

Immunotherapy for colorectal cancer

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SUMMARY

Immunotherapy includes techniques to boost natural immune resistance to tumours with both vaccines and biologic response modifiers, primarily cytokines involved in modulating immune responses. The occasional dramatic disappearance of widespread metastases is probably attributable, in most cases, to a brisk and successful immunologic response. The occasional patient who survives in the face of known metastatic disease for a decade or more is probably also a testament to an effective immunologic host response. These occasional examples demonstrate the power of the immune system to arrest or even cure what appears to be a hopeless case. Clearly, we would like to be able to achieve this result deliberately in all cancer victims. The key to achieving predictable and significant immune responses in cancer patients lies in a better understanding of the nature of tumour immunity.

INTRODUCTION

Generally speaking there are two broad types of anti-tumour immune responses. One involves the humoral arm of the immune system and the other involves the cellular arm of the immune system. An important aspect of both is the ability of antigen-presenting cells to process and present tumour-related peptide antigens that are the primary basis for immune recognition of tumour cells. Tumour antigens that have been phagocytosed and partially digested by antigen-presenting cells are presented as peptides bound to MHC type II receptors on the

surface of antigen presenting cells. Examples of such antigen-presenting cells include macrophages, epidermal Langerhans cells, other types of dendritic cells and B-cells. The MHC class I cell surface receptors that are the basis for HLA tissue typing are present on all nucleated cells in the body including tumour cells. These receptors semi-randomly present examples of peptides present within the cell. MHC class I receptors also present tumour-specific peptide antigen on the tumour cell surface, giving the opportunity for the properly sensitized immune system to react to the tumour.

The antibody-mediated arm of tumour immunity

Antibody-dependent mechanisms of tumour immunity include antibody-dependent cell-mediated cytotoxicity (ADCC), complement dependent cytotoxicity (CDC) and opsonization. These mechanisms depend on the ability of the immune system to create antibodies to tumour cell surface antigens that in this case, do not have to be presented on class I MHC receptors, as with the T-cell-mediated responses to be discussed later.

Antibody-dependent cell mediate cytotoxicity (ADCC)

ADCC involves the attachment of tumour-specific antibodies to tumour cells and the subsequent destruction of the tumour cell by immunocompetent cells. Fc receptors on immunocompetent cells recognize the Fc portion of antibodies adhering to surface tumour antigens. Most commonly, the effector cell of ADCC is a natural killer (NK) cell. Following recognition and attachment via its Fc receptors, the NK cell can destroy the target tumour cell through release of granules containing perforin and granzym B and/or activation of the FAS/FAS ligand apoptosis system in the target cell. Perforin molecules make holes or pores in the cell membrane, disrupting the osmotic barrier and killing the cell via osmotic lysis.

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Complement-dependent cell-mediated cytotoxicity (cdc)

Complement-dependent cell-mediated cytotoxicity involves the recognition and attachment of complement-fixing antibodies to tumour-specific surface antigens, followed by complement activation. Sequential activation of the components of the complement system ultimately lead to the formation of the membrane attack complex (MAC) which forms transmembrane pores that disrupt the osmotic barrier of the membrane and lead to osmotic lysis. The MACs function similarly to the perforin molecules released by cytolytic T cells and NK cells, killing cells by osmotic lysis.

Opsonization

Opsonization is the process in which tumour-specific antibodies attach to their target antigens on tumour cell surfaces, thus marking them for engulfment by macrophages. This can also lead to processing and presentation of new tumour-specific antigens by the macrophage in addition to direct destruction of the tumour cells.

The cell-mediated arm of tumour immunity

Cell-mediated tumour defenses include cytolytic T-lymphocytes, NK cells and macrophages. Cytolytic (CD8 positive) T-cells destroy tumour cells via T-cell receptor recognition of tumour-specific antigen presented on MHC type I receptors at the tumour cell surface. Tumour antigen-specific T-cells bind to the MHC I receptor-tumour antigen complex and destroy the tumour cell via the release of granules containing granzyme B, perforin and via induction of FAS pathway apoptosis.

