REVIEW

BCG: A throwback from the stone age of vaccines opened the path for bladder cancer immunotherapy

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MORALES A. BCG: A throwback from the stone age of vaccines opened the path for bladder cancer immunotherapy. *Can J Urol* 2017;24(3):8788-8793.

Introduction: *It is 40 years since the initial documentation of the efficacy of bacille Calmette-Guérin (BCG) in the management of non-muscle invasive bladder cancer (NMIBC) and probably an opportune a time as any to retrace the origins of this development and to reflect on the progress that has occurred on the use of immune modifiers in the treatment of NMIBC.*

Materials and methods: *A PubMed search for publications on the history of BCG was conducted, and those related to the development of the vaccine for protection against tuberculosis as well as those published in the last 40 years related to its use for treatment for NMIBC were selected for review. A manual search was also carried out for recent articles on immunotherapy for NMIBC failing to respond to BCG. Publications were selected for their usefulness in exemplifying the development of BCG as an antineoplastic agent, elucidating its mechanisms of action of BCG or introducing significant modifications in treatment regimens resulting in enhancement of its efficacy. Alternative innovative immunotherapeutic approaches were chosen to illustrate current trends in the management of this disease.*

Results: *Well thought-out modifications of the original protocol resulted in enhanced efficacy of the vaccine, which currently ranks as the best-known and most-used and investigated agent for high risk NMIBC. Despite its efficacy, a considerable number (30%-40%) of these tumors fail to respond to BCG. In addition, as a live bacterium it carries the potential for serious adverse effects and some patients are unable to tolerate it. These shortcomings have created the need for new agents. These range from other mycobacteria and viruses to monoclonal antibodies alone or in combination with other agents currently at various stages of development.*

Conclusion: *After 4 decades of use, BCG remains the most effective agent against high risk NMIBC, but it still holds substantial drawbacks. The enduring use of immunotherapy for NMIBC has created a propitious environment to search for better alternatives. There are an increasing number of promising in vitro, animal and early human clinical trials to anticipate a significant therapeutic alternative in the foreseeable future.*

Key Words: immunotherapy, cancer vaccines, bladder cancer, bacillus Calmette-Guérin

Introduction

Bacille Calmette-Guérin (BCG) therapy was not only the first confirmed successful immunotherapy against an established solid human cancer, but has enjoyed an enduring dominance over all other forms of medical treatments for intermediate and high risk non-muscle invasive bladder cancer (NMIBC).[1] From the original reports showing that administration of BCG decreases the frequency and number of recurrences of bladder cancer[2] and exhibits considerable antineoplastic activity against carcinoma in situ (CIS),[3] investigators across the globe have made concerted efforts to understand the exact mechanisms of BCG anti-cancer activity. In broad terms, following intravesical administration, BCG orchestrates a vigorous immune response involving T lymphocytes, activated macrophages and their cytokines resulting in the killing of cancer cells. But more recently, new avenues of research are showing effective immunotherapeutic approaches with less associated adverse effects. Herein the historical perspective and expectations for novel immune modifiers for treating non-muscle invasive bladder cancer (NMIBC) are reviewed.

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Anchored in the past and primed for the future

The prevalence and mortality rate of tuberculosis was high at the end of the 19th century. At the time of Confederation (1867) it was the leading cause of death and most serious health problem facing Canadians.[4] This was also a time when a great deal of interest existed in the development of protective vaccines against microorganisms. Following Koch demonstration of tuberculosis as an infectious disease and pointing as the causative agent to be *Mycobacterium tuberculosis* (*M. tuberculosis*), Albert Calmette and Camille Guérin at the Institute Pasteur in Lille, France, started the long process of developing an agent against the disease. *Mycobacterium bovis* (*M. bovis*) is closely related to *M. tuberculosis*, and they found that, by repeated sub-cultures over many years, it would lose a great deal of its virulence. The modified strain of *M. bovis* was subsequently referred to as bacille Calmette-Guérin or simply BCG. After demonstration of its safety and efficacy in animals, the vaccine was first administered to neonates in 1921 with significant success in regards to safety and efficacy[5] leading to widespread use in Europe. Ten years later, dozens of infants in Lübeck, Germany died after receiving an accidentally contaminated BCG preparation. This tragedy, highly publicized in the lay press,[6] created a great deal of suspicion about the safety of the vaccine that persisted for several decades and still resonates.

