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Biological therapy of colorectal cancer

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Abstract

In this review, the immunogenicity of colorectal cancer (CRC) and the results of clinical and recent preclinical studies are discussed. Evidence for immune reactivity has been found in several preclinical models and the prognostic value of some of these immune responses have been reported. The possible mechanisms are discussed. Treatment with monoclonal antibodies is still experimental; as previously described benefit of treatment with monoclonal antibodies could not be confirmed. Labelled monoclonal antibody therapy has produced mixed results and also need further investigation. Several antigens are used in active specific immunotherapy (ASI). Its targets and modifications are discussed, as are their use in clinical studies. Although some of the results are promising, the results still have to be confirmed in larger studies. Since there is sufficient evidence for immune reactivity in CRC, further research on immunotherapeutic strategies is justified and will be focused on the development of humanised antibodies, the search for other relevant T-cell epitopes and ways to induce a more effective T cell response. © 2002 Published by Elsevier Science Ltd.

Keywords: Colorectal cancer; Immune therapy; Clinical/preclinical models

1. Introduction

Although CRC has never been considered a very immunogenic tumour, there is evidence for immune reactivity, both in clinical and preclinical models. In the following paragraphs, we will review the immunogenicity of colorectal cancer (CRC) and discuss the results of clinical and some recent preclinical studies that may serve as a basis for future clinical immunotherapeutic research.

2. Immunogenicity and immune evasion in colorectal cancer

Several authors have reported on the prognostic value of T cell infiltrates (TIL) in CRC tissues [1–3]. Specific cytotoxic T cell (CTL) responses against CRC epitopes may be induced both *in vitro* and *in vivo*, this has been reviewed in Ref. [4]. Furthermore, there is evidence of natural occurring cellular and humoral responses directed against tumour-associated antigens (TAAs) in patients with CRC [5,6].

Several mechanisms have been identified that may explain why CRC can develop despite the presence of cellular and humoral immune responses. First, many tumour cells fail to present antigen due to the loss or reduction of human leucocyte antigen (HLA) class I expression, necessary for peptide presentation to CTL. Tumours that lose expression of major histocompatibility (MHC) class 1 molecules may, however, be more susceptible to natural killer cell activity [7,8]. Second, mutations in peptide transporting molecules (TAP) have been observed in CRC which may affect the presentation of T cell epitopes and lead to suppressed cellular immunity [7]. Third, CRC cells may express FasL more frequently in metastases compared with primary tumours [9]. Binding of FasL to Fas (Apo-1/CD95) on the surface of T cells results in events leading to the apoptotic cell death of activated T-cells.

Fourth, a decreased expression of CD3 zeta chains in the T cell receptor in peripheral blood lymphocytes and TIL has been demonstrated in patients with CRC [10]. This is known to result in signal transduction defects. However, whether this fully explains a decreased

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immunogenicity is uncertain since interleukin-2 may restore CD3 zeta chain expression, but not the ability to lyse target cells [11]. Fifth, CRC cells have been shown to produce transforming growth factor β (TGFβ) [12] and interleukin 10 [13], cytokines that may suppress the cellular immune response. Recently, a TGFβ-mediated downregulation of TGFβ receptors, as well as effects on stroma formation and angiogenesis was implicated in the development of CRC [14].

To what extent these escape mechanisms play a role in an individual patient is largely unknown, and this will be one of the major challenges of future research since this information will significantly contribute to the design of more relevant immunotherapeutic strategies.

3. Non-specific immunotherapy

In non-specific immunotherapy, immunomodulating agents are administered with the aim of inducing a generalised immune response without attempting to direct the activity towards a specific antigen. Results of this treatment modality have been reviewed previously in Ref. [15].

In summary, many of these agents, mostly derived from microbial sources like Bacille Calmette Gúerin (BCG), OK432 or *Corynebacterium parvum*, have been tested in laboratory and clinical trials, usually in combinations with chemotherapy. Anecdotal reports of antitumour responses have been published, but overall the results were disappointing. A recent update of a large study that included treatment arms with surgery alone and BCG plus surgery showed a significant survival benefit for the addition of BCG at 10 years. However, it was uncertain whether this benefit could be attributed to an antitumour effect [16].

Interferon-alpha and interleukin-2 used as single agents and in combination with 5-fluorouracil (5-FU), have been tested in small studies in patients with CRC, with marginal efficacy.