In addition to being the principal effector cell for ADCC, NK cells participate in tumour immune responses in another way. All nucleated cells express MHC I receptors on their surface. The primary purpose of the MHC I receptor is to present endogenous peptide (self) antigens on a cell's surface. When a foreign peptide antigen such as a tumour-specific antigen is presented by the MHC I surface complex, cytolytic T-cells reactive to the antigen can recognize the foreign peptide with their T-cell receptor and kill the cell. Another function of MHC I receptors is to inhibit the innate tendency of NK cells to bind to and kill cells. Tumours may attempt to avoid immunosurveillance by downregulating expression of MHC I receptors, thus avoiding T-cell recognition. However, because MHC I expression is necessary to inhibit natural killer function, tumour cells that downregulate expression of MHC I receptors target themselves for natural killer attack.

Recent modalities for colorectal cancer immunotherapy

The aim of this review is to update gastroenterologists on recent advances in the field of colorectal cancer immunotherapy. Several approaches have been considered. Active nonspecific immunotherapy aims to stimulate the immune system, without targeting any specific tumour antigen. Diphtheria toxoid, Bacille Calmette-Guerin (BCG) levamisole, and cytokines such as interleukin-2 (IL-2) are all examples of this modality. This is different from active specific immunotherapy where vaccines based on anti-idiotypic antibodies, DNA, tumour-associated antigens and its peptides, and heat shock proteins all prime the immune system to target individual tumour antigens. This review also covers monoclonal antibodies developed to target antigen, and adoptive immunotherapy.

Active nonspecific immunotherapy

Active nonspecific immunotherapy aims to augment the body's immune response, without directing it against any specific tumour antigen. Approaches include BCG, IL-2, levamisole, and diphtheria toxoid.

Intraperitoneal administration of BCG to patients with advanced colorectal cancer was associated with minimal toxicity and a median survival of 13,2 months¹. A significant prolongation of disease-free interval and overall survival was seen in 83 patients with Dukes C tumours, randomized to receive BCG ± 5-fluorouracil (5-FU)². BCG in conjunction with autologous colorectal tumour cells in patients with Dukes B and C cancers, however, showed no survival benefit³. Other work using BCG in patients with stage II and III disease has confirmed this lack of benefit, in terms of overall and disease-free survival.⁴

IL-2 is a 15,5 kDa glycoprotein that plays a central role in immune regulation.⁵ Administration of the cytokine to 155 patients with advanced malignancy showed objective response rates of 22% and 24% in renal cell adenocarcinoma and melanoma, respectively, but no regression of any colorectal cancer metastases⁶. Four patients died of therapy-related complications, and many experienced nausea, vomiting, and malaise. IL-2 has been given preoperatively to 50 patients with metastatic colorectal cancer leading to a prolonged survival time.⁷ Administration of IL-2 in combination with IL-1 to 14 patients with advanced disease showed objective responses in 7, with toxicities similar to those described above.⁸ A combination of IL-2 with IL-4, and use of the killer cell growth factor IL-12 have also been proposed as potential forms of ac-

tive nonspecific immunotherapy.^{9,10}

Levamisole is minimally toxic and has been shown in vitro to augment the immune response by potentiating T cell, macrophage, and neutrophil function. Results in the clinical setting have, however, been disappointing, with two randomized trials showing no survival benefit when compared with placebo.^{11,12}

Diphtheria toxoid may act as an immunostimulatory agent in patients with colorectal cancer¹³. Significant increases in serum levels of IL-2, IL-6, IL-8, and tumour necrosis factor (TNF)- α were seen following administration, with only 2 of the 22 patients showing evidence of recurrence at 5 years.

Adoptive immunotherapy

Adoptive Immunotherapy is a treatment approach in which cells with antitumour reactivity are administered to a tumour-bearing host, in whom they mediate, either directly or indirectly, the regression of the established tumour.¹⁴ There are, broadly speaking two strategies. The first involves removing mononuclear cells from the peripheral blood, stimulating them with IL-2, and infusing them into the patient. The second requires lymphocytes to be separated from fresh tumour specimens, stimulated in IL-2, and then infused back into the patient.

