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Review

Colorectal cancer vaccines

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Background Advances in molecular pathology have enabled a number of colorectal cancer antigens to be identified and characterized. The commonest investigated include 17-1A, 791Tgp72 and carcinoembryonic antigen. Vaccines have been developed that stimulate the immune system to target these antigens. This paper reviews current areas of research in this field.

Methods and Results Relevant articles were obtained on vaccines for colorectal cancer from Medline and the Bath Information Data System. A number of approaches are currently being evaluated in Phase I, II and III trials. These include anti-idiotypic antibody immunization, DNA vaccines, mucin and heat shock protein-based vaccines, oncogenes and viral vectors.

Conclusion Evidence is accumulating to suggest that immune responses may be generated against colorectal cancer using these approaches. While the concept of vaccination against this malignancy is essentially experimental, surgeons should be aware of current advances.

Colorectal cancer is the second commonest cause of cancer death in the UK, accounting for approximately 16000 deaths per year[1]. Despite advances in surgery, chemotherapy and radiotherapy, only modest improvements in overall mortality rate have been achieved in the past 30 years. Cancer vaccines offer a potentially new treatment option. Encouraging results were originally noted in a number of cancers using autologous tumour and the cytokine interleukin (IL) 2[2]. This approach was, however, hampered by toxicity and the limitations of processing the patient's own tumour. Specific antigens on tumour cells have now been identified and a variety of approaches have been developed to induce immune responses against them. The aim of this review is to evaluate critically current strategies aiming at active vaccination against colorectal cancer.

Tumour-associated and tumour-specific antigens

Immune responses may be induced either by B cells or T cells. The former is a humoral response that leads to antibody production, while the latter is termed cell-mediated immunity. Animal studies have shown that tumour regression is more commonly associated with the induction of cellular rather than humoral immunity[3]. For T cells to recognize antigen, the antigen must be taken up and digested by specific antigen presenting cells (APCs). It is then bound to glycoproteins, encoded by the major histocompatibility complex (MHC), and presented on the surface of the APC in conjunction with co-stimulatory molecules. Tumour cells are poor immunogens, as they lack co-stimulatory molecules, and have low expression of the necessary MHC molecules. Vaccines aim to target APCs more effectively than tumour cells, thereby stimulating a T cell response.

There are two main types of T cell. Cytotoxic T lymphocytes (CTLs) express the surface marker CD8 and recognize peptides presented by APCs bound to class I MHC glycoproteins. Once stimulated they will proliferate, migrate to the tumour site and kill any cell that expresses the same peptide. The second type, CD4-positive helper T cells, provide the cytokines necessary to activate and allow CTL proliferation. Recognition of antigen in their case is in association with class II MHC glycoproteins (*Fig. 1*). An effective tumour vaccine should stimulate both cytotoxic and helper responses.

Two categories of antigen, or epitope, are present on the surface of colorectal cancer cells: tumour-associated antigen (TAA) and tumour-specific antigen (TSA). The former may occur as a result of overexpression of a normally expressed antigen, re-expression of antigens normally repressed in differentiated tissue, or an antigen may be expressed on tissue where it is not normally present. They are not confined to a single type of malignancy; 791Tgp72 is, for example, a TAA that occurs on osteosarcomas as well as colorectal and ovarian tumours. TSAs such as K-RAS form either through cellular mutations or by the expression of viral glycoprotein envelopes on cell membranes[4].

This review concentrates on the most widely investigated antigens present on the surface of colorectal cancer cells, 17-1A, 791Tgp72 and carcinoembryonic antigen (CEA), and the ways in which immune responses have been generated against them. Vaccination involves anti-

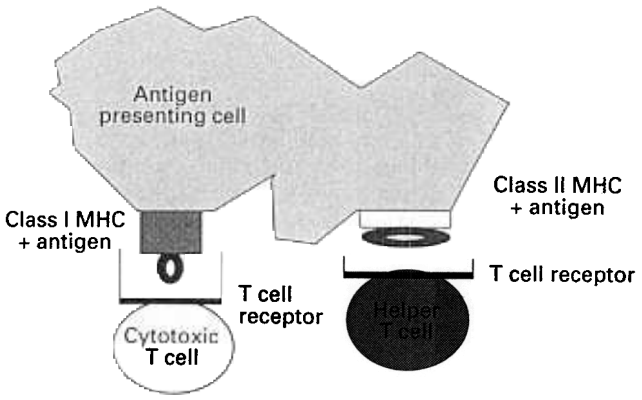

Fig. 1 Antigen presentation to cytotoxic T cells and helper T cells. MHC, major histocompatibility complex

