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Induction of controlled prostatic tissue necrosis by bacille Calmette-Guérin derivatives*

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Summary. Intraprostatic administration of live bacille Calmette-Guérin (BCG) in humans has been found to produce tumor necrosis; unfortunately, the number of severity of complications have made its clinical use prohibitive. Previous studies have shown that soluble and microparticulate components present in the supernatants obtained after centrifugation of a reconstituted BCG preparation exhibit similar immunogenicity to the one shown by live bacteria. The supernatants, however, are not associated with disseminated infection of the progressive regional tissue destruction observed with the use of viable vaccine.

Experiments were conducted to determine the effect of intraprostatic injection of BCG and its supernatants. Adult dogs, after positive conversion to protein purified derivative (PPD), were randomly assigned to three groups. Under direct vision and with digital rectal control, intraprostatic injections of various agents were given as follows: group I, normal saline; group II, live BCG; group III, 200 μg of BCG supernatants. Two months later the animals were sacrificed, and the prostates removed in toto and submitted for a thorough histological examination. Extensive but variable tissue necrosis was noted in groups II and III. No histological alterations were present in group I. The histological picture of the animals receiving BCG supernatants conclusively demonstrated circumscribed necrosis of the gland. Side effects and complications were present in animals receiving live BCG but conspicuously absent in the ones receiving supernatants. The observed effectiveness and safety of BCG supernatants for intraprostatic administration in an experimental system may lead to a simple, safe, and efficacious therapeutic modality for localized carcinoma of the prostate in humans.

Various groups have employed bacille Calmette-Guérin (BCG) for the treatment of adenocarcinoma of the prostate in humans. Robinson et al. [10] administered live BCG directly into the primary prostatic tumor of 14 patients using a transrectal approach. They documented "macrophage granulomata in the prostate with local destruction of tumor cells" 1 week after treatment. Unfortunately, there were severe complications in most of the cases. These patients, however, were preterminal and resistant to other therapies (6 patients) or had very advanced disease and were receiving a variety of concomitant treatments. Furthermore, these investigators employed the Glaxo BCG which has been recognized as a strain of low immunogenicity and associated with an inordinate number and severity of adverse reactions [2]. Merrin et al. [4] also employed intraprostatic injection of live BCG (Connaught strain) in two patients: No effect was noted in one while the second exhibited necrosis of the primary tumor as well as the soft tissue metastases receiving the vaccine; untreated lesions did not show histological changes. Again, very significant adverse effects were noted (i.e., lung granulomata, prostatorectal fistula). Taken as a whole, these and other similar small studies on patients with cancer of the prostate suggest that live intratumoral BCG produces local tumor destruction, but the severity and number of complications preclude its widespread clinical use. It is evident that the administration of killed BCG or its antigenic components should prevent the self-perpetuating and, consequently, more severe complications of live vaccine. Such an alternative has not been tried in prostatic cancer, but it has produced only limited success in other tumor systems [12].

Extensive and detailed studies by Mackaness and his group [2,5] clearly established the parameters conditioning the immunogenicity of BCG. Thanks to their work we know which preparations exhibit the best antitumor activity. An exceedingly important but largely forgotten observation made by them was that soluble and microparticulate components which are present in the supernatants after centrifugation of a reconstituted vaccine exhibit immunogenicity similar to the one shown by live bacteria

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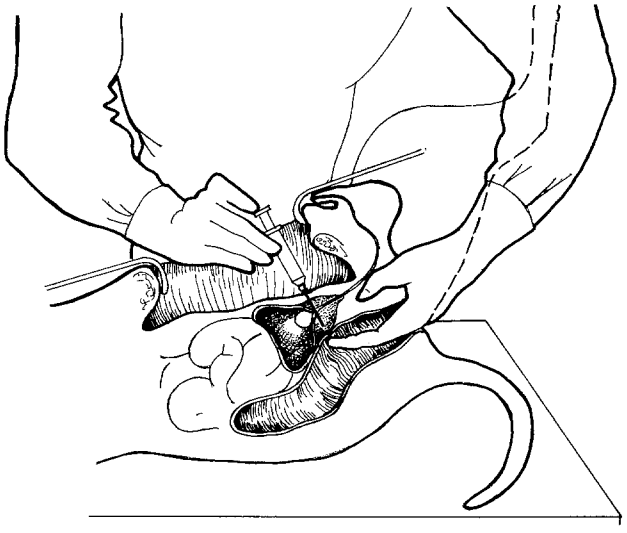


Fig. 1. Technique for the intraprostatic administration of BCG supernatants. The procedure is based on the one employed for prostatic brachytherapy

of the best strains. The relevant point is that these supernatants contain no live bacteria, and their local administration, therefore, should not be associated with disseminated infection or the progressive regional tissue destruction observed with the live vaccine. We have tested this hypothesis by injecting live BCG or its microparticulate material from supernatants into the prostate glands of dogs.