Incubation of human peripheral blood lymphocytes with IL-2 generates lymphoid cells capable of lysing fresh natural killer (NK)-resistant tumour cells. These have been termed lymphokine-activated killer (LAK) cells. Infusion of LAK cells in combination with IL-2 caused regression of pulmonary and hepatic metastases from MC-38 murine colon adenocarcinoma.¹⁵ Partial responses in 3 of 26 colorectal cancer patients receiving LAK + IL-2 have been observed, with toxicity confined to hypotension, weight gain, and oliguria. Further work has confirmed these findings, with 1 complete, and 4 partial responses seen in a total of 30 patients.¹⁶

The second of the two approaches involved infusing lymphocytes separated from fresh tumour specimens with cyclophosphamide and IL-2. Sixty-six patients were treated with this regime, of whom 2 had colorectal cancer. Objective responses were seen in up to 50% of patients, all of whom had melanomas and renal cell carcinoma.

Attempts have been made to assess the effector cell population involved in tumour cell killing following adoptive immunotherapy. Work involving intraperitoneal administration of rIL-2-expanded tumour-infiltrating lymphocyte (TIL) in patients with advanced epithelial

ovarian cancer showed that such TIL were primarily CD3+CD4+TCR $\alpha\beta$ +, while another study showed they were predominantly CD4+ and CD8+^{17,18}. These findings have been confirmed in TIL from patients with colorectal cancer¹⁹. In addition, CD3- CD56+ and CD3+ CD56+ phenotypes were also seen. The efficacy of adoptive immunotherapy was challenged, however, when work showed that expanded TIL become trapped in liver, lungs, and spleen rather than having any effect at the tumour site.²⁰

Monoclonal antibody therapy

Monoclonal antibodies (MAb) may be used alone as therapeutic agents to cause tumour cells to be destroyed. They do this by a variety of mechanisms, including apoptosis (programmed cell death), complement-dependent cytotoxicity, and antibody-dependent cell-mediated cytotoxicity. They have also been used in conjunction with radioactive sources and cytotoxic agents, and as antibody-dependent enzyme prodrug therapy, although these areas will not be covered in this review.

Probably the most extensively investigated antibody is 17-1A, a murine IgG2a MAb against a 26kDa polypeptide tumour-associated antigen known as GA 733-2 (or CO 17-1A).²¹ A review of 8 trials using 17-1A MAb in over 200 patients with colorectal cancer showed a response rate of around 5%. The effect was short-lived, although associated toxicity was low.²² A further 5 of 24 patients with metastatic colorectal cancer showed evidence of tumour regression.²³ As antibody-dependent cell-mediated cytotoxicity is one of the effector mechanisms for tumour cell death, the action of the antibody should be potentiated by granulocyte macrophage colony-stimulating factor (GM-CSF). This was thus tested on 20 patients with metastatic cancer. Two patients achieved complete remission, and 1 showed a minor response. A further 2 patients had stable disease.²⁴ The 17-1A antibody has been used as postoperative adjuvant therapy in 189 patients with Dukes C tumours. Patients receiving 17-1A had a 30% and 27% reduction in death and recurrence rate, respectively.²⁵ An update of this work has confirmed reductions in mortality rate and tumour recurrence by 32% and 23%, respectively, after a median follow-up of 7 years.²⁶ These data are currently being tested in the United Kingdom in a randomized, multicenter phase III study, recruiting patients with Dukes C tumours to one of three arms: 5-FU and FA, m17-1A and 5-FU, and FA and M17-1A.

The original work using this approach used murine monoclonal antibodies, which may be recognized as for-

eign, leading to human antimouse antibodies and reducing overall efficacy.²⁷ Formation of chimeric antibodies, which are less immunogenic, is one approach, although recent work has shown how single-chain Fv antibodies against CEA may be used.²⁸ These consist of light and heavy chain variable regions bound by a peptide bridge, and have been shown to penetrate further into tumours, while removing the effect of nonspecific Fc binding.

