced which can then be exposed to antigen (usually recombinant peptide delivered with a carrier, although more recently whole tumour lysates have been used effectively in experimental systems). Although the logistics of this approach could present many problems, culturing dendritic cells in vitro for one week would not be difficult in a unit which routinely does autologous bone marrow transplantation, often after separation of CD34 stem cells. Presently, this may offer the best help for immunotherapy in patients with solid tumours.

Interestingly, results of studies of transgenic mice and of patients suggest that tolerance to an auto-antigen (MUC1) has been broken. This may be because T cells binding low affinity peptides are not eradicated during thymic maturation. It is also possible that the synthetic MUC1 peptides used for immunisation, being devoid of carbohydrate, do not resemble a native peptide and thus, the immune system does not recognise them. The possibility of the existence of autoimmunity has not been intensively studied as there are such difficulties in inducing immune responses in patients with cancer. Indeed, immunotherapeutics might even be encouraged by these responses, as they may be seen to be indicative of a biological effect of therapy, as opposed to finding CTLs, antibodies, and no tumour responses. As a result, occasional tumour responses have been noted in melanoma; vitiligo also occurs due to autoimmune reactions in the skin. Although such superficial effects might be acceptable in melanoma, the occurrence of severe pancreatitis would not. As many of the antigens present in pancreatic cancer (table 1) are also found in a normal pancreas, continued monitoring for autoimmune pancreatitis would be sensible. It is, however, expected that cancer would be the preferential target as a tumour may express 10–100-fold more of the antigen than normal tissue.

We know about the antigens of the different carriers used, the cytokines that generate an appropriate Th1 CTL response, and how this can be augmented with cytokines. The most popular approach would seem to be the ex vivo exposure of dendritic cells to one or more of the antigens listed in table 1, perhaps with a cytokine in vitro, followed by in vivo use, possibly with another cytokine such as IL-12. Such trials are currently in progress, principally in melanoma and breast cancer; several pancreatic cancer trials are also ongoing.

Pancreatic cancer should be receiving more attention for immunotherapy as the prognosis for this disease is so poor. The low survival rate allows for more efficient determination of the effectiveness of treatment; within one year of completion of a trial, it should be possible to tell whether a new, biologically based therapy has been substantially beneficial. Furthermore, there are few successful, competing therapies for pancreatic cancer. Information is already available on the ability, in an experimental setting, to generate immune responses in pancreatic cancer to antigenic peptides present and overexpressed. CTLs or proliferative T cells have been found in pancreatic cancer reactive to MUC1,[15] Her2/neu,[16] carcinoembryonic antigen,[17] p53,[18] and ras.[19] Clinical immunotherapy programmes are also in place with mutant ras peptides.[20 21] These studies include a controlled trial on the use of mistletoe which shows that this alternative drug has no effect.[22] The mention of such a trial may seem frivolous but sophisticated genetic engineering techniques have yet to lead to the development of any treatments that would have a substantial effect on tumour growth in patients with advanced solid tumours.

Currently, we can be cautiously optimistic that immunotherapy will have some value in the future. This value would be based on identifying and producing antigens and the ability to induce CTLs in transgenic animals and in patients—that is, to seem to break tolerance, especially with low affinity binding peptides. Yet, there is no convincing evidence that the presence of CTLs to one or more peptides correlates with a substantial degree of tumour shrinkage, even though responses have been noted in patients with melanoma.

The trials, however, are in early stages and as more phase I studies are done, and the safety of the treatments demonstrated, patients with early stage disease will be studied. These patients will have the advantage of being healthier, with better immune responses, and smaller tumours for the immune system to eradicate. Finding such patients may be difficult as pancreatic cancer presents late. The current plethora of agents will take many years to sort out, but better responses will be generated by GM-CSF before blood harvest, IL-4 and GM-CSF for dendritic cells, possibly

with IL-7, and followed by IL-12 both in vitro and in vivo. The poor prognosis of pancreatic cancer means that it should be high on the list of diseases examined in special immunotherapeutic centres where in vitro and in vivo treatment and testing can be done on sufficient numbers of patients to determine the best course of action.

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