Materials and methods

Animals

Ten adult mongrel dogs (over 5 years of age) were tested with purified protein derivative (PPD) by the footpad technique and were found to be PPD negative. They then received 2 weekly subdermal injections of BCG in the back of the neck and were successfully converted to PPD positive. They were then randomly divided into three groups. Under general anesthesia a laparotomy was performed, and the prostate gland was dissected. Under direct vision and with digital rectal control (Fig. 1) using a technique similar to the one employed for implantation of I^{125} seeds, intraprostatic injections of various agents were given as follows: group I (2 dogs), normal saline; group II (4 dogs), live BCG 40 mg (2.8×10^7 organisms) per cc; group III (4 dogs), BCG supernatants 200 μg/cc. The animals were examined daily. Two months after surgery they were sacrificed, a laparotomy done, the pelvis explored, and the prostate glands removed in toto and submitted for serial histological examination. The pathologist (P.N.M.) was blinded as to the treatment given to the specimens under examination.

Vaccine

The BCG vaccine was obtained from the Institute Armand Frappier (Montreal) in lyophilized form and reconstituted in normal saline.

Supernatants

Reconstituted BCG from several 120 mg ampules was placed in 50 ml plastic centrifuge tubes and spun at 40,000 *g* for 30 min. The cell pellet was discarded and the supernatants pooled and spun again at 40,000 *g* for 10 min. The protein concentration of the supernatants was measured by the method of Lowry [3]. Sterility of the final preparations was tested by culture in blood agar plates. Stains and cultures [11] for *Mycobacteria* were done to rule out the presence of viable organisms in the final specimen.

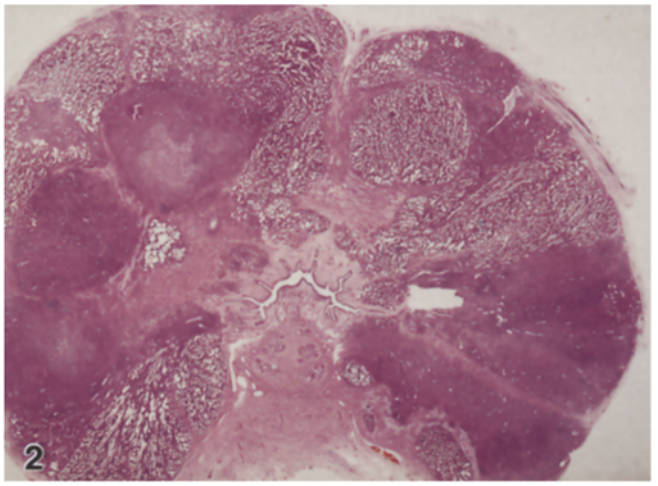
Results

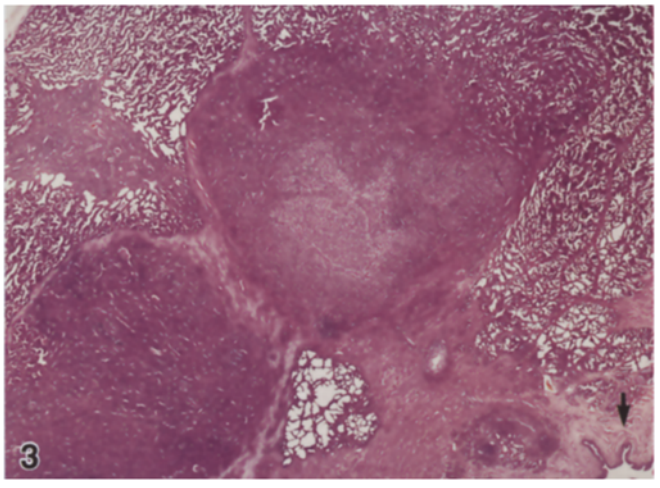
Adverse effects were noted only in two of the animals receiving live BCG (group II). Both dogs developed ulcers at the site of previous BCG immunizations in the neck. In addition, one of these animals was sick and manifested a loss of appetite for 3 days after surgery.

The removal of the prostate 2 months after treatment was accomplished without difficulty as there was little periprostatic scarring or induration. The gross appearance of the prostate glands was similar in all three groups. The histological appearance, however, was strikingly different in the control group as compared with the treatment groups. In group I the glandular pattern was normal. In the treatment groups, on the other hand, an extensive acute and chronic inflammatory reaction with necrosis of prostatic tissue was evident around the sites of injection (Figs. 2–4). The distribution of the tissue necrosis correlated well with the injection sites and needle tracks. However, the inflammatory reaction and tissue destruction lacked homogenicity, leaving "cold" areas reflecting the rather crude technique employed for the intraprostatic administration of the vaccine.