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idiotypic antibodies that mimic tumour antigen, and polynucleotide-mediated immunization where DNA or RNA encoding epitope is inserted into the cell genome. Vaccines may also be based on peptides encoded by oncogenes. In addition, this review considers mucin and heat shock proteins (HSPs) as vaccines.

17-1A antigen

The 17-1A antigen was first isolated from a colon carcinoma cell line by immunoprecipitation with the 17-1A monoclonal antibody[5]. It is a 37–40-kDa cell surface glycoprotein present on over 90 per cent of colorectal cancers. It exists in various configurations, depending on the level of glycosylation, and is thought to be involved in cellular adhesion. Passive serotherapy using monoclonal antibodies against this antigen prolongs survival in patients with primary tumours[6]. These results are currently being tested in a multicentre phase III study in patients with stage III (Dukes C) tumours. There are, however, a number of ways in which active immunity and T cell responses can be elicited against CO17-1A.

Immunization with anti-idiotypic monoclonal antibodies may offer an alternative immunological approach to tumour therapy. The theoretical basis of this treatment modality is outlined in the network hypothesis of Lindenmann and Jerne[7]. The premise is that antibodies (Ab1) against TAAs have specific idiotypes in their variable regions. The anti-idiotypic monoclonal antibody (Ab2) is an antibody against this idiotype and must therefore 'mimic' the antigen on the surface of the tumour cell (*Fig. 2*). The concept of the anti-idiotypic antibody acting as an 'internal image' of the antigen suggests that this novel presentation of tumour epitope should elicit an immune response[8–10]. This approach has a number of advantages over other forms of immunotherapy. Anti-idiotypic monoclonal antibodies may be presented by APCs in the context of class I and II MHCs, thus eliciting both cytotoxic and helper T cell responses. Presentation of the epitope in a different molecular environment may also act to break any tolerance that may have developed to the weakly immunogenic TAAs[11]. Anti-idiotypic monoclonal antibodies have a longer half-life in the peripheral blood and are resistant to proteolytic digestion. They can also be used when the TAA is either unknown, or difficult to purify in the quantity required. In addition, there is *in vitro* evidence that the anti-idiotypic antibody may be more effective in eliciting an immune response than the actual antigen[12,13]. The former is also free from the potential dangers of retroviruses and genetic manipulations.

