DEVELOPMENT OF AN AUTO-VACCINE + BCG and its possible application in cancer treatments

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The scientific research project developed by our team is centered on developing and restructuring an autovaccine as a supplementary resource for the treatment of cancer, to be added to the other procedures currently in use (surgery, chemotherapy, radiotherapy, radium therapy).

As an overview, we shall refer to our team’s experience of more than 30 years in the diffuse cutaneous leishmaniasis (DCL) disease that we presented to the XI International Dermatology Conference in Stockholm, Sweden in 1957, for which we were awarded the “National Scientific Research Award” on January 23, 1960. We have selected this disease as a comparative model with cancer (1).

The DCL that was then described corresponded to isolated cases but has increased to more than 60 documented cases, the majority involving children and starting in adults currently affected in their early childhood.

The studies made during their treatment were discouraging due to the relapses after the treatment. We used anfo-B pentavalent antimonials and more recently, miltefosine in these studies.

Additionally, we used inmunotherapy with the vaccine made up of pasteurized promastigotes and amastigotes like forms without any results.

A few years ago, still under the effect of the failures resulting from using drugs that were available then, we tried to develop an autovaccine whose results were successful in three adults with CDL, but the observation was very irregular due to the frequent absences of the patients, which prevented us from determining the success that we later observed and analyzed in the light of its importance.

For several years we studied the possible development mechanisms of DCL, as well as the influence of the components of the autovaccine mentioned above, which has been very useful in the last year of our work.

We defined DCL as a specific immunodeficiency antigen. From these studies we concluded that the disease was caused by a metabolic defect of the macrophagic cell, which allows for an exaggerated multiplication of the leishmania parasite within the cell. But the fundamental fact that we wish to emphasize derived from the study of the macrophage of the BALB/c - comparable to DCL - is that the problem lies in excessive enzyme, arginase, whose action on the arginine with formation of urea and ornitine favors the parasite’s multiplying.

We studied this fact in the DCL (2) which allowed us to determine that this disease is caused by a metabolic defect of the macrophage which in turn causes an exaggerated multiplication of the parasite as a secondary effect of the macrophagic defect.
As a consequence of these results, and also based on the clinical record, histopathology, immunology and evolution of this disease and the way it spreads in the patient via lymphatic system as well as the blood vessels, we can consider the DCL macrophage as a cell with tumor characteristics that breaks away from the rest of the organism’s cellular elements, possibly due to the influence of genetic, environmental and other factors.

Regarding the development of the autovaccine composed in the DCL mainly of macrophagic cells and other nominal elements of the lesions caused by this disease together with Calmette-Guerin bacilli (BCG), we renewed its application in DCL cases (3-5).

Among these cases, due to the significance of the response obtained and the permanence to this day of the results, is the treatment of L.R., an 11-year old boy whose disease had not responded to the application of any of the drugs used until now. With the consent of his parents, we administered a dose of autovaccine composed of cells and material extracted from the arm, with abundant parasites and mixed with BCG, to which we added a suspension of amastigotes like.

The results of L.R.’s treatment are extraordinary and have remained constant for more than four months to this day, which is why we wish to emphasize certain highlights of the treatment.

After 30 days of having received the autovaccine dose, we noted that all the lesions reappeared in the form of scars, that the parasites had disappeared and a particularly important development: the positivization of the test to leishmanine (Montenegro reaction) which had always been negative.

We have applied the use of BCG as an adjuvant as well as in the autovaccine based on our experience and the bibliography consulted.

There is a vast amount of literature on the use of BCG in the immunotherapy of various tumors. It is not our purpose to review it here, but two studies are worth mentioning, one due to the great number of patients assessed and the other due to its connection with the current proposed study.

Han and Pan (6) have reviewed 176 tests of the use of BCG in treating superficial bladder cancer after the transurethral resection, finding that it is effective in preventing relapses. A combination of chemotherapy + BCG proved no better than BCG alone. For this meta-analysis, 25 tests with information on the recurrence in 4,767 patients were selected. The results regarding solid tumors were not so conclusive and are less encouraging. However, in a small group, 31% of the patients with inflammatory breast cancer treated with chemotherapy, surgery and vaccination composed of allogenic tumor cells + BCG were reported to have survived more than 10 years, although the role of the vaccine therapy was not precisely defined.

Evidently, the ethical aspects of the use of BCG have been taken into account in these studies. Despite isolated reports of adverse effects in individual cases, a minimum percentage of patients has shown adverse reactions related to the immunotherapy with BCG and its use in tumor patients; apparently it would not be contraindicated except in extremely severe cases of generalized immunosuppression. Probably, the presence of reactivity to the tuberculine could be sufficient proof to evaluate the immune competence.

Our experience with a vaccine as immunotherapy for localized cutaneous leishmaniasis (LCL) composed of pasteurized promastigotes + BCG, developed in the Biomedicine Institute and administered for more than 15
years, showed us the absence of secondary phenomena and favorable healing outcomes in more than 95% (7-9) of the cases.

It should be kept in mind that the BCG has been used by the World Health Organization (WHO) in more than 200 million people without significant side effects.

There are several studies on the immunological mechanisms involved in the response to BCG, in infectious diseases as well as tumors. According to one study, the phagocytosis of the BCG results in the release of pro-inflammatory cytokines (IK -1.6 and 8). Then a Th1 reaction develops mediated by CD4+ lymphocytes, with the release of IL-2 e IFNγ. Finally, some populations of cytotoxic cells capable of killing CD8+ lymphocytes-tumor cells, macrophages, natural killers (NK), lymphokine activated killer (LAK) and BCG activated killer, are amplified.

