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REVIEW ARTICLE

The response of urological tumours to immunotherapy

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Summary

The use of immunotherapy to attempt to treat cancer is not new. At the end of the last century, William Coley observed that the tumour of a patient with a sarcoma who developed streptococcal erysipelas regressed. This led Coley to develop a collection of heat-killed bacteria, known as Coley's toxins, which he used to activate the immune system, with some reported tumour regressions. Subsequently, several investigators used BCG to treat solid tumours. When used by intralesional injection, BCG induced regressions of melanoma skin metastases in some patients, but without affecting survival. In 1976, Morales described the use of intravesical BCG to treat superficial transitional cell carcinoma (TCC) of the bladder. The efficacy of intravesical BCG remains the most successful example of cancer immunotherapy to date.

Key words: bladder cancer, immunotherapy, prostate cancer, renal cell carcinoma, urological tumors

Introduction

Tumor immunology

According to the immune surveillance theory of Burnet, the immune system is responsible for eliminating newly transformed cells and therefore the emergence of a tumour is a failure of the immune system [1]. Cases of spontaneous tumour regression, reported in melanoma and renal cell carcinoma (RCC), support the idea that the immune system is sometimes capable of delaying tumour progression and on rare occasions can eliminate the tumour completely.

Candidate cells for involvement in antitumour immunity include cytotoxic T lymphocytes, macrophages, natural-killer (NK) cells and antibody-dependent cell-mediated cytotoxicity. Evidence is emerging that, at least in solid tumours, antitumour immune responses are mediated more effectively by T-cells rather than antibodies [2].

The recognition of cancer cell as foreign cell depends on several factors, including the expression of tumour-specific antigens, and the normal expression of class I MHC antigens are required for antigen presentation. The activation of cytotoxic T lymphocytes (CD8+) requires two signals. The cytotoxic T lymphocytes are class I restricted, and a short peptide sequence of the antigen must be presented in conjunction with the class I MHC of the tumour cell. The second signal involves lymphokines produced by helper T lymphocytes (CD4+). The latter are activated by the presentation of endocytosed antigens in conjuction with class II MHC on "professional" antigen-presenting cells such as macrophages, dendritic cells and B lymphocytes. One possible mechanism by which tumour cells may avoid recognition by immune cells is if antigen presentation is defective because the expression of MHC is absent or poor, or by the absence of co-stimulatory molecules.

Absent or reduced class I HLA expression has been reported for a large proportion of prostate, bladder and RCC [3-5].

The concept of active immunotherapy of cancer

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is based on the theory that tumours possess specific antigens which can be recognized by the immune system. Several tumour-associated antigens have been described in the last few years, containing peptide sequences that can be recognized by specific cytotoxic T lymphocytes.

Immunotherapy strategies

There are several categories of immunotherapy which have been tried for the management of cancer:

(i) passive non-specific immunotherapy, e.g. transfer of lymphokine-activated killer (LAK) cells,

(ii) passive specific immunotherapy, e.g. transfer of specific antibodies or transfer of specific immune cells such as cytotoxic T-lymphocytes,

(iii) active non-specific immunotherapy, e.g. BCG, IFN-α and IL-2 and,

(iv) active specific immunotherapy, e.g. immunization with a variety of therapeutic vaccines.

Each of these strategies has been applied to the management of one or more of the urological malignancies, the exception being germ cell tumours, where the results of current chemotherapy regimens are excellent, even in advanced disease.

Bladder cancer

Intravesical immunotherapy of superficial TCC of the bladder using BCG is the only non-surgical therapy that has been shown to alter the progression of superficial bladder cancer. The process for attenuating *mycobacterium bovis* was devised over several years by Calmette and Guerin, and *M. bovis* BCG vaccine was developed in the 1920s as a treatment and prophylaxis for tuberculosis. Subsequently, it was assessed for efficacy against several cancers before Morales described its use in superficial bladder cancer [1].

