Hunterian Lecture

Studies using the anti-idiotypic monoclonal antibody 105AD7 in patients with primary and advanced colorectal cancer

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The mortality from colorectal cancer has not changed appreciably in the last 30 years and new treatment avenues, such as immunotherapy, are being explored. 105AD7 is a form of active specific immunotherapy that aims to stimulate specific T-cells to target tumour specific antigens on colorectal cancer cells. Results indicate that the cancer vaccine 105AD7 is non-toxic and is capable of stimulating T-cells to target tumour specific antigens, become activated, and kill tumour cells by apoptosis. These immune responses have no effect on survival on a cohort of immunosuppressed patients with advanced disease, but results from a case-control study in patients with minimal residual disease suggest they may confer a slight survival advantage on patients receiving 105AD7.

Key words: Immunotherapy – Colorectal cancer – Anti-idiotypic antibody, 105AD7

Attempting to generate an immune response against cancer is nothing new. Work in the late 19th century by Hericourt and Richet showed that sera could be raised against tumours in dogs, and Ehrlich proposed in 1900 that 'magic bullets' or antibodies could be used in the fight against malignancy. A New York surgeon called Coley prolonged survival of patients with osteogenic sarcoma using extracts from haemolytic streptococci and *Bacillus prodigiosus*,¹ and Besredka in 1935 showed that mice could be inoculated intradermally with cancer cells, resisting a subsequent tumour challenge.²

Immunotherapy for the treatment of colorectal cancer has, however, advanced dramatically in recent years. Work originally attempted to stimulate the immune system in a

non-specific fashion similar to those above, using agents such as Bacille-Calmette-Guérin,³ diphtheria toxoid,⁴ and levamisole.^{5,6} Work by Rosenberg took it a step further. He used the cytokine IL-2 to stimulate both extracted peripheral blood leukocytes, and tumour cells. Encouraging results were seen in renal cell cancer and melanoma, though rarely in colorectal cancer.^{7–10} Advances in molecular biology meant that it became possible to characterise tumour specific and tumour associated antigens (TSA and TAA), against which immune responses could be generated – so-called active specific immunotherapy (ASI). A number of approaches come under this heading, including anti-idiotypic antibodies, polynucleotide mediated immunisation (DNA and RNA vaccines), viral vectors, heat shock

Abbreviations: APC, antigen presenting cell; ASI, active specific immunotherapy; CEA, carcinoembryonic antigen; IL, interleukin; Mab, monoclonal antibody; NK, natural killer; PBL, peripheral blood lymphocyte; TAA, tumour associated antigen; TSA, tumour specific antigen

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proteins, and monoclonal antibodies specifically directed against TAA.¹¹⁻¹⁴ The cancer vaccine 105AD7 is one such anti-idiotypic antibody, 'mimicking' the TAA CD55 present on up to 80% of colorectal cancer cells.^{15,16} Because it is presented to the immune system via antigen presenting cells (APC), which tumour antigen is not, it is theoretically capable of stimulating T-cell responses against cancer cells expressing CD55. Preclinical animal studies suggested that 105AD7 was capable of inducing such an immune response.^{15,17} Thirteen patients with advanced colorectal cancer were then immunised as part of a phase I study.¹⁸ No toxicity was seen, and patients receiving the vaccine lived approximately 3 times as long as a contemporary group of unimmunised patients. More importantly, 9 of the 13 patients showed evidence of T-cell responses, either in terms of IL-2 production, or evidence of T-cell blastogenesis.

In view of the lack of toxicity, it was decided to assess the vaccine's efficacy as adjuvant therapy in patients with primary colorectal cancer. The aim of this work was initially to assess whether lymphocytes primed by the cancer vaccine 105AD7 were capable of targeting the TAA that the vaccine mimicked on colorectal cancer cells. If they were, then we needed to assess if they were activated, and capable of causing tumour killing by apoptosis (programmed cell death). The final question we set out to answer was whether administration of the vaccine had any effect on overall survival.

