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The Role of Biological Response Modifiers in the Management of Patients with Colorectal Cancer

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Biological response modifiers are agents which exert their antitumour effects indirectly via modulation of the host's response to the tumour. Immunotherapeutic approaches which fall into this category can be divided into specific and non-specific. An example of the latter is the combination of 5-fluorouracil (5-FU) with levamisole which improves survival of patients with colorectal carcinoma when utilised in an adjuvant setting. Specific immunotherapy using active specific immunisation is attracting much attention. This is in part due to improvements in survival seen in one randomised clinical trial in an adjuvant setting, and also to exciting advances in the fields of tumour immunity and vaccine development. The development of more effective vaccines promises much for patients with early colorectal carcinomas.

Key words: colorectal carcinoma, biological response modifiers, immunotherapy
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BIOLOGICAL RESPONSE modifiers can be operationally defined as agents which exert their antitumour effects indirectly via modulation of the host's response to the tumour. This broad definition can encompass a number of different approaches to therapy. These include immune modulation, inhibition of metastases, inhibitors of angiogenesis and interruption of autocrine/paracrine growth factor loops. The latter three categories are subjects of other papers in this issue, and I will, therefore, concentrate on giving an overview of immunotherapeutic approaches in this paper.

Immunotherapeutic approaches may be broadly divided into two main categories, namely non-specific and specific. Non-specific immunotherapy may be defined as the use of immunomodulating agents which are administered with the aim of inducing general stimulation or suppression of the immune system without attempting to direct the activity towards a specific antigen. In the last few years, a long list of these agents has been investigated in both laboratory and clinical trials. The majority of these agents are derived from microbial sources. Such agents include Bacille Calmette-Güerin (BCG), *Corynebacterium parvum* and OK432, whilst more recently cytokines, produced by recombinant DNA technology, have attracted much attention. The majority of the former group are generally believed to mediate their activity via stimulation of so-called innate or natural immunity. Whilst there are many anecdotal reports of this class of agent producing antitumour responses, when they have been tested in randomised clinical trials, usually in combination with chemotherapeutic agents, the results have been singularly disappointing. Perhaps a major exception to this rule is the combination of levamisole with 5-fluorouracil (5-FU) as an adjuvant therapy for patients with colorectal carcinoma.

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In 1989, Laurie and colleagues [1] reported that both levamisole alone and levamisole plus 5-FU reduced the probability of recurrence of colorectal cancer, with the latter therapy being more statistically significant ($P = 0.003$) than the former ($P = 0.05$). Data from the larger and much publicised Intergroup trial in the U.S.A. [2] demonstrated a significant improvement in survival for patients treated for 1 year with a combination of 5-FU and levamisole, whilst levamisole alone was no better than the observation control group. The mechanism of this interaction has not been clearly elucidated. Recently, levamisole has been shown to enhance cytotoxicity of Kupffer cells within the liver whilst its effects are apparently not mediated via biochemical modulation of 5-FU metabolism (Beelen and Peters, personal communication). Unfortunately, immunological monitoring has not been reported for patients in the two large trials mentioned above and the mechanism, of the effects of levamisole remains undetermined.

The interferons have also been tested in patients with colorectal carcinomas. Only 2 of 101 patients obtained partial responses when interferon was used as a single agent [3–7]. Surprisingly, the combination of interferon with 5-FU appeared to possess higher activity than 5-FU alone. In phase II trials, a response rate of 32% was observed in 111 patients [8, 9]. The triple combination of 5-FU, leucovorin and α-interferon gave a response rate of 44% in 31 patients [10]. This enhanced activity was not, however, the result of immune modulation but of biochemical modulation of 5-FU metabolism, with both enhancement of the inhibition of thymidylate synthase [8, 9] and alteration of the pharmacokinetics of 5-FU [10] being described.

Interleukin-2 (IL-2) first entered clinical trials in the mid-1980s and was then regarded as a cytokine with much clinical potential. Table 1 summarises the results of clinical trials carried out with IL-2 in patients with colon carcinoma. Although the overall response rate is low at 7%, in four of these reports [11–14] responses were observed. It is also important to mention that

Table 1. Interleukin (IL)-2 in colon carcinoma

Author	Reference	IL-2 schedule	Other agents	No. of responder/ no. treated	Response rate (%)
Rosenberg	11	HDB	Alone	0/12	0
Rosenberg	12	HBD	Alone	0/10	0
Rosenberg	11	HDB	+LAK	5/30	17
Rosenberg	12	HDB	+LAK	2/8	25
Dillman	13	HDCIV	+LAK	0/35	0
Oldham	14	HDCIV	+TIL	0/8	0
Dillman	13	HDCIV	+TNF	0/16	0
Oldham	15	HDCIV	+IFN-α	1/10	10
Hamblin	16	HDCIV	+5-FU	2/7	29
Atzpodien	17	SC IL-2	+IFN-α	0/8	0

HDB, high-dose bolus; HDCIV, high-dose continuous infusion; LAK, lymphokine-activated killer cells; TIL, tumour-infiltrating lymphocytes; TNF, tumour necrosis factor; IFN, interferon; 5-FU, 5 fluorouracil.

only three of these reports [11, 13, 15] included sufficient patients to allow an accurate assessment of the antitumour efficacy of IL-2. In my view, IL-2 has not been adequately evaluated in patients with colorectal carcinoma. An interesting regimen which would perhaps be worth testing in these patients is that described by Atzpodien [16] in renal carcinoma patients incorporating interferon, IL-2 and 5-FU.