Mode of action of BCG

The detailed mechanism of action of intravesical BCG has not yet been elucidated fully. However, it has been shown that it is necessary for BCG to bind to the urothelium, possibly by attachment to fibronectin, an adhesion molecule present in the bladder wall [6]. Bladder cancer cells can internalize BCG and BCG antigens are expressed on the surface of the tumour cells [7]. During intravesical treatment there is a local inflammatory response involving mononuclear cells, particularly T helper cells, and MHC class II expression is upregulated [8]. Several different cytokines are released into the urine after the administration of BCG, including IFN-γ, TNF-α, IL-2, 6, 8, 10, and 12, and granulocyte macrophage colony stimulating factor (GM-CSF). It is hoped that a particular cytokine profile might define effective treatment and predict which patients will fail to develop the proper immune response to treatment. It has recently been shown that low levels of urinary IL-2 correlated with tumour recurrence within 6 months [9]. A more recent study showed that inducibility of IL-2 gene expression, but not IFN-γ, in peripheral blood lymphocytes following intravesical BCG was a powerful predictor of remission [10]. Such a marker could be developed and used as a prognostic test.

Efficacy of BCG

In a recent review of all the randomized trials of intravesical chemotherapy, Lamm et al. [11] concluded that although intravesical chemotherapy has shown a statistically significant reduction in short-term tumour recurrence (2-3 years) in 13 of 22 studies, none of the long-term studies showed a reduction in either tumour recurrence or progression. Intravesical single-dose mitomycin C does seem to have a positive short-term effect given as adjuvant after transurethral resection [12]. The lack of a durable response with mitomycin C supports the hypothesis that intravesical chemotherapeutic agents probably act by preventing tumour cell implantation on resected areas and not by altering the tumour neogenesis of the urothelium.

In contrast to the short-lived reduction in tumour neogenesis seen with intravesical chemotherapy, evidence is accumulating that BCG can alter the natural history of superficial bladder cancer in the long-term. In randomized trials of BCG against intravesical chemotherapy, there was a statistically significant reduction in the recurrence rate in favour of BCG against thiotepa, doxorubicin and mitomycin C [13,14].

Perhaps of greatest importance is the impact of BCG immunotherapy on disease progression. Several studies have shown a reduction in tumour progression, from 8-17% in the control arms to 3-4% in the BCG treatment arms [15] and other studies have corroborated this by showing that there was a resultant decrease in mortality from 32% in controls to 14% in those receiving BCG [16].

Optimizing BCG therapy

There are several important variables in BCG immunotherapy which need to be clarified; the optimal

preparation, the route of administration, the frequency and duration of administration, the possible value of maintenance therapy, the lowest effective dose and the host response. The preparation of BCG vaccine is still much the same as the original process described by Calmette and Guerin in 1908, and consists of live attenuated bacilli, dead bacilli and subcellular debris. There is variability not only between suppliers but even between different lots from the same supplier. Strains of BCG include Evans (Glaxo), Pasteur, Annmand Frappier, Connaught, RIVM and Tice, and no clear evidence has been presented which shows one to be superior in the treatment of superficial bladder cancer [17].

The standard course of BCG comprises weekly intravesical administration for 6 weeks, while some studies suggest that the long-term results may be improved by an additional 6-week course (maintenance therapy) [18]. Lower doses of BCG have been assessed with a view to reducing the incidence and severity of side-effects. The results are conflicting, with some studies suggesting equivalent efficacy with a half-dose [15] while others suggest a significantly worse response [19]; further results are awaited. An interesting proposal is to combine BCG with chemotherapy, the idea being that the cytotoxic drug leads to widespread de-epithelialization and thus might facilitate the uptake of BCG by the submucosal tissues. A phase II EORTC study using mitomycin C followed by BCG showed the combined treatment to be effective against a marker lesion in the bladder; phase III trials are now required [20].

The importance of host response has yet to be evaluated fully in BCG therapy of bladder cancer. It is well known that BCG is a highly effective vaccine for tuberculosis in some parts of the world, e.g. the UK, but has poor efficacy in other areas; within populations, individuals respond differently to BCG immunization. Might there be a similar variation when BCG is used as a cancer immunotherapeutic agent? [21].

Adverse effects of BCG

The main drawback of BCG therapy is that the frequency and severity of side-effects is reported to be higher than for conventional intravesical chemotherapy, with up to 90% of patients having cystitis [22]. However, in a review of complications in 2602 patients in different centres receiving different strains of BCG, a much lower incidence of side-effects was reported, with 95% of patients having none. The incidence of complications was: fever in 2.9%, haematuria in 1%, granulomatous prostatitis in 0.9%, pneumonitis/hepatitis in 0.7%, arthralgia in 0.5%, sepsis in 0.4%, rash in 0.3%, ureteric obstruction in 0.3%, contracted bladder in 0.2%, renal abscess in 0.1% and cytopenia in 0.1% [23].