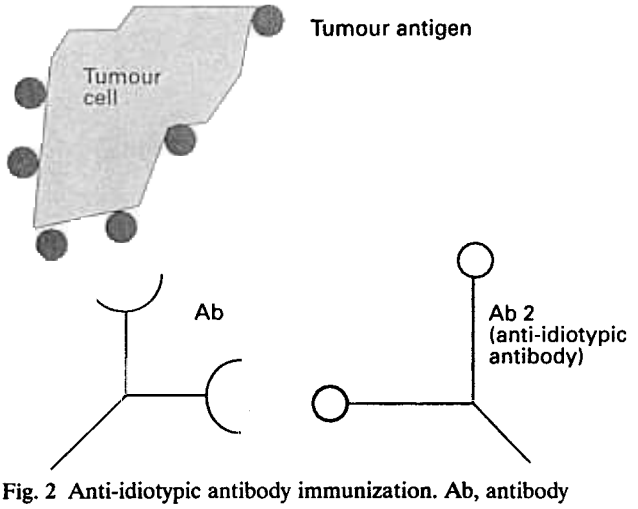

Fig. 2 Anti-idiotypic antibody immunization. Ab, antibody

A polyclonal anti-idiotypic antibody to CO17-1A has been developed, and 30 patients with advanced colorectal cancer have been immunized[14–16]. Humoral responses were noted, and all had evidence of Ab3 production. This antibody showed identical binding of tumour cells as that observed with Ab1. Six patients had partial clinical remission and a further seven had arrest of metastases following treatment. Of these 13 patients, nine also received chemotherapy, making conclusions about the efficacy of Ab2 contentious. A follow-up trial used a different goat polyclonal antibody in 12 patients who had undergone resection of the primary tumour[17]. Six of these patients developed antibodies against the anti-idiotypic antibody and two had antigen-specific T cells, which proliferated in culture on stimulation with the CO17-1A antigen. In addition, seven of the original 12 had tumour remission lasting from 1·1 to 4·1 years after immunization. Cellular immunity has been noted in a further patient with advanced colorectal cancer[18] following immunization with SCV106, a goat anti-idiotypic monoclonal antibody that also mimics the TAA 17-1A. This patient had two lung metastases from a previously resected colonic carcinoma which were removed after completion of the antibody course. Antibodies eluted from the resection specimen were confirmed to be against the tumour antigen in a conventional enzyme-linked immunosorbent assay, and immunohistochemical analysis of tissue showed massive infiltration of T helper and cytotoxic T cells.

Recent work has shown how passive immunotherapy with unconjugated monoclonal antibodies may give rise to an idiotypic network response that correlates with clinical outcome[19]. Twenty-four patients with metastatic colorectal cancer were treated with monoclonal antibody 17-1A (Ab1). After completion of therapy, five of the patients had peripheral blood T cells specifically recognizing human antimonoclonal antibody 17-1A idiotypic antibodies. These same five patients were the only ones in the study who had any objective tumour regression following monoclonal antibody therapy. The association between the presence of anti-idiotypic reactive T cells and clinical response was statistically significant.

Clinical responses have clearly been demonstrated using anti-idiotypic antibodies. The major disadvantage of this approach is that the tumour itself must express the antigen that the antibody is mimicking. If it does not, a vaccine based on this approach will not work.

Tumour-associated antigen 791Tgp72

The anti-idiotypic monoclonal antibody 105AD7 mimics the TAA 791Tgp72, present on 80 per cent of colorectal cancers[20]. Thirteen patients with liver metastases from colorectal cancer were recruited to a phase I study. No treatment-related toxicity was observed and patients receiving the vaccine lived for a median of 12 months, significantly longer than a contemporary group of patients[21]. In addition, nine of the 13 showed either a T cell blastogenesis response against cell lines expressing 791Tgp72 or evidence of IL-2 production. Those patients who had the best immune response lived the longest. A

randomized, double-blind survival study in patients with advanced colorectal cancer has recently closed, recruiting 162 patients to either 105AD7 or placebo arms; results are awaited. 105AD7 has also been used in an adjuvant setting and given to patients with primary colorectal cancer before surgery. Enhanced killing of autologous tumour by natural killer (NK) cells and non-NK effectors has been observed[22]. Increased tumour infiltration by lymphocytes expressing CD4, CD8, CD56 and CD25 has also been noted[23,24].

Evidence is accumulating that anti-idiotypic antibody therapy induces an immune response against antigens present on tumour cells. The low toxicity and the lack of requirement for processing of autologous tumour are advantageous. However, tumour cells not expressing the antigen that the anti-idiotypic antibody mimics may escape killing, thereby reducing the efficacy of the vaccine.

Carcinoembryonic antigen

CEA is one of the best characterized tumour marker antigens in terms of its tissue distribution, biochemistry and molecular structure[25,26]. It is expressed extensively in humans on the majority of colorectal, gastric and pancreatic carcinomas, as well as on approximately 50 per cent of breast cancers and 70 per cent of non-small-cell lung cancers. CEA is also expressed to some extent on normal colon epithelium and in some fetal tissues[27]. At the amino acid level CEA shares approximately 70 per cent homology with non-specific cross-reacting antigen (NCA) which is found on normal granulocytes[28,29]. However, CEA is highly overexpressed on tumour cells and is therefore a potential target for active immunotherapy. The immunogenicity of CEA in humans is at best controversial. Several studies claim to have detected antibodies to CEA in patients, while others have questioned the validity of such assays[30]. The first evidence that T cells from patients with cancer could recognize and respond to CEA was demonstrated *in vitro* by immunization with an anti-idiotypic antibody which mimics CEA[31].