Targeting of tumour antigen by primed lymphocytes

The preclinical and phase I studies had suggested that 105AD7 was capable of inducing T-cell responses. If this were so, then we should, in theory, see significant infiltration of the primary tumour by lymphocytes. To test this hypothesis, 16 patients with primary colorectal cancer were recruited prospectively. The diagnosis of colorectal cancer was made either histologically and/or on the basis of a double-contrast barium enema. Exclusion criteria included autoimmune disease, on-going infection, pregnancy, and renal/hepatic failure. The study was performed under the auspices of the Cancer Research Campaign UK Phase I/II Clinical Trials Committee, and clinical grade monoclonal antibody was prepared as previously described.¹⁷ All patients received at least two immunisations with 105AD7 prior to surgery, though the amount was varied as part of on-going work aiming to optimise dose.

Samples of normal bowel and tumour edge/centre were taken from the resection specimens, and snap frozen in liquid nitrogen, prior to cutting into 5 µm sections. These were labelled immunohistochemically using a standard avidin-biotin complex method, as previously described.¹⁹

The Mabs used included anti-CD4 (T helper cells), anti-CD8 (cytotoxic T-cells), anti-CD56 (natural killer cells), anti-CD68 (macrophages), anti-T-cell receptor ξ chain, and an IgG₁ negative control. Human tonsil was used as a positive control. Analysis of tumour sections was performed blind by one observer initially, and then subsequently by another to confirm that the technique was reproducible (data not shown). This technique itself allowed quantification of lymphocytes expressing the relevant CD antigen as a pixel count. All tumour sections from trial patients were matched to those from an unimmunised control group according to site, stage, and differentiation of tumour, as well as sex and approximate age of patient. This was possible as tumour samples are taken from all tumour sections electively removed at the Queen's Medical Centre.

Median infiltration of CD4, CD8 and CD56 expressing lymphocytes was higher at the tumour edge and centre in trial patients relative to controls, when results were combined (0.92 *versus* 0.65; $P = 0.021$). Infiltration by CD4⁺ lymphocytes was similarly significantly higher in trial patients (1.17 *versus* 0.81; $P < 0.05$). Median scores for trial patients' sections labelled with Mab against CD8, CD56, CD68 and TCR- ξ chain were 0.81, 0.82, 1.10 and 0.56, respectively. The corresponding median infiltrations for control patients were 0.75, 0.44, 1.02 and 0.62, respectively. There was no significant difference between any of these groups. Scores for the IgG₁ negative control antibody were 0.20 and 0.18 respectively (not significant).

Ratios of pixel scores obtained from the edge and centre of the tumour, and normal bowel were also calculated. The ratio for the combined results of CD4, CD8 and CD56 expressing lymphocytes were significantly higher in trial patients (0.93 *versus* 0.78; $P = 0.04$).

These initial experiments have recently been repeated in a further 6 patients.²⁰ The results found above have been confirmed and, in addition, expression of CD56 on natural killer cells was found to be independently statistically significant in trial patients relative to controls.

T-cells are one of the most effective ways of targeting tumour cells immunologically,²¹ and a number of anti-idiotypic monoclonal antibodies have been shown to induce such responses. Patients with advanced colorectal cancer have been immunised with an anti-idiotypic antibody (3H1) that mimics CEA, and shown evidence of T-cell proliferation.^{22,23} Development of CD4⁺ T lymphocytes has been seen in a study similar to ours where a goat polyclonal anti-idiotypic antibody mimicking the TAA GA733 was administered to patients with primary colorectal cancer. There are a number of other studies from the same group suggesting that T-cell responses may be generated following administration of an anti-idiotypic antibody that mimics the TAA 17-1A.²⁴⁻²⁷ In the clinical setting, 105AD7 was originally shown to produce

T-cell responses in patients with advanced colorectal cancer.¹⁸ This work adds further support to our previously published findings, suggesting that T-cells stimulated in the blood stream by 105AD7 are capable of targeting TAA on colorectal cancer cells. If they target TAA at the site of the primary tumour, then it may well be that they are capable of targeting TAA on circulating micrometastases.