The most severe complication of BCG is generalized BCG infection; at least 7 deaths associated with the use of intravesical BCG have been reported worldwide, on the basis of which the risk of death has been estimated as <1 per 12500 patients [23]. Most of these cases were associated with intravenous absorption of BCG due to traumatic catheterization or failure to withhold BCG until one week had elapsed after transurethral resection or biopsy. Therein lies the key to reducing the risk associated with this form of therapy. In addition, its use is contraindicated in immunocompromised patients, in pregnancy and lactation, and in patients with active tuberculosis and intractable urinary tract infections. The currently suggested optimal therapy for systemic BCG infection is 300 mg isoniazid, 600 mg rifampicin and 40 mg prednisolone daily [24]. There should be a low threshold for initiating anti-tuberculous therapy if sepsis is suspected.

Indications for intravesical BCG

On the one hand, most clinicians agree that BCG is not justified for patients with low-stage (Ta) or grade (G1) disease, for primary tumours, unifocal tumours or in an adjuvant setting. On the other hand, few would dispute that BCG should be first-line therapy for aggressive disease such as carcinoma *in situ*, although the role of BCG in T1/G3 bladder cancer is more controversial. It is for those patients with multiple frequent recurrences that many clinicians are dissuaded by the higher incidence of side-effects with BCG and by rare systemic complications, despite the apparent therapeutic advantage over intravesical chemotherapy. Although 60-80% of patients complain of local symptoms such as dysuria and bladder spasms, it has been shown that this does not seem to significantly impair their quality of life [25].

Other immunotherapies for bladder cancer

Keyhole limpet haemocyanin (KLH) is a blue respiratory glycoprotein from the primitive gastropod mollusc *Megathura crenulata*. The intravesical administration of KLH is characterized by a marked increase in CD4+ T cells in the bladder submucosa [26] and it has been reported to reduce the recurrence rate in superficial bladder cancer [27] but further evaluation is necessary. Intravesical IFN-α2b and IL-2 [28] have shown some promise in phase II trials in superficial

bladder cancer, but more data are required. One of the most exciting possibilities is to use gene therapy to modify the BCG vaccine to induce the secretion of cytokines or the expression of bladder tumour-associated antigens, with a view to eliciting a stronger, more specific and more reproducible immune response.

Renal cell carcinoma

The spontaneous regression of metastases following resection of the primary tumour makes RCC an attractive target for immunotherapeutic interventions. The incidence of spontaneous regressions is <1% [29], too low to justify performing a radical nephrectomy in the hope of attaining regression of metastatic disease.

Interferons

The first agent to be used in clinical trials in metastatic RCC was IFN-α [29], the effects of which include a direct antiproliferative action on tumour cells *in vitro*, stimulation of host lymphoid cells and macrophages, upregulation of the MHC I and, therefore, enhanced antigen-presenting capability. Several non-randomized studies have shown response rates of 15-20% [30]. Unfortunately, the complete response rate is near to 2% and there is uncertainty as to whether response merely selects patients with better initial prognoses or actually leads to increased survival. The median survival of treated patients was 11-49 months. Patients with pulmonary metastases seem to be most likely to respond to IFN-α [31].

Interleukin-2

Il-2 is a cytokine produced by lymphocytes and is a growth and activation factor for both T cells ans NK cells [32]. In a series of 149 patients with metastatic BCC treated with a high-dose bolus IL-2, 10 (7%) patients had complete regression and 20 (13%) patients had partial regression. More significantly, 7 of 10 patients with complete response had a durable response with no recurrence at a median follow-up of 22 months [33]; similar responses have been confirmed at other centres.

IL-2 toxicity affects the cardiovascular, renal, hepatic and neurological systems because vascular permeability is increased and its initial use was associated with a mortality of 4%. With better management of the adverse effects and improved selection of patients, treatment-related deaths have largely been eliminated in recent studies [33]. Provided the responses to low-dose IL-2 prove to be durable, in future IL-2 may be safely given on an out-patient basis, with a large reduction of adverse effects. IL-2 is the only therapy for metastatic RCC approved by the USA FDA.