Active specific immunotherapy

Reasons proposed for why colorectal cancer cells are incapable of eliciting an immune response include an inability to process epitope, absent adhesion, or costimulatory molecules, the presence of inhibitory cytokines, or the fact that these tumours have low expression of MHC molecules.²⁹ Active specific immunotherapy attempts to stimulate the immune system to target a specific tumour antigen by presenting epitope in a different form. A number of different approaches have been adopted. Anti-idiotypic antibodies mimic antigen and elicit T cell responses. Polynucleotide vaccines (DNA and RNA) encode the tumour antigen, whereas vaccines based on viral vectors provide an alternative way of altering the host genome. Oncogene products may act as tumour-associated antigens, against which vaccines may be developed, and autologous tumour may be processed to form mucin or heat shock protein-based vaccines.

Heat shock protein preparations from a patient's tumour contain antigenic peptides bound to heat shock protein molecules.^{30,31} Immunization with this type of vaccine removes the need to identify all the antigenic epitope on the cancer cells, as heat shock proteins are naturally complexed with the entire repertoire generated in the cell. Immune responses are therefore against all antigens present in the tumour. As the vaccine is autologous, no material is inoculated into the patient that they haven't already been exposed to, thus reducing the chance of toxicity. Murine studies have shown that injection of apparently homogeneous heat shock protein preparations can confer resistance to a tumour challenge,³²⁻³⁵ and phase I studies are currently ongoing.

Glycoprotein mucins protect the underlying gastrointestinal mucosa. They consist of a large number of O-glycosylated tandem repeat domains that vary in number, length, and degree of glycosylation.^{36,37} Tumour mucins have shorter sugar side chains, thus exposing peptide antigens against which immune responses may be generated.³⁸

A phase I study using a mucin peptide admixed with

BCG has recently been undertaken in patients with advanced colorectal cancer.³⁹ Delayed type hypersensitivity responses were seen against mucin-specific peptides, though only 2 patients had stable disease.⁴⁰ Patients with advanced colorectal cancer have been immunized with Theratope sialyl-Tn-KLH (keyhole limpet haemocyanin) cancer vaccine in Detox adjuvant, following low-dose cyclophosphamide therapy.⁴¹ This study showed that patients with higher antibody titres following vaccination survived longer than patients with lower titres, thus suggesting a response that might confer an advantage on immunized patients.

Peptide vaccines can bind to class I and II major histocompatibility complex (MHC) molecules and elicit immune responses. Somatic point mutations of *r* α s oncogenes occur in approximately 45% of colon adenocarcinoma. Activation of the *r* α s oncogene occurs most commonly at codon 12 or codon 61 and results in corresponding single amino acid substitutions within the p21 *r* α s protein. Mutated, the p21 *r* α s proteins are not expressed by normal tissue, and thus represent cancer-specific proteins. Several studies have shown that T cells from patients with colorectal cancer can recognize peptides that span the mutated segment of mutated *r* α s protein.^{42,43} In a recent study Gjertsen et al⁴⁴ presented data from a clinical phase I/II trial involving patients with adenocarcinoma of the colon and pancreas vaccinated by intradermal injection of synthetic *r* α s peptides in combination with granulocyte-macrophage colony-stimulating factor. Forty-eight patients (10 surgically resected and 38 with advanced disease) were treated on an outpatient basis. Peptide-specific immunity was induced in 25 of 43 (58%) evaluable patients, indicating that the protocol used is very potent and capable of eliciting immune responses even in patients with end-stage disease. Patients followed up for longer periods showed evidence of induction of long-lived immunological memory against the *r* α s mutations. CD4+ T cells reactive with an Arg12 mutation also present in the tumour could be isolated from a tumour biopsy, demonstrating that activated, *r* α s-specific T cells were able to selectively accumulate in the tumour. Vaccination was well tolerated in all patients. Patients with advanced cancer demonstrating an immune response to the peptide vaccine showed prolonged survival from the start of treatment compared to non-responders (median survival 148 days vs. 61 days, respectively; $p=0.0002$). Although a limited number of patients were included in that study, the association between prolonged survival and an immune response against the vaccine suggests that a clinical benefit of *r* α s peptide vaccination may be obtained for this group of patients.