The T-cell receptor ξ chain is involved in signal transduction and subsequent T-cell activation.²⁸ Work has shown that levels increase following administration of a synthetic peptide vaccine containing mucin antigenic epitopes.²⁹ Our results support those of others suggesting that levels of ξ chain are higher in adjacent normal bowel than the tumour itself,³⁰ though no up-regulation of ξ chain was seen in immunised patients.

Macrophages are monocyte-derived cells that infiltrate tumours in varying numbers.^{31–33} As well as expressing the surface antigen CD68, which we have assessed in this work, they also have CD14 and MHC Class II molecules, and are thought to exist in three distinct phenotypes: suppressive, stimulatory and phagocytic.^{34–36} The exact one that predominates depends on cytokines such as IL-4, IL-10 and IFN- γ , and our initial hypothesis was that the significant infiltration of CD4⁺ T helper cells might recruit phagocytic macrophages to the tumour site. Though overall infiltration was higher in trial patients, the difference did not reach statistical significance.

Lymphocyte activation

Having established that lymphocytes were capable of targeting TAA on the tumour cell surface, the next step was to determine whether they were activated and, at least potentially, capable of causing tumour cell death by apoptosis. Nineteen patients with primary colorectal cancer were immunised pre-operatively with varying doses of 105AD7.¹⁹ Samples from 16 of these patients were labelled immunohistochemically with a Mab against the activation marker CD25 (the α subunit of the IL-2 receptor). In addition, fresh tumour samples from 14/19 patients were disaggregated, and labelled with CD25 prior to flow cytometric analysis. Both groups were compared with matched controls, and analysis performed blind. Median pixel counts on immunohistochemically labelled sections were higher in trial patients (0.75 *versus* 0.50; $P = 0.06$), significantly so when ratios of tumour:normal bowel infiltration were taken into account.¹⁹ The percentage of CD4⁺ T-cells expressing CD25 (activated helper T-cells) was also significantly higher in trial patients relative to controls (10% *versus* 5%) confirming these results. Increased expression of CD25 as described above has also been confirmed in a separate cohort of patients immunised with 105AD7.³⁷

Contact between primed T lymphocytes and tumour antigen induces synthesis of IL-2 and its receptor. Released IL-2 has been shown to promote activation, growth and differentiation of a variety of leukocytes including T lymphocytes, B-cells, NK cells and monocytes, and in addition promote further receptor formation.^{38–40}

This autocrine/paracrine expression of this receptor, therefore, suggests lymphocyte activation.^{41,42} These results show that there are more activated lymphocytes at the tumour site of patients receiving 105AD7 than matched controls.

Tumour cell killing by apoptosis

The work above suggested that lymphocytes were capable of targeting TAA on the colorectal cancer cells, and become activated. The next step, therefore, was to show whether these lymphocytes were causing tumour cells to die by apoptosis (programmed cell death). To do this, we used a monoclonal antibody (APO2.7) that labels a mitochondrial antigen (7A6) expressed early in cells undergoing apoptosis. Samples of fresh tumour were taken from a separate group of 13 immunised patients and a number of controls, and disaggregated. These were then labelled with APO2.7 and analysed blindly by flow cytometry. The proportion of tumour cells undergoing apoptosis was 22% in trial patients as compared with 7.8% in controls ($P = 0.0023$).⁴³

In addition to assessing apoptosis at the tumour site, we used chromium release assays to determine if peripheral blood lymphocytes were capable of killing autologous tumour. Six patients with primary rectal cancer were immunised with 105AD7 pre-operatively.⁴⁴ Peripheral blood lymphocytes (PBL) were taken prior to immunisation, and tested against tumour cells extracted from biopsies also obtained pre-immunisation, and against NK-sensitive target cells. Cryopreserved lymphocytes taken before and after tumour immunisation, PBL taken immediately prior to surgery, and lymphocytes from tumour-draining lymph nodes were tested against autologous cells from the resected specimen or NK-sensitive target cells. Significant killing of autologous tumour cells, which was not due to NK activity, was seen with cryopreserved lymphocytes, or lymph node cells of three patients at 1–2 weeks post-immunisation with 105AD7, but not on pretreatment biopsies. Enhanced NK activity was seen 2–3 weeks post-immunisation in 3/6 patients.