4. Antibody therapy

4.1. Unlabelled monoclonal antibodies

The murine IgG2a monoclonal antibody (MoAb) 17–1A (Edrecolomab) binds to the cell surface glycoprotein CD17–1A antigen (GA733–2) that is preferentially expressed on adenocarcinomas, but also is found on normal epithelial cells. Naturally occurring autoantibodies to GA733–2 are found in a high incidence in patients with CRC, but do not appear to be correlated with the stage of disease and/or survival [6]. Clinical responses with edrecolomab therapy have been demonstrated. The exact mechanism of action of this antibody is unknown, both T cell and humoral immune reactivity through activation of the idiotypic network have been described [17,18]. Augmented immune responses have been described by combining edrecolomab with cytokine therapy [19]. Treatment with edrecolomab raised great expectations when in a randomised adjuvant setting versus observation a significant survival benefit was demonstrated in 189 patients with resected Stage III CRC. Edrecolomab was administered intravenously at an initial dose of 500 mg followed by four monthly doses of 100 mg. After a median follow-up of 7 years, the antibody-treated group had a reduced mortality of 32% and decreased recurrence rate of 23% [20]. Unfortunately, in a large international phase III study with over 2700 stage III colon cancer patients, single agent therapy with edrecolomab proved significantly worse compared with 5-FU and leucovorin, and the addition of edrecolomab to 5-FU and leucovorin offered no advantage over chemotherapy alone [21]. There was no obvious explanation for the lack of efficacy of the addition of edrecolomab to chemotherapy, since human-anti-mouse antibody formation (HAMA) by edrecolomab with chemotherapy was less than without. Since this study did not contain an observation arm, a small beneficiary effect of edrocolomab cannot be ruled out. The results of a large US phase III study with similar design are still pending.

Other less extensively tested murine monoclonal IgG2A antibodies are L6, which binds to an antigen expressed on >90% of CRC cells [22], and D612, also binding to a membrane glycoprotein expressed on gastrointestinal tumours and showing enhancement of antibody-dependent cellular cytotoxicity [23].

In summary, the current results of treatment with murine MoAb have not been very promising. Their weak immunomodulatory effects and the induction of HAMA are considered to be instrumental in this.

4.2. Radiolabelled monoclonal antibodies

Radiolabelled MoAbs have been used in patients with CRC as diagnostics, i.e. [^{111}In]-labelled B72.3 MoAb and [^{99}mTc]-labelled IMMU-4, the Fab′ antibody fragment of an anti-carcinoembryonic antigen (CEA). Both techniques were shown superior to conventional methods in detecting metastases of CRC [24,25]. Many radioisotopes are potentially suitable for therapeutic use. Instead of gamma rays used for imaging, beta particles from isotopes such as ^{90}Y, ^{131}I and ^{67}Cu travel only a few millimetres to centimetres in tissue, thus killing antigen-positive and negative cells. Another targeting strategy makes use of the high affinity non-convalent binding between avidin and biotin. After injection with biotinylated anti-CEA MoAb, cold avidin was administered to remove non-tumour bound antibodies. A third injection with labelled biotin was given

after 48 h, thus producing images with high tumour: background ratios [26].

Administration of the TAG-72 MoAb CC49 conjugated to streptavidin followed by the administration of 90Y-Dota-biotin allowed a 9-fold increase in the radioactive dose administered [27].

Several studies with [^{131}I]-labelled MoAbs have been performed in patients with CRC, however usually with disappointing results [28–30]. Several modifications have been made to increase the concentration of radiolabelled antibody in the tumour. Using smaller F(ab′)2 AB fragment instead of intact anti-CEA antibody resulted in faster penetration into tumour masses, thus enabling higher maximum dose rates to be given to the tumour [31]. In a murine CRC model, monovalent Fab fragments were also shown to be superior to intact IgG, as well as to chemotherapy with 5-FU and leucovorin [32]. This last group also demonstrated that [^{131}I]-labelled anti-CEA Moab in a murine model was most effective in eradicating limited disease compared with more advanced tumours, and they observed clinical responses in a small phase I study with CRC patients [33].

5. Active specific immunotherapy

The goal of active specific immunotherapy (ASI) is to evoke a tumour-specific immune response resulting in the destruction of tumour cells, as well as a memory response against the relevant antigens. Initially, this was performed by using tumour cell preparations, but advances in molecular biology have led to the use of more sophisticated vaccine preparations such as gene-modified tumour cells, purified TAAs, DNA-encoding protein antigens and protein-derived peptides.

5.1. CEA as a target for immune reactivity

CEA, an adhesion molecule playing an important role in the metastatic process, is highly expressed in CRC and is the most well-known target antigen for immunotherapy in this disease. Although the immunogenicity of CEA has been questioned for quite some time, there is a potential role for this epitope in the immunotherapy of CRC. Recently, natural T cell responses to CEA, as well as to her-2/neu, were reported in CRC patients with metastatic, but not with localised disease [5]. A stable CEA-specific CTL line has been generated from the peripheral blood of a CRC patient treated with a recombinant CEA vaccine [34]. Novel HLA-A24- and HLA-A3-restricted CEA peptides have been identified which may be applied in peptide-based immunotherapy of CRC [35,36].