Polynucleotide-mediated immunization involves the intramuscular delivery of DNA or RNA vaccines. Such an approach has been shown to lead to gene expression in myocytes and myofibroblasts and continuous intracellular production of protein antigens that may be presented in association with MHC molecules.^{45,46} The immune response generated against the tumour-associated antigen may be further enhanced by adding genes for cytokines such as IL-2, IL-6, IL-7 or GM-CSF^{47,48}. Mice immunized with a plasmid encoding the full length of cDNA for CEA developed cellular and humoral responses against the glycoprotein⁴⁹. A minigene encoding a single antigen from mutant p53 has also been shown, in a mouse model, to elicit cytotoxic T-lymphocytes.⁴⁷ Phase I studies in patients with B cell lymphoma and malignant melanoma has suggested that T cell responses may be generated.^{51,52}

Viruses may be used to transfect cells with genes encoding tumour-associated antigens. The aim is to co-present a weak immunogen, such as CEA, with a highly immunogenic viral protein in order to enhance the immune response. DNA encoding CEA is inserted into viruses, such as baculoviruses, retrovirus, herpes, pox, adenovirus, and vaccinia viruses. Infected cells express a protein product, recognized by anti-CEA antibodies. Work in animals has shown that effective humoral and cell-mediated responses can be generated that correlate with delayed tumour growth.⁵³⁻⁵⁵ Phase I studies have used vaccinia encoding CEA in patients with colorectal cancer.⁵⁶ Local reactions were seen at the injection site, although cytotoxic T-lymphocyte responses were generated using this approach.^{57,58} The tumour-associated antigen 17-1A has recently been cloned, expressed in baculovirus, and administered to patients with colorectal cancer.⁵⁹ Evidence of antibody responses were seen.

Monoclonal antibodies (Ab1) against tumour-associated antigens may themselves induce antibody formation *in vivo*. These Ab2s are directed against the variable regions of Ab1s, and are themselves termed anti-idiotypic antibodies⁶⁰. Essentially they "mimic" the tumour-associated antigen, and may be presented by antigen-presenting cells in the context of class I and II MHC, thus eliciting both cytotoxic and helper responses. The most commonly investigated Ab2s mimic tumour-associated antigens 17-1A, 791T/gp72, and CEA.

Thirty patients with advanced colorectal cancer were immunized with a goat anti-idiotypic polyclonal antibody, mimicking 17-1A⁶¹. Evidence of a humoral response was seen, and 6 patients showed partial clinical remission and a further 7, arrest of metastases following treatment. A

follow-up trial used a different goat polyclonal antibody in 12 patients who had undergone resection of their primary tumours, and showed similar results.⁶² Cellular immunity has been seen in a further patient with advanced colorectal cancer⁶³ immunized with SCV106, a goat anti-idiotypic monoclonal antibody that also mimics 17-1A.

105AD7 is an anti-idiotypic monoclonal antibody that mimics the tumour-associated antigen 791T/gp72, present on approximately 80% of colorectal cancer cells. No toxicity was seen with the vaccine, and patients who received it lived significantly longer than a contemporary nonimmunized group⁶⁴. In addition, evidence of T-cell responses were seen in 9 of the 13 patients. The vaccine has also been used in patients with primary colorectal cancer, where increased tumour infiltration of CD4-, CD8-, and CD56-expressing lymphocytes has been seen, as well as enhanced expression of CD25.^{65,66}

3H1 is an anti-idiotypic monoclonal antibody that mimics carcinoembryonic antigen. A phase I study showed humoral and cellular responses developing in patients with advanced disease, with minimal toxicity.⁶⁷

GENE THERAPY

The overall objective is to enhance the effectiveness of tumour vaccines through genetic engineering and in this way to increase their ability to induce immune responses capable of destroying cancer cells. Clinical trials in cancer patients comprising immunizations with a mixture of irradiated skin cells genetically modified to express the gene for an immunostimulatory substance, termed IL-2, and irradiated tumour cells are in progress. IL-2 gene transfer has resulted in significant anti-tumour immune responses in several animal tumour models. In these studies, the transfer of IL-2 genes into tumour cells has reduced or abrogated tumour formation after implantation into animals. Successful anti-tumour immunity has been induced in an animal model of colorectal carcinoma by immunization with a mixture of irradiated tumour cells and IL-2 transduced skin cells⁶⁸. Immunization with a mixture of irradiated tumour cells and IL-2 modified cells induced anti-tumour immunity capable of rejecting a subsequent live tumour cell challenge. Repeated immunizations with a mixture of irradiated tumour cells and IL-2 modified cells abolished established, visible tumours in a subset of the treated animals. Several clinical laboratories have chosen to initiate the evaluation of this novel therapy in patients with colorectal cancer. Colorectal carcinoma is one of the most common cancers in the United States and Europe with an