Concurrent with the development of BCG, there was the remarkably visionary work of William Coley who systematically treated cancer patients with bacterial preparations, initially with a mixture of *Streptococcus erysipelas* and *Bacillus prodigiosus*[7] and later on using heat-inactivated *Streptococcus pyogenes* and *Serratia marcescens*. Although he reported intriguing numerous anti-tumor responses, his work was largely ignored. Much credit must be given to his daughter Helen Coley Nauts for publicizing[8] and continuing his work[9] as well as her founding of the Cancer Research Institute of New York, a pioneer in support research into cancer immunotherapy.

Contemporary with these events and based on autopsy observations there was a belief that tuberculosis exerted a protective effect against the development of cancer in humans.[10] The availability of BCG led some investigators to determine its profound effect in the reticuloendothelial system and initiate cancer treatment with the vaccine.[11] Unfortunately, events such as the Lübeck tragedy and World War II intervened to interrupt further research in this promising area. It was not until the end of the war that interest on BCG as anti-neoplastic agent was rekindled. Old et al demonstrated significant activity against transplanted autologous cancer in rodents.[12] In the late 1960s and early 1970 there were numerous publications by pioneers using BCG in the treatment of leukemia[13] and melanoma,[14] including a case report of a melanoma metastatic to the bladder responding to intralesional injection of BCG.[15]

Development of the regimen for intravesical BCG therapy and initial results

A confluence of various studies created a propitious environment for developing a regimen of BCG use in NMIBC. In 1966, Coe and Feldman[16] reported that a strong delayed hypersensitivity reaction could be elicited in outbred guinea pig bladders. By 1970, Zbar et al[17] had established the criteria for successful BCG therapy for experimental cancers. They include: a) an immuno-competent host, b) a sufficient number of viable mycobacteria, c) close proximity of the vaccine and the cancer cells and d) a limited tumor load. It was therefore evident that NMIBC ideally fulfilled these criteria for BCG therapy. In addition, it was already known that repeated administration was needed to induce a delayed hypersensitivity reaction. Finally, the adverse reactions of bladder irritability as well as the cutaneous response to the intradermal route largely subsided after 1 week, thus a weekly schedule was deemed most suitable. Anecdotally, the vaccine form the Institut Armand Frappier (Montreal, QC, Canada) was packed containing six vials per box, thus prompting the use of a 6 week treatment protocol. Although purely a serendipitous decision, it was later on proven to be the right choice.[18] At the time it was also believed that the intradermal administration (by Heaf gun) was important to enhance an immunological systemic response and to easily observe the local skin reaction as a credible reflection of the vesical response.

It was not until 1976 that the first publication appeared of a systematic (albeit small) study documenting that the intravesical and percutaneous administration of BCG weekly for 6 weeks resulted in a 12-fold reduction in bladder cancer recurrence.[2] These findings generated sufficient interest that the National Cancer Institute (NCI) sponsored two control human clinical trials requiring adherence to the original protocol. The studies were conducted at the University of Texas in San Antonio (UTSA) and at the Memorial Sloan-Kettering Cancer Center (MSKCC) in New York. They both confirmed the efficacy of BCG. The UTSA trial showed a significant reduction in the recurrence rate of the treated patients;[19] the MSKCC trial further supported these findings but also provided evidence that the beneficial effect was durable and prevented

progression of the disease.[20] The relevance of the 1976 publication has been recently recognized as "one of the most impactful papers published by the *Journal of Urology* in the last century.[21]

Direct antineoplastic activity of BCG against an existing bladder cancer first appeared in 1980 with the publication of an observational study of 7 patients with histologically documented CIS.[3] The treatment regimen was identical to the one used for the prevention of recurrences. This report was remarkable not only for showing the efficacy of BGC against an existing solid malignancy but because the complete response rate was 71% (7/9 patients) with a mean follow up of 22 months, but also because the same response rate of CIS to BCG has been consistently found in multiple subsequent larger, controlled randomized studies[22] and across different BCG strains. The report of Lamm et al[15] showing a clear superiority of BCG over doxorubicin was largely instrumental in the approval of BCG by the U.S. Food and Drug Administration (FDA) in 1990.