Dendritic cells (DC) pulsed with a HLA class I-restricted *CEA*-derived peptide or CEA RNA were shown to induce a CEA-specific CTL reactivity in patients with CEA-expressing tumours [37]. These authors subsequently demonstrated the safety and feasibility of the administration of CEA peptide-pulsed autologous DC in this patient group [38]. Therapy with DC-based vaccines has attracted much attention in recent years since objective remissions have been described in a minority of patients with various tumour types. Research on improving DC vaccines is ongoing, and one approach may be the administration of Flt3 ligand which was shown to mobilise DC in the peripheral blood, as well as to increase DC infiltration into the tumour tissue of CRC patients [39].

5.2. Tumour cell vaccines

Immunisation with the use of whole tumour cells has the advantage that all potential TAAs are presented to the immune system. Since this tumour evidently has not evoked a relevant immune response in the patient, the immunogenicity of such a vaccine should be increased. This may be achieved for instance by the route of administration and/or the addition of adjuvants. Obtaining fresh tumour cells is complicated by the laborious procedure and the limited availability of tumour cell lines that are HLA typed and express high levels of MHC antigens. In a prospective randomised trial, 98 patients with Dukes' stage B2 and C colon or rectal cancer were treated by surgical resection alone or resection plus active specific immunotherapy. This approach was based on successful tests in a guinea pig model [40]. Vaccine administration began 4–5 weeks after tumour resection, beginning with one intradermal vaccination per week for 2 weeks consisting of 10^7 viable irradiated autologous tumour cells and 10^7 viable BCG organisms. In the third week, patients received one vaccination of 10^7 irradiated tumour cells alone. After a median follow-up of 6.5 years, no differences in recurrence rate or survival were observed. Subgroup analysis showed a trend for a benefit in patients with colon cancer [41]. No correlations with immune responses were reported. In a second study, 412 patients with stage II and III colon cancer were treated with the same vaccination regimen versus control, and after a median follow-up of 7.6 years, there was again no significant difference in clinical outcomes between the treatment arms [42]. Subgroup analysis showed a positive trend in overall survival for immunised patients who completed treatment and who developed a delayed type hypersensitivity (DTH) response to the third vaccination of $\geqslant 5$ mm. There was a correlation between the magnitude of DTH response and survival. In a third adjuvant trial, 254 patients with stage II and III colon cancer received in addition to this schedule a fourth vaccination at 6 months with irradiated tumour cells alone, and were randomised versus observation. After a median follow-up of 5.3 years, there was no significant

difference in the 5-year overall survival. Subgroup analysis showed a significant longer recurrence-free survival for patients with stage II disease; 60% versus 80% ($P=0.011$) [43]. This study was also superior to previous studies in terms of quality control of the vaccine. Taken together, these results are indicative for clinical efficacy which appears to be mediated through a cellular immune response. However, no significant benefit was demonstrated in any of these studies in the intent-to-treat population. Therefore, these results have to be confirmed in a multicentre-setting with a larger number of patients before applying this technique as a standard procedure in patients with stage II or III colon cancer. In stage III colon cancer such a randomised study of adjuvant 5-FU and leucovorin with or without ASI (OncoVAX^R) is currently ongoing.

In another small non-randomised study, 48 patients with stage II and III CRC received autologous colon cancer cells inactivated by irradiation and infected with Newcastle disease virus (NDV) and 9 patients received tumour cells mixed with BCG. The 2-year survival rates were 98% for patients treated with the NDV cells compared with 67% for the BCG cells with less side-effects [44]. The concept of the use of paramyxovirus NDV instead of BCG for vaccine modification seems to have a number of advantages, among which are an enhanced expression of viral antigens and the induction of local cytokines production with T cell stimulating effects. Obviously, the size of this study was too small to draw definite conclusions.

A polyvalent allogeneic tumour cell vaccine, Cancervax^R, combined with BCG has been used in a phase II trial in 28 patients with stage IV CRC. Preliminary results show that overall survival was better for patients who demonstrated a significant cellular immune response [45].

5.3. Genetically modified tumour cells

The introduction into tumour cells of foreign genes encoding for cytokines like interleukin 2 and granulocyte macrophage-colony stimulating factor (GM-CSF) has the potential to enhance immunogenicity [46]. Gene transfer can by established by transfection of plasmid constructs or transduction using a viral vehicle such as retroviruses and adenoviruses. Genes can be transferred using viral vectors either to cultured tumour cells *in vitro*, or directly to tumour cells *in vivo*. Although some evidence of specific immune reactivity has been observed, these approaches are limited by technical problems and their use is still highly experimental. CEA-expressing tumours may also be targeted by engineering proteins within the viral envelopes binding to surface CEA [47]. This approach has been successful in mice models and is being tested in freshly isolated tumour cells from patients [48].