These results suggest that 105AD7 is capable of priming lymphocytes in the peripheral blood, and targeting antigen on the tumour cell surface. They then become activated and destroy tumour cells by apoptosis. If it is happening at the tumour site, then it may well be happening to circulating micrometastases expressing the TAA that 105AD7 mimics. If this is the case, then survival may be prolonged in patients receiving the vaccine.

Survival analysis

An assessment of survival was performed in both patients with advanced disease, and those with primary colorectal cancer who had received the vaccine pre-operatively.

A prospective, randomised, double-blind, placebo-controlled survival study in patients with advanced colorectal cancer was performed in order to test the survival results seen in the phase I study.⁴⁵ A total of 162 patients were enrolled in one of four trial centres (Nottingham, Leeds, Hull and Newcastle) between April 1994 and October 1996. All had a life expectancy greater than 3 months, and none had received chemotherapy in the preceding 3 months. Patients attended at trial entry, and at 6 and 12 weeks, where they received 105AD7 or placebo. Blood tests, weight and performance status were also recorded at each visit. Study groups were comparable in terms of patient demographics, and time from diagnosis of advanced colorectal cancer (277.1 *versus* 278.6 days). Baseline disease was similar, with 50% of patients having malignancy in at least 2 anatomic sites. Compliance with treatment was poor, with only 50% of patients receiving 3 planned vaccinations. Median survival from randomisation date was 124 and 184 days in 105AD7 and placebo arms, respectively ($P = 0.38$), and 456 and 486 days from the date of diagnosis of advanced disease ($P = 0.82$). 105AD7 vaccination was well tolerated, with no evidence of toxicity. It did not, however, prolong survival in patients with advanced colorectal cancer relative to controls. The reasons for lack of efficacy are unclear, but may reflect the high tumour burden in the patient population and concomitant immunosuppression. With half of all patients receiving only 1 or 2 doses of 105AD7, it seems likely that insufficient vaccine was actually given to effect survival.

A further survival analysis was, therefore, performed. This was a non-randomised case-control study in which 23 patients with primary colorectal cancer receiving 105AD7 before surgery were compared with a contemporary group of unimmunised patients undergoing surgery. The control group consisted of patients randomly selected from the Trent Colorectal Cancer Audit. This database was originally established on 1 August 1992 to document outcome in patients with colorectal cancer, seen in the ensuing 12 months, in hospitals in Trent.⁴⁶ The audit was extended to cover patients treated in Wales between January 1993 and December 1993. In all, 52 hospitals were covered and the notes of 3520 patients scrutinised. Trial patients and control patients were matched according to the following variables: site, stage, differentiation of tumour, approximate age, ASA grade, and whether or not they had chemotherapy or radiotherapy. In all, 96 control patients were identified. At 2-year follow-up, there was no significant difference between trial and control groups in terms of overall survival. In the

immunised group, 17/23 (74%) patients were alive and disease-free, 2/23 (9%) had recurrent disease, and 4/23 (17%) had died from all causes. This compared with the control group in which 67/96 (70%) patients were alive and free from disease, 14/96 (14.4%) had recurrent disease, and 15/96 (15.6%) who had died.

Conclusions

The mortality from colorectal cancer has not changed appreciably in the last 30 years and new treatment avenues, such as immunotherapy, are being explored. 105AD7 is a form of ASI that aims to stimulate specific T-cells to target TAA on colorectal cancer cells. Our results have shown that the cancer vaccine 105AD7 is non-toxic. In addition, we have demonstrated that the vaccine is capable of stimulating T-cells to target TAA, become activated, and kill tumour cells by apoptosis. These immune responses have no effect on survival on a cohort of immunosuppressed patients with advanced disease, but results from a case-control study in patients with minimal residual disease suggest they may confer a slight survival advantage on patients receiving 105AD7. Further work will concentrate on improving the delivery of 105AD7 using a more potent adjuvant, and looking at the 5-year follow-up of patients from the non-randomised case-control study.

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