Combination therapies

Because IFN-α and IL-2 act in different ways, the use of combination therapy was considered early in their development. This strategy was supported by the results in animal models, which suggested synergistic antitumour effects using the two agents together [34]. However, although initial trials in humans showed some promise, prospective randomized studies have reported no significant increase in response rates with this combination [35].

The combination of immunotherapy with chemotherapy has yet to be assessed in randomized trials. A response rate of 35% was reported with IFN-α and 5-fluorouracil (5-FU) [36] and the response rate with IL-2, IFN-α and 5-FU has been reported to be 47-49% [37].

Cellular therapy

Cellular therapy involves the infusion of the patient's immune cells which have been stimulated *in vitro* by cytokines such as IL-2. The main strategies are to use LAK cells, tumour-infiltrating lymphocytes (TIL) and autolymphocyte therapy (ALT).

1. LAK cell therapy. This involves harvesting the patient's peripheral blood lymphocytes (PBLs) by pheresis and activating them by incubation with IL-2. The cells are then re-infused into the patient combined with high-dose IL-2. Most LAK activity is mediated by NK cells. Unfortunately, in a randomized trial of IL-2 against IL-2 plus LAK cells, no statistically significant difference in response or survival between the groups was detected [38]. Therefore, the addition of LAK cells does not improve the efficacy of IL-2 alone.

2. TIL cellular therapy. The immune cells infiltrating the tumour are both cytotoxic (CD8+) and helper (CD4+) T cells and have been shown to possess specific antitumour activity, presumably because they recognize specific tumour-associated antigens [39]. TILs are harvested mechanically and enzymatically, preparing a single-cell suspension from the radical nephrectomy specimen, and expanding the cells *in vitro* in the presence of IL-2 [40]. After 2 weeks, the tumour cells perish but TILs continue to proliferate, and after about 6 weeks there are sufficient numbers of cells to be infused into the patient together with IL-2.

Phase I/II trials of TIL plus IL-2 show response rates of 0-33% [41] but the number of patients in these trials was low. Current randomized studies are not expected to confirm any benefit from adding TIL to IL-2.

3. ALT cellular therapy. The basis of this therapy is to use antibodies to the CD3 component of the T cell antigen-receptor to activate memory T cells, the idea being that some of these memory cells have been exposed to tumour-associated antigens. Like LAK cells, PBLs are harvested by pheresis and then incubated with anti-CD3 monoclonal antibody, which only activates T cells previously exposed to antigens, and clonal expansion occurs mediated by IL-2. After irradiation to reduce the activation of suppressor T lymphocytes, the cells are infused into the patient. The initial study of ALT in metastatic RCC showed a survival advantage of 21 months in the ALT treatment arm, compared with 8.5 months with cimetidine alone [42]. Cimetidine was used as it is thought to block expansion of suppressor T cells clones. Two other reports support this apparent survival benefit with ALT [43]. As with TIL therapy, ALT needs to be compared in a randomized trial against IL-2 alone.

Cancer vaccines

The idea of injecting live irradiated whole tumour cells as therapeutic vaccine was proposed some time ago. The advances in molecular biology have enabled investigators to genetically modify tumour cells and increase their immunogenicity; the genotypic content of the cell is modified by the addition of a functional gene whose product is then expressed. The use of tumour vaccine cells which have been genetically manipulated to produce a variety of cytokines has shown promising results in some animal models [44]. The intention is to produce high concentrations of cytokines local to the tumour cells such that antigen presentation of tumour-specific antigens by tumour-specific lymphocytes is enhanced. The local production of cytokines produces a vigorous inflammatory response at the site of vaccine injection and avoids the toxicity associated with their systemic administration.

Several cytokines have been transfected; the best results in urological tumours have been achieved with tumour vaccines secreting IL-2 or GM-CSF. The vaccine may be either autologous, i.e. using the patient's tumour cells, or allogeneic, using a bank of standard tumour-cell lines which are not necessarily HLA-matched with the patient. The autologous approach is currently most widespread and has the theoretical attraction that the vaccine is HLA-matched to the patient and should contain the same antigenic repertoire as the patient's tumour. It necessitates obtaining the patient's tumour cells at the time of surgery and establishing a cell line *in vitro*. The cells are transfected with the required gene, e.g. GM-CSF, and their number increases. Following γ-irradiation to ensure the cells do not replicate, thus rendering them non-tumorigenic, they are stored in liquid nitrogen until required; the cells are then injected intradermally into the patient at regular intervals.