A phase I clinical trial in patients with advanced colorectal cancer, using the anti-idiotypic antibody 3H1, demonstrated anti-CEA antibody responses in nine of 12 patients, with four showing T cell proliferation against CEA[30]. Toxicity was limited to local reaction with mild fever and chills. Studies are now focusing on treating patients with minimal residual disease.

Instead of using anti-idiotypic antibodies as surrogate antigens, the CEA gene has been cloned in baculovirus and recombinant protein has been used as the immunogen. This may be advantageous as the patient's APCs can process, present and select the most appropriate T cell epitopes. It has, however, the disadvantage that immune responses may be generated to the region of CEA which is homologous with NCA, causing granulocyte toxicity. Two clinical studies have been carried out using recombinant CEA. Two of five patients with breast cancer showed CEA-specific proliferative responses, with one having a CEA-specific delayed type hypersensitivity (DTH) response[32]. In a follow-up study, the addition of the cytokine granulocyte–macrophage colony-stimulating factor (GM-CSF) to CEA enhanced the proliferative responses to CEA from two of six patients to six of six[33]. No toxicity was observed.

A new approach to vaccination, which has been very successful in infectious diseases, is polynucleotide immunization. DNA or RNA can be administered by intramuscular injection, allowing myocytes to take up the DNA and express the gene product. The released protein is taken up by APCs which migrate to the draining lymph nodes and present antigen to the T cells[34]. An alternative route of immunization is intradermal injection; it is presumed that the DNA is taken up by Langerhans cells, which then act to present the antigen. This leads to a continuous intracellular production of protein antigens that may be presented in association with class I MHC molecules, thereby eliciting CTL responses[35,36].

The advantages of DNA vaccines are numerous. They can be easily purified, coated on gold particles and introduced directly into tissues by gene gun (bolistics). DNA may also be combined with genes for cytokines, such as IL-2, IL-6 or IL-7, or GM-CSF, in order to enhance the immune response generated[37].

Mice may be immunized with a plasmid encoding the full length complementary DNA (cDNA) for CEA[28]. Evidence of humoral and cellular responses against the glycoprotein was found in all of five mice immunized and three generated CEA-specific memory T cells. In addition, a further two had IL-2/IL-4 release in response to CEA. Clear evidence exists to support this approach as a potential vaccine strategy and approval has been granted for a phase I trial in colorectal cancer. In the light of recent negative publicity, it remains to be seen how patients will react to the concept of gene therapy for their cancer.

Recombinant vaccinia viruses are being considered for use in the treatment of cancer because it has been shown, in animal models, that co-presentation of a weak immunogen with the highly immunogenic vaccinia proteins can elicit a strong immune response against the inserted gene products[38]. Animals developed good antibody and cell-mediated immune responses to CEA when they were immunized with complete cDNA of CEA inserted into the vaccinia genome (rV-CEA). A phase I study utilizing this approach in patients with metastatic carcinoma showed for the first time that it was possible to induce cytolytic T cell responses to CEA which killed tumour cells[29]. However, the immune response to the vaccinia inhibited replication of the recombinant virus at subsequent immunizations, making it impossible to boost the primary immune response to CEA. Animal studies have suggested that this problem may be solved by priming with rV-CEA and then boosting with either recombinant CEA or specific CEA peptides. Clinical trials are planned.

Oncogenes

Peptide vaccines can bind to MHC molecules and elicit immune responses. Generation of CTLs would be further enhanced if the peptide was presented by an APC, such as a dendritic cell[39]. A murine model has shown that antigen-specific CTLs may be generated following subcutaneous administration of irradiated bone marrow-derived dendritic cells, pulsed with OVA peptide *in vitro*[40]. These results have been confirmed in a separate study, in which β-galactosidase acted as the TAA[41]. Immunization of mice with mutant p53 peptide-pulsed dendritic cells, generated from stem cells of other tumour-bearing mice, can induce effective antitumour CTL responses and lead to significant antitumour effects[42]. If the T cell epitope is as yet

undefined, as is the case for a number of cancers, CTLs may still be generated using unfractionated acid-eluted tumour peptides in conjunction with the method outlined above[43].