5.4. Peptides, mucins and carbohydrates

As an alternative to the abovementioned vaccines, purified TAAs may be used either as whole protein or as peptides, thus acting as immunogens. Whole proteins may be more immunogenic than peptides, while peptide vaccines are more easily synthetically generated.

Mucins such as MUC1 are heavily glycosylated high molecular weight proteins and are overexpressed and aberrantly glycosylated in many cancers such as CRC. Expression of mucin on the cell membrane can mask surface immunoregulatory molecules and inhibit interaction between tumour and immune cells. MUC1 expression on tumour cells, or shedding of MUC1 by tumour cells may also suppress the immune system through the induction of T cell apoptosis [49]. MUC1 is also known to suppress CD4+ helper T lymphocytes *in vitro* [50]. However, mucin can become a target for CTL activity by changes in glycolysation. Vaccination with MUC1 results in the production of antibodies and mucin-specific CTL precursors, indicating specific activation of the immune system. The results of clinical studies with MUC1 vaccination have been disappointing so far, this has been reviewed by Ref. [4]. In a recent study with Theratope^R, a mucine carbohydrate vaccine in 45 patients with stage IV CRC no responses and seven disease stabilisations were observed, with a median overall survival of 14.2 months. A randomised trial is planned [51].

Immunisation against tumour-associated carbohydrate antigen has also been attempted. TF and sTn antigens are disaccharides and in normal tissues these antigens are restricted to the luminal surface of secretory cells, thereby largely inaccessible to the immune system. Altered glycolysation leads to exposure of these core structures in malignant tissues. However, the antibodies that were induced by this approach showed only low affinity for their natural antigens [52].

5.5. Recombinant vaccines expressing CEA

In order to enhance immunogenicity, combination with a strong immunogen such as the vaccinia virus has been tested [53] or avianpox virus (ALVAC-CEA) [54,55]. In both studies, CEA-specific CTL responses were found without clinical responses. Further clinical trials are ongoing.

5.6. Anti-idiotype antibodies

Several anti-idiotype antibodies that mimic TAAs on CRC cells have been reported. CeaVac^R (3H1) is a murine monoclonal anti-idiotype antibody that mimics a highly tumour-restricted CEA epitope, and was shown to have antitumour effects in a murine model [56]. Polyclonal antibody responses were demonstrated in 17

out of 23 patients with advanced CRC; in 13 patients this concerned true anti-CEA responses. Five patients had specific T-cell responses to CEA. The concomitant use of chemotherapy did not impair this immune response [57]. In an adjuvant setting, CEAVac^R was shown to generate high titres of polyclonal anti-CEA and anti-idiotype specific T-cell responses. Randomised studies in the therapeutic and adjuvant setting are being planned [57,58]. 105AD7 is a human MoAb that mimics the gp72 antigen which is expressed in 80% of CRC cells, and the induction of a DTH reaction against human tumour cells has been demonstrated. In a phase I study with 13 CRC patients, 105AD7 was administered intramuscularly, and increased levels of IL-2 and a lymphocyte proliferative response was observed after stimulation *in vitro* by gp72 antigen-positive cells. A clinical benefit was suggested by the authors after comparison with historical controls, and in order to confirm this a double-blind randomised trial has been initiated [59].

5.7. New targets for cellular immune reactivity

Several novel candidate target antigens or epitopes have been described in CRC. These include the tumour-specific SART-1, which was found to be a target for an HLA-A24-restricted T cell response [60]. The lck protein, a tyrosine kinase, also is expressed on metastatic cells of CRC and is recognised by HLA class I-restricted and tumour-specific CTL [61]. Moreover lck-derived peptides were shown to augment CTL reactivity in the peripheral blood of CRC patients. Her-2/neu is highly expressed in CRC, both in primary as well as in metastatic lesions. An HLA-A24-resticted T cell epitope from the her-2/neu antigen with a high-affinity HLA-A24 binding peptide have recently been characterized [62]. Lastly, aberrant expression of p53 frequently occurs in CRC, as well as in many other tumours. A novel HLA-A24-restricted p53-derived peptide has been characterised that was capable of specific CTL induction [63].

6. Conclusions

There is sufficient evidence for immune reactivity in CRC in order to justify further research on the development of immunotherapeutic strategies in this disease. Current research is focused on the development of humanised antibodies, the search for other relevant T-cell epitopes and more efficient ways to induce an effective T cell response.

As is probably true for immunotherapy in general, positive results, if any, are most likely to be expected in patients with limited tumour burden such as in the adjuvant setting. This has the obvious drawback of necessitating randomised trials with large number of patients, since relevant surrogate markers for the desired immune response are still not available. Several of such trials have been initiated in order to confirm the promising results of smaller studies.

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