There is also a macrophagic activation by the IL-2-e-IFN lymphokines that probably plays an important role in the destruction of intracellular parasites through the synthesis of nitric oxide and other free radicals.

The possibility that the induction of apoptosis by BCG could play a role in its anti-tumor and anti-parasite activity has recently been raised.

Thus, BCG activates multiple mechanisms; some are active on an intracellular level (cellular infections) and others exert a cytolytic activity against cells with abnormal antigens on their surface (tumor cells and probably cells infected with microorganisms that convey new antigens on their surface).

From this review we could perhaps draw several preliminary conclusions:

- The activity of the BCG in diseases such as leishmaniasis in its diverse forms is related to the anti-tumor activity of the BCG, featuring a diversity of immunological mechanisms. Therefore, it seems valid to extrapolate the observations in the treatment of leishmaniasis, particularly in its diffuse cutaneous form, to the treatment of some tumors.

- It appears that the ethical issues associated with the use of BCG in human patients have been settled in view of the significantly high number of published studies (10-18) featuring a minimum frequency of negative side effects.

Evidently, the studies would complement the usual practices with chemotherapy, surgery or prior radiation to reduce the load of abnormal cells and later treatment in accordance with the practices in use.

For example, if an auto-vaccine + BCG is introduced in breast cancer treatment, it would be important to determine in which phase the vaccine would fit in the sequence of use of the other forms of treatment in different types of patients. For example, in the case of multiple lymph node metastasis, the use of the autovaccine could follow surgery.

An attempt to explain the action mechanism of an autovaccine + BCG, in DCL as well as in cancer, could be useful in the analysis of both comparative models.
In DCL, the fact that in 30 days all the lesions had reappeared, as in the case of L.R., the possible action mechanism would be as follows: death by apoptosis of the specific immunodeficient cells, taking into account the total lack of response of cellular necrosis, which we haven’t observed in the use of the BCG + *M. leprae* in leprosy immunotherapy where we observed reactional responses, some very active.

This comparison allows us to determine that the mechanism of cellular death by apoptosis lacks reactional phenomena and only presents a very discreet two-day rise of temperature.

In the DCL, in addition to apoptosis, a Th1 type response would develop expressed in the patient by a shift to the positivity of the Montenegro reaction (leishmanine).

The fact that the apoptosis phenomenon occurs combined with a Th1 response and that the simultaneous occurrence of the two phenomena does not cause a secondary effects response in the macroscopic host, shows that the autovaccine + BCG is a harmless, but deeply effective therapeutic resource in correcting the problem of immunodeficiency observed in some diseases.

Regarding cancer, the autovaccine composed of the patient’s tumor cells + BCG could unleash a mechanism similar to the one proposed for DCL, namely a tumor cell apoptosis phenomenon, therefore without negative side effects, to which a type Th1 response could be added as mentioned hereinabove.

The autovaccine + BCG was administered in a few cases of advanced cancer without observing reactional phenomena even though more than one dose of the autovaccine was administered. This was performed as an ethically approved attempt, but due to the advanced state of the cases in which the tests were performed, it only allowed for partial observation to determine the lack of reactional phenomena.

**Autovaccine + BCG in cancer treatment research projects.**

The proposal is to carry out a comparative study, which shall be blind on the part of the observer, to evaluate the efficiency of the autovaccine + BCG in cancer treatments, using patients from the Vargas and Razetti hospitals in Caracas.

To minimize the size of the study and facilitate the comparison, it was proposed that they be paired in groups by age, gender, location and type of primary tumor, the presence or absence of detectable metastasis and the application of antineoplastic treatment.
The patients who are recipients or not of the autovaccine will be randomly distributed within each pair.

The requirements for inclusion are:

- Patients diagnosed with cancer in any stage, preferably in intermediate or advanced stages.
- Patients over the age of 20 and under 75.
- Agreement to participate voluntarily in the study and sign the informed consent form. The text of the informed consent is omitted because of space limitations. The readers who are interested may request the form by e-mail to: jconvit@telcel.net.ve.
- Commitment to receiving the antineoplastic treatment prescribed and in the prescribed mode.

Exclusion factors:

- Patients with cancer which have proved to have a viral etiology (i.e. cervical cancer)
- Immunodeficiency.

Size of the study: the initial size could involve 30 patients in each group. In the event encouraging results are obtained, the size will be increased in order to analyze the benefits obtained by subgroups (type of cancer, location, gender, age, etc.)

Application of the autovaccine.

The autovaccine shall be prepared from a fragment of the patient’s tumor, either primary or a metastasis, according to the following procedure:

The sample must be taken applying rigorous asepsia.

The tumor fragment received is started in a laminar flow hood to preserve sterility.

It is then irrigated in normal saline with penicillin-streptomycin.

The sample is placed in a homogenizer with 1mL of normal saline without an antibiotic.

It is homogenized in 10 steps, passing through the grid and samples are taken for:

a) microbiological study
b) staining using hematoxylin and eosin
c) mix with BCG

This product shall be mixed with BCG in doses according to the results of the PPD of the patient, and pursuant to the following scheme:

0 to 9mm - 0.0175 PPD
20 to 29mm - 0.005 PPD
PPD > 30 mm - 0.0025

Up to seven doses shall be administered at an interval of four to eight weeks between doses.

The control group shall receive normal saline only.

Patient follow-up

All the patients must be subjected to a continuous follow-up and weekly control for at least two years from the start of the treatment.

The changes in the tumor (remissions and survival) as well as the emergence or remission of metastatic lesions shall be assessed.

Analysis

Comparison of proportions of change of each of the (study and control) groups
Determine whether there are differences in the survival time measured by survival analysis.
If required, analyze by controlling associated variables.

REFERENCES