Adjustments of the original protocol

Following the FDA's approval of BCG for the treatment of CIS of the bladder, there was an explosion of interest not only in improving the efficacy of the treatment aspects, but also in the elucidation of its mechanisms of action.

Clinically, a number of modifications to the original regimen were soon introduced. The initial studies of BCG in bladder cancer called for both percutaneous and intravesical administration. Later on, the percutaneous route was discontinued on the basis of several studies showing that it did not add to the efficacy of the intravesical route alone.[23,24] Although it eliminated an inconvenience to both the patient and the physician, this issue merits a revisit. It was our view that the delayed cutaneous reaction was a reliable indicator of the response taking place in the bladder mucosa but also a booster to a vigorous anti-tumor response. A variety of schedules for the intravesical administration were introduced. The original induction phase of six weekly treatments remains as the most efficacious.[18] However, a great deal of controversy developed over the years about the need for a maintenance schedule and a number of them were proposed with contradictory results.[25-27] Currently the American[1] and the European[28] Associations of Urology recommend a maintenance schedule. Although not universally accepted, it is generally agreed that the Southwest Oncology Group Study[29] offers the best prospect for a durable response. Some believe that a shorter maintenance period is appropriate for those patients with a satisfactory initial response. Finally, it is now firmly established that BCG administration is not indicated for low grade tumors with low recurrence potential.[1]

Anticipating a decrease in adverse effects, investigations were carried out to determine the efficacy and safety of keeping the 6 week induction course, but decreasing the amount of vaccine administered. Reducing the dose by one half or one third lessened the number and frequency of adverse effects but also decreased the efficacy, particularly in the more aggressive neoplasms.[30,31] Despite the vast clinical experience and 4 decades of clinical use, the optimal dose and duration of treatment remains controversial.[32]

Mechanisms of action

The fact that BCG is a living organism and that its mechanisms of antineoplastic activity are immunologically mediated, creates great challenges in understanding how it works. One of the first issues to be elucidated was the demonstration that a fundamental, initial step is the attachment of the microorganism to urothelial cells (both normal and malignant) by fibronectin and integrin receptors.[33,34] It has long been established that this stage induces a local granulomatous inflammatory reaction[2] with infiltration of granulocytes, macrophages and lymphocytes (helper T cells). Following internalization of BCG, the response is also characterized by the induction of cytokines such as interleukin (IL)-1, IL-2, IL-6, IL-8, IL-12, IL-18, interferon-γ, TNF-α and granulocyte-macrophage colony stimulating factor (GM-CSF)[35-37] as well as chemokines.

Cellular immune mechanisms also play a critical role in the BCG action. The role of lymphocytes was established with the demonstration that athymic mice do not respond to BCG administration and the requirement of $CD4^+$ and $CD8^+$ T cells.[38] Granulocytes and macrophages are an important component of the inflammatory response to the vaccine and the latter are cytotoxic to bladder cancer cells.[39] The participation of NK cells[40] as well as dendritic cells[41] has been deemed essential for the antineoplastic response induced by BCG.[42] Finally, tolls-like receptors expressed in all these cells as well as normal and malignant urothelium are believed to play a role in the complex immunological cascade elicited by intravesical administration of BCG.[43]

Defining a path to the future

A large body of literature has now accumulated on the clinical and basic aspects of BCG in the treatment of bladder and other genitourinary malignancies.[6,43,44] Although BCG remains the standard in the treatment of high grade NMIBC, as a live organism it carries the

potential for serious and lasting adverse effects. A major limitation is the absolute contraindication for the use of intravesical BCG immediately following endoscopic removal of the tumors and the need to wait for treatment until the bladder mucosa has healed. Even with the necessary precautions, serious adverse events are associated with therapeutic use of BCG.[44] In addition, a significant number (30%-35%) of these cancers fail to respond initially or recur at a later date following therapy. These drawbacks have led to an interest in modifying BCG or to use different mycobacteria as alternatives or as salvage for BCG-unresponsive cancers.