Discussion

Several observations became apparent during this study. Firstly, intraprostatic administration of liquid agents is indeed a simple procedure, not much different from the clinical techniques of prostatic brachytherapy [1, 13]. Secondly, previously immunized dogs tolerate well the intraprostatic administration of live BCG or its supernatants. Thirdly, the local response to these two agents is quite similar and characterized by an intense inflammatory reaction and tissue destruction within the prostatic capsule. Fourthly, extreme care must be used to ensure homogeneous distribution of the vaccine as to avoid "misses": Our initial belief that the fluid will be distributed throughout the interstitia was wrong since there is compartmentalization of the gland. In this regard, it should be noted that compartmentalization of the prostate is less marked in humans and, frequently, not evident in the presence of prostatic cancer. It must be emphasized that the experiments described here were carried out in prostate glands of mature dogs without evidence of cancer. The distribution of the vaccine in glands harboring cancer or in transplanted experimental prostatic tumors remains to be determined. Fifthly, the inflammatory reaction and tissue destruction appear to be largely contained by the





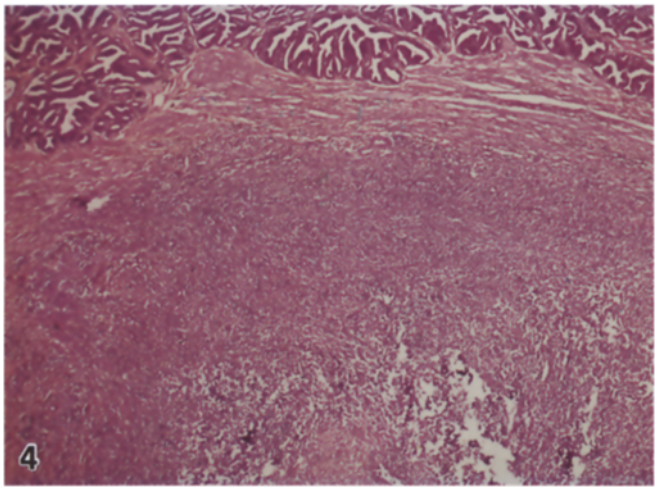


Fig. 2. Complete section of a prostate gland 2 months after administration of BCG supernatants. There are well-defined areas of necrosis. The distribution of tissue damage, however, is not homogeneous

Fig. 3. Microphotograph demonstrating the area of necrosis produced by the supernatants which extends to but does not penetrate a septum which separates this area from normal glandular tissue. The urethra (*arrow*) is intact

Fig. 4. Higher magnification microphotograph showing the septum separating the area of necrosis from the "cold spot" of normal prostatic tissue

prostatic capsule. In none of the animals was there evidence of significant periprostatic or urethral damage, and none of them developed urinary retention. Finally, there was no significant difference in the intensity of the inflammatory reaction or the degree of tissue destruction when the live vaccine and its derivatives are compared. These observations support previous clinical work demonstrating that intraprostatically administered live BCG induces necrosis of a primary tumor [4, 10]; of particular relevance, however, is the finding that the microparticulate material obtained from BCG supernatants induces a similar local response but does not appear to be associated with the severe and unpredictable local effects reportedly induced by viable organisms.

The nature of the supernatants has not been established. It is doubtful that they represent cell skeletons because these were not observed on microscopic examination. Cell wall fragments could be the principal component. The lack of activity of heat-inactivated BCG reported in previous studies [9] has induced us to explore other possibilities. We have reported [7] that live BCG administered to humans produces an exopolysaccharide glycocalyx capsule which provides the most intimate contact between the bacteria and the host's immune system. Although still unproven, it appears reasonable to postulate that this exopolysaccharide mediates the antigenic and the inflammatory reactions and ensuing tissue damage observed in these experiments [6]. More recently, we have developed an extract or derivative of growing BCG cells that appears to be more immunogenic than a simple supernatant solution of reconstituted lyophylized BCG. The derivative evokes the same immunogenic response as BCG when the two compounds are instilled in the bladder of rabbits [8].

We are presently testing the effectiveness of this derivative as well as of other BCG extracts in an experimental prostatic cancer model (the Dunning R3327 H tumor). If the initial observations are confirmed, the use of these derivatives of the vaccine may have an application in the treatment of localized carcinoma of the prostate. Although still theoretical, transrectal, ultrasound-guided injection of chemotherapeutic and/or immunological compounds directly into a prostatic tumor nodule is an exciting possibility, and a BCG derivative may prove to be a very appropriate agent.

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