The group at John Hopkins recently described the results of a phase I trial of autologous GM-CSF secreting vaccine in 16 patients with stage IV RCC undergoing nephrectomy [45]. No dose-limiting toxicity was encountered and there was dose-dependent induction of T cell-mediated delayed-type hypersensitivity (DTH). One patient who had the largest DTH response had partial clinical remission but there was no clinical response in the rest of the patients.

Preclinical studies suggest that vaccines are most effective in minimal disease; therefore, it may be that their eventual role will be adjuvant therapy after radical nephrectomy to destroy or control micrometastatic disease, which is present in over one-third of patients undergoing "curative" surgery [46].

The role of cytoreductive nephrectomy

Although it is thought that immunotherapy is most likely to be effective in low-volume disease, the role of debulking by nephrectomy in patients with stage IV RCC is still controversial. Certainly, there appears to be no role for nephrectomy outside of immunotherapy protocols, except for palliation, as the median survival is only 4 months. Some studies suggest that patients who have had a nephrectomy have a better outcome from systemic therapy [47]. However, it has been reported that up to 40% of patients fail to survive long enough postoperatively to receive their immunotherapy [48]. If cellular therapies or vaccines prove to have superior efficacy to the current "gold standard" IL-2, then nephretomy will be necessary to reduce tumour bulk and to provide cells should autologous prove to be superior to allogeneic vaccines.

Prostate cancer

Prostate cancer is a slow-growing tumour of low immunogenicity and spontaneous regressions are unknown. At one time it was thought that the prostate may be an immunologically privileged site because it had no lymphatics and was therefore not amenable to immunotherapy [49]. However, there is now evidence

to suggest that the immune response may be important in prostate cancer. In Finland, a series of 325 prostate adenocarcinomas with long-term clinical follow-up were examined for the density of TILs. Patients with absent or low density of TILs were at high risk of tumour progression and this was independent of other risk factors such as tumour grade [50].

Immunotherapy for prostate cancer was previously tried using BCG, with a statistically significant increase in survival in one study and no effect in another [51]. The genetic modification of whole tumour cell vaccines has allowed the immunotherapy of prostate cancer to be considered seriously and is one of many gene therapy approaches now being tried in this tumour [52]. Two groups showed dramatic responses in the Dunning rat model of prostate cancer using tumour vaccines secreting either IL-2 or GM-CSF [53]. The autologous vaccine approach is being evaluated in a phase I/II study at John Hopkins in patients with capsular disease following radical prostatectomy, and the allogeneic approach is being evaluated in patients with advanced prostate cancer in a NCI phase I/II study at the Memorial Sloan-Kettering Institute using MHC class I matched allogeneic cells transduced to use IL-2 and IFN-γ (NCI-V95-0629).

Recently, specific cytotoxic T lymphocytes which recognize peptide sequences of PSA were described [54]. Dendritic cells possess all the necessary antigen-presenting capabilities and are arguably the most efficient antigen-presenting cells known. It has been shown that dendritic cells can be propagated *in vitro* from precursors in the peripheral blood of patients with prostate cancer [55].

Conclusion

BCG is likely to remain the most widely used immunotherapy in the urological armamentarium in the foreseeable future. Several long-term studies have confirmed its place as the only therapy which reduces the incidence of disease progression and mortality from superficial bladder cancer. It is likely that new intravesical immunotherapeutic agents or genetically modified BCG will become available in the future.

Immunotherapy offers the best response and survival rates compared with other treatments in metastatic RCC. Interleukin-2 is currently the "gold standard" although it should be emphasized that relatively few patients benefit from this treatment. Cellular and vaccine therapies are of unproved benefit at present. The management of advanced RCC requires close cooperation between urologists and medical oncologists so that patients with good performance status can be offered cytoreductive surgery and, ideally, be randomized into clinical trials.

The ability to genetically modify tumour cells *in vitro* to produce immunogenic vaccines has shown promise in animal models and is being assessed in clinical trials in many tumours, including renal, bladder and prostate cancer.

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