annual incidence of greater than 150,000 in either U.S. or Europe. Most patients are treated with tumour resection and do not have a clinically detectable tumour following surgery. However, the majority of patients have microscopic metastases and eventually relapse with clinically overt disease in the liver or abdominal cavity. Encouraging results have been obtained with a tumour vaccine as an additional therapy following tumour resection. Immunization with tumour preparations resulted in a significant increase in disease free and total survival. These findings, combined with the demonstration of enhanced anti-tumour immunity following tumour immunizations with cells genetically modified to express IL-2 in several animal tumour systems, provided the rationale for using IL-2 gene transfer in clinical studies⁶⁹. Patients received immunizations with increasing doses of IL-2 modified skin cells.

A clinical study of this and related approaches in patients with colorectal and brain cancers has been completed.⁷⁰ The patients treated to date have received at least 3 subcutaneous immunizations at 2-4 week intervals. There have been no significant changes in complete blood counts, serum chemistries or urinalyses compared to pre-treatment values. Delayed type hypersensitivity skin reactions at the sites of the second or subsequent vaccinations were observed in 8/11 patients implying the induction of immunological memory responses.⁷¹ Biopsies of the vaccination sites after the third immunization revealed subcutaneous and dermal perivascular lymphocytic and eosinophilic infiltrates. Anti-tumour immune responses mediated in part by cytotoxic T cells have been demonstrated in 3/5 patients analyzed to date. Clinically, 2 patients have had stabilization of previously rising CEA tumour marker levels during the course of therapy. The patient with the most dramatic skin reaction has had stabilization of previously enlarging abdominal metastases on computerized tomography (CT) scan.⁷⁰⁻⁷¹ Tumour necrosis was observed by CT scans in a patient with a glioblastoma brain tumour. In an additional colon cancer patient treated by direct tumour injection of IL-2 transduced fibroblasts, tumour destruction was documented by CT scan. These findings suggest that these forms of IL-2 gene therapy are well tolerated and warrant further clinical evaluation.

Additional gene modification approaches to enhance the effectiveness of tumour vaccines are also now under investigation. Many tumour cells secrete high levels of the immunosuppressive factor transforming growth factor- β (TGF- β). The transplantable rat 9L gliosarcoma secretes TGF- β and serves a useful model for evaluating

tumour vaccines⁷². Using the 9L model, it is important for testing the hypothesis that genetic modification of tumour cells to block TGF- β expression may enhance their immunogenicity and make them more suitable for active tumour immunotherapy. Subcutaneous immunizations of tumour bearing animals with 9L cells genetically modified to inhibit TGF- β expression with an antisense plasmid vector resulted in a significantly higher number of animals surviving for 12 weeks (11/11, 100%) compared to immunizations with control vector modified 9L cells (2/15, 13%) or 9L cells transduced with an interleukin-2 (IL-2) retroviral vector (3/10, 30%) ($p < 0.001$ for both comparisons). Histologic evaluation of implantation sites in sacrificed animals performed 12 weeks post-treatment revealed no evidence of residual tumour.⁷³⁻⁷⁷ These results indicate that inhibition of TGF- β expression significantly enhances the effectiveness of tumour cell vaccines and supports future clinical evaluation of TGF- β antisense gene therapy for TGF- β expressing tumours. Incorporation of genetic inhibition of TGF- β in future gene therapy clinical trials in patients with colorectal, prostate, breast and lung cancers, is also in progress.

CONCLUSION

Immunotherapy is rapidly developing as a potential treatment option for colorectal cancer. Certain modalities, such as 17-1A MAb, are already in phase III studies, while others are clearly less well advanced and may ultimately not fulfill their early promise. Gastroenterologists need to be aware of the advances in this field, and keep an open mind on their efficacy.

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