Mutations in codon 12 of K-*ras* are frequently found in pancreatic adenocarcinomas[44]. Mutant p21 ras is, therefore, a TSA that can be recognized by human T cells[45]. Synthetic *ras* peptides have been used in conjunction with APCs as a vaccine for pancreatic cancer, with encouraging results. This approach could also be applied to colorectal carcinomas, which also show mutations in codon 12 of K-*ras*. As an alternative to peptide vaccination, it is possible to clone the peptide epitope as a minigene and use this DNA as the immunogen. Minigenes coding for a single epitope derived from mutant p53 have been demonstrated to elicit CTLs in a mouse model[46].

Mucins

Human epithelial mucins are a family of high molecular weight glycoproteins that lubricate and protect the underlying gastrointestinal mucosa. They are characterized by a large number of *O*-glycosylated tandem repeat domains which vary in length, number and extent of *O*-glycosylation[47,48]. Novel mucin epitopes are expressed by tumour cells owing to aberrant glycosylation of pre-existing mucins[49,50]. This results in shorter sugar side-chains, with concomitant exposure of peptide antigens in the intestinal crypts. Evidence has accumulated to show that T cells specific for native epitopes on the mucin polypeptide core tandem repeat can be expanded *in vitro*[51,52]. Further work has also shown that a humoral response may be generated, with B cells recognizing the mucin tandem repeats[53]. Antibodies have, therefore, been detected in the blood of patients with colonic carcinomas, breast and pancreatic tumours[54].

A vaccine has been formed by transfecting the gene for the TAA (MUC-1) into Epstein Barr virus-immortalized B cells[55]. The latter act as APCs priming cytotoxic T cell precursors and DTH responses occurred in the two chimpanzees immunized. A phase I study using a 105-amino-acid MUC-1 peptide admixed with Bacillus Calmette-Guérin has been used recently in 30 patients with advanced colorectal cancer[56]. A number experienced ulceration at the injection site, and systemic symptoms such as fever, rigors and malaise. Immunologically, DTH responses were noted against mucin-specific peptides, and seven of 22 patients tested showed a twofold to fourfold increase in CTLs. Clinically, only two patients had stable disease. Eleven 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[57]. This phase II study demonstrated that patients with higher anti-sialyl-Tn immunoglobulin G antibody titres following vaccination survived longer than patients with lower titres, suggesting an immune response. Data are accumulating suggesting a potential role for mucins as cancer vaccines, although toxicity and the problems associated with processing autologous tumour may preclude their use.

Heat shock proteins

HSPs are a group of proteins present in all living cells. HSP preparations contain a broad array of peptides tightly bound to HSP molecules[58,59]. They offer a number of advantages as cancer vaccines. If a lasting therapeutic effect is to be conferred by a vaccine, a CTL response must be generated[60–62]. Vaccination with HSP–peptide complexes circumvents the need for identification of the antigenic epitopes of cancer cells, as HSPs are naturally complexed with the entire repertoire generated in the cell. Another advantage of such an approach is that an immune response will be generated against all antigens present in the tumour. Furthermore, HSPs require no adjuvants to elicit a CTL response and the complexes can be purified rapidly. As the vaccine is autologous, no material is inoculated to which the patient has not already been exposed thereby reducing the chance of toxicity.

A number of studies have shown that injection of apparently homogeneous HSP preparations from a given tumour into syngeneic rats or mice renders the animals resistant to that particular tumour[63–65]. For this treatment modality to be successful, each vaccine would need to be 'custom built' for individual patients, using autologous tumour. Although toxicity would be minimal with this approach, it may nevertheless prove technically difficult, excessively time consuming or expensive. If so, it will not be viable as a vaccine for colorectal cancer. Despite these limitations, phase I studies are currently ongoing.

Conclusion

This review has highlighted how immune responses may be generated against the three most commonly investigated colorectal cancer antigens. It has also considered vaccines based on oncogenes, mucins and HSPs. A number of experimental approaches are apparent, some of which are currently being evaluated in phase I/II studies. It is probable that a number of strategies will fail to become established as treatment options. Surgeons, however, should be aware of these areas of current research and have some understanding of this rapidly evolving field.

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