A further interesting and more specific approach is that of active specific immunotherapy (ASI). In 1993, Hoover and associates [17] reported the results of a prospective randomised control trial of ASI with an autologous tumour cell plus BCG vaccine. With a median follow-up of 93 months, there were significant improvements in disease-free ($P = 0.039$) and overall survival ($P = 0.02$) for patients who received ASI. An editorial in the same issue of the *Journal of Clinical Oncology* pointed out some serious deficiencies in the analysis of this trial and, therefore, counselled caution in accepting the results and conclusions of this study. In the Netherlands, this trial is being repeated under the auspices of the Amsterdam Comprehensive Cancer Centre. As of March 1995, 219 patients have been randomised in this trial. Although patient accrual is continuing and, therefore, therapeutic results are not yet available, this trial does illustrate some of the problems with autologous tumour cell vaccination. In only 530 of the 722 (73%) patients' tumour dissociations was it possible to obtain sufficient tumour cells for the preparation of four vaccines. Of these 530 patients, 311 (59%) were not randomised for the reasons given in Table 2. Thus, only 31% of patients initially registered for the study were ultimately included. This logistic problem is a major drawback for this approach. The problem of too few tumour cells can be overcome by using allogeneic human colon carcinoma cell lines, but these must be HLA-matched and must, of course, express tumour-associated antigens present on the patient's tumour. A great advantage of ASI compared with adjuvant chemotherapy with 5-FU plus levamisole is mild toxicity associated with the former therapy. If the Amsterdam trial confirms the results achieved by Hoover and colleagues [17], then a logical next step will be to compare 5-FU plus levamisole with ASI.

The use of autologous tumour cells as vaccines relies on the tumour cells themselves as being able to present antigen to cytotoxic T lymphocytes. Since tumour cells are not professional antigen-presenting cells, this may lead to suboptimal activation of the cytotoxic T cells. This failure to initiate an immune response may be due to a number of factors, and insight into these events might suggest strategies which could overcome the problem. The first question to be addressed is what are the antigens which the immune system might recognise upon tumour cells? It has now become clear that the process of carcinogenesis involves multiple mutations in genes involved with normal cell proliferation and differentiation. These mutations frequently lead to the production of intracellular proteins of an abnormal structure. Examples include p53 and ras amongst others. It turns out that these abnormal proteins can be processed within the cell and peptide fragments from them presented at the cell surface with major histocompatibility complex (MHC) class I molecules where they would, in principle, be able to activate the lytic machinery of cytotoxic T lymphocytes (CTL). If these antigens exist then the question naturally arises as to how it is that tumours emerge at all. Induction of unresponsiveness or tolerance is often seen in tumour-bearing hosts and this is usually due to altered or ineffective antigen presentation. Deficient antigen processing would lead to a failure of immune recognition [18], whilst lack of the presence of co-stimulatory molecules at the cell surface

Table 2. Reasons for exclusion from randomisation in the Amsterdam randomised trial (n = 311) *of active specific immunotherapy with autologous tumour cells plus BCG in patients with Dukes' stages B2 and C colon carcinoma*

	Percentage of successful tumour dissociations
Patient refusal	35
Dukes' stage A, B1 or D	26
Poor physical/mental condition	14
Prior malignancy	7
Non-colon tumour	6
Late consult	4
Benign lesion	3
Residual disease	3
Died before	1
Technical freezing problem	1

might lead to failure in tumour immunity despite the presence of antigen associated with MHC class I molecules [19, 20]. Indeed, in some systems the transfection of genes coding for these co-stimulatory molecules (for example, B7) resulted in effective CTL responses against tumours that would otherwise have grown progressively [19, 21–24]. It has recently been realised that the cytokine milieu at the site of antigen presentation is also of paramount importance for efficient immune activation. Cytokines such as granulocyte–macrophage colony-stimulating factor (GM-CSF) potently enhance antigen presentation in unprimed systems [25, 26]. In animal models, vaccination with GM-CSF gene-transfected tumour cells has been shown to produce better protection against tumour cell challenge than vaccination with non-transfected tumour cells [27, 28]. This effect is believed to be mediated by GM-CSF-stimulated accumulation and activity of professional antigen-presenting cells at the vaccination site. Transfection of other cytokine genes is now being actively explored and should lead to the development of more efficient vaccines; indeed vaccines utilising these principles are currently beginning to be evaluated in clinical trials.

The realisation that antigen is presented to the immune system as small peptides incorporated into MHC molecules has led to the search for such tumour-derived peptides. The hope is that vaccination will be able to be performed with these small artificially produced peptides thus obviating the need to obtain autologous tumour cells with all their incumbent problems (see above). Indeed, in this regard, considerable advances have been made in the last few years [29].

In summary, important advances are being made in our understanding of tumour immunity and this knowledge will undoubtedly lead to novel therapeutic strategies in the coming years. It remains to be seen how great a role immunotherapy will play in the management of patients with colorectal cancer, but there is certainly room for considerable optimism.

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