The seriousness of failing to respond to BCG has been brought to recent attention by Mmje at al[45] showing that recurrence of low grade papillary tumors allows further BCG treatment and a conservative approach while a high grade recurrence should be treated as early invasive disease due to its comparable progression rate. These patients and those who fail BCG at a later date have very few proven safe alternatives. Radical cystectomy would offer the best opportunity for cure but carries significant mortality (2%-5%) and morbidity (17%-32%) rates, as well as life-altering changes.[46] Although a number of novel immune-oncology agents alone or in combination with established modalities have shown activity against these neoplasms, none has yet received approval for this specific indication.[47]

In order to circumvent the safety issues, a number of investigators explored the antineoplastic activity of killed BCG or its cellular fragments with limited clinical success. Surprisingly, little heed was given to the pioneering work of Ribi et al[48] and Gray et al[49] conclusively showing anticancer activity of bacterial cell-wall skeletons preparations from *Mycobacterium phlei* (*M. phlei*), a ubiquitous mycobacterium found in soil, on plants, and in drinking water and acknowledged as a non-pathogen for amphibians, birds, fish or mammals.[50]

We became interested on the use of *M. phlei* and over the last 2 decades have conducted extensive basic and clinical investigations utilizing cellular components of this microorganism. It was documented that different *M. phlei* preparations exhibit significant antineoplastic activity in vitro,[51] in experimental animal cancers[52] and in human NMIBC.[53] In an open label study of a 129 patients with a high risk of recurrence or progression of NMIBC and who had failed treatment with BCG, it was found that the *M. phlei* formulation was better tolerated than BCG and resulted in a disease-free survival rate ranging of 21.0% to 35.1% with durable response of 31.9 months or more which is better than any other current treatment for this very challenging population.[54] The FDA however would not approve the compound in the absence of a controlled trial, although the study fulfilled the recent consensus recommendations from the International Bladder Cancer group.[55]

Recently a similar concept was explored by Noguera-Ortega et al[56] who found significant activity of another non-pathogenic mycobacteria (*M. brumae*) against bladder cancer. The research however has not yet reached human evaluation.

On a related issue, the bladder mucosa response to microorganisms is an important factor that needs consideration in assessing non-specific bacterial or viral response as an anti-cancer weapon. Issues of innate defenses of the urothelium ranging from umbrella cells and their uroplakins to the resident immune cells (macrophages, dendritic cells, γδ T and stem cells).[57]

Immunotherapy for cancer in general is now widely accepted and numerous agents have been introduced in the therapeutic armamentarium. A variety of microorganisms have been investigated with variable degrees of success. Bacteria as agents for cancer therapy are enjoying a renaissance, a topic exhaustively reviewed by Felgner et al.[58] Similarly, different viruses have long been known to augment cytotoxicity against cancer cell lines[59-62] but, again their efficacy and safety in humans remain to be established. A general review of the topic[63] and a more specific one dealing with virotherapy for urological cancers have been recently published.[64]

A significant recent advance in bladder cancer immunotherapy taking a different tack is represented by atezolizumab, a fully humanized, engineered monoclonal antibody of IgG1 isotype against the protein programmed cell death-ligand (PD-L1) that reduces activation of cytotoxic T cells. By blocking this inhibitory effect it significantly enhances an anti-tumor response and preliminary clinical results are very promising.[65] Allogeneic vaccines have also been proposed for high grade NMIBC with vesigenurtacel recently receiving fast track designation.[66] These novel approaches represent innovative and auspicious developmental paths for immunotherapy of bladder cancer. We're a long, long way from the baby steps of the post-World War II era.

Conclusion

2016 marked 4 decades since BCG was reported to be effective in the prevention of recurrence and the treatment of NMIBC. Although it still maintains its pre-eminent position in the armamentarium, new immune modulators are emerging with the promise of superior efficacy and safety. Immunotherapy for cancer has reached an important place in urological oncology and it is already offering significant results. New agents have reached FDA approval and conclusive results on their efficacy and safety are forthcoming. □

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