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Analysis of the effect of intravesical treatment with interferon-α2b on the clinical evolution and on the *in vivo* function of T lymphocytes and natural killer cells in patients with superficial bladder tumors

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Following complete transurethral resection, patients with superficial transitional cell carcinoma (TCC) were treated with intravesical interferon-α2b. *Ex vivo* analysis of peripheral blood mononuclear cells (PBMNC) both during and after the treatment period showed an enhancement of natural killer (NK) activity which fell to basal levels upon cessation of therapy. Simultaneous analysis of T-cell activity revealed a similar pattern of variation in the response to the polyclonal mitogen phytohemagglutinin. Both findings point to an alteration in systemic immunoreactivity as a result of intravesical treatment. Further work will be aimed at determining whether this is relevant to the therapeutic value of such treatment and whether prolonged treatment will maintain these effects.

Key words: Bladder cancer, intravesical therapy, interferon-α2b, natural killer cells, T lymphocytes, immunoreactivity

Introduction

The tumoral transformation of a cell is a complex process that involves several genetic, biochemical and metabolic factors. The local growth and systemic dissemination of the tumor is a similarly intricate process, based on the intrinsic characteristics of the tumor cells and on their interaction with the host's immunologic mechanisms.[1]

There is increasing evidence that the immune system plays an important role in surveillance against the growth and dissemination of neoplasias[2–4] and while several immune cells are involved in this protective activity, the function of T lymphocytes and natural killer (NK) cells appears to be crucial. T lymphocytes have two major subsets: T helper (T_H) cells, which regulate the proliferation and maturation of other immune cells, such as B cells and NK cells, and cytotoxic T cells (T_C). NK cells are large granular lymphocytes that can lyse tumoral cells in a non-major histocompatibility (MHC)-restricted fashion,[5,6] while lysis mediated by T_C cells is MHC-restricted.

The activation, proliferation and maturation of such T lymphocytes and NK cells are mainly regulated by different cytokines, amongst which interferon (IFN)-α plays an important role.

In patients with transitional cell carcinoma (TCC) of the bladder, several alterations in the immune system have been reported,[8,9] although we have found normal NK activity in peripheral blood mononuclear cells (PBMNC) from patients with superficial TCC of the bladder. However, the recurrence and progression of the disease are associated with a functional impairment of this effector population. In patients with infiltrative, local or metastatic disease, the lytic activity of the quantitatively normal NK cells present in peripheral blood is very low or absent.[10] There is also evidence that T lymphocytes present a similar clinical pattern of alterations in patients with TCC of the bladder.[11]

The aim of immunotherapy of cancer patients at present is to increase the activity of the specific and nonspecific effector mechanisms of the immune system directed against the neoplasia,[12] and in this respect it has been shown that intravesical treatment with IFN-α2b and other immunomodulators is effective in the prevention of recurrence of superficial bladder TCC (T_a, T_1 and T_{is}).[13–18]

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We are involved in a prospective clinical and immunologic study of intracavitary IFN-α2b instillation in patients with superficial TCC of the bladder in order to assess its biologic basis and clinical effectiveness, with the complementary aim of achieving the prospective biologic identification of responder patients through sequential analysis during a 1 year follow up. To this end, the *ex vivo* function of NK cells and T lymphocytes of the IFN-α2b-treated patients has been studied.

Materials and methods

Up to April 1991, 37 randomized patients with superficial TCC of the bladder (stages T_1 and T_{is} and grade II–III) (UICC) have undergone intravesical treatment, after complete transurethral resection of all visible tumors. Twenty received 30 mg mitomycin C (MCC) dissolved in 30 ml of sterile water, and the other 17 received 50 MU of IFN-α2b dissolved in 50 ml of saline. The two groups of patients were comparable and the treatment regimen was carried out for 12 weeks with no maintenance treatment being used. PBMNC of the patients were obtained sequentially during treatment and 6, 9 and 12 months after terminating the intravesical installations. The mean follow up was 12.5 months (5–18 months) in the IFN-α2b group and 13.2 months (5–18 months) in the MCC group, since only patients with a follow up of more than 4 months' duration were considered for evaluation.

Results

In the 11 patients from the IFN-α2b group selected for this study, we found normal NK activity in PBMNC under basal conditions, prior to initiating intracavitary instillation of IFN-α2b.

The different *ex vivo* analyses of the NK activity of PBMNC performed during the 3 months of intracavitary treatment with IFN-α2b disclosed an enhancement of cytotoxicity. Thereafter, a progressive decrease in the NK cytotoxic activity was observed in the PBMNC of these patients, reaching levels similar to those found prior to beginning the instillation (Figure 1).

It is interesting to note that in one patient, in the eighth month of follow up, we did not observe the aforementioned enhancement of NK activity of PBMNC, but rather a marked diminution with respect to basal levels (Figure 2).

Simultaneously, we studied the proliferative response of T lymphocytes in PBMNC from these patients to the polyclonal mitogenic signal of a plant lectin (phytohemagglutinin), finding a pattern of variation in response to the mitogen similar to that found in the lytic activities of NK cells (Figure 3).

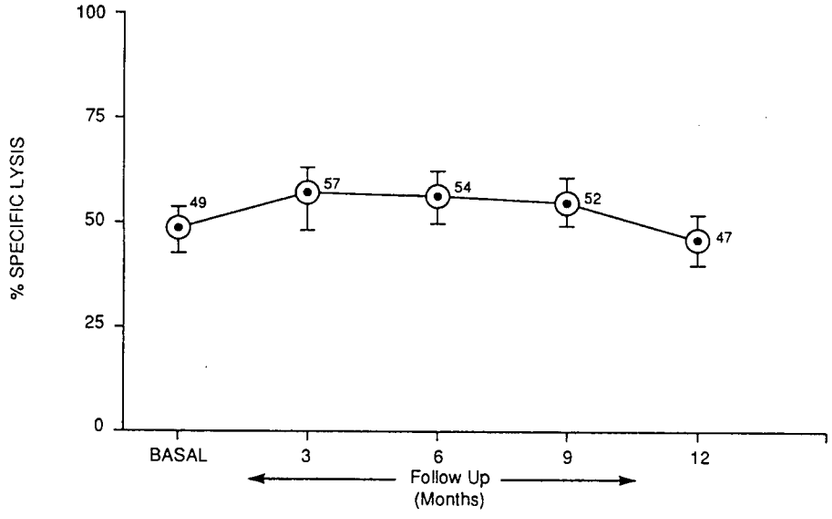

Figure 1. PBMNC from 11 patients with superficial TCC of the bladder, treated with complete transurethral resection of all visible tumors and intracavitary instillation with IFN-α2b, were obtained at different times during follow up. These PBMNC were used as effectors against ^{51}Cr-labeled K562 target cells in standard cytotoxic assays. Results represent the means of the different triplicate cytotoxic assays performed in each patient at a 50:1 effector-to-target cell ratio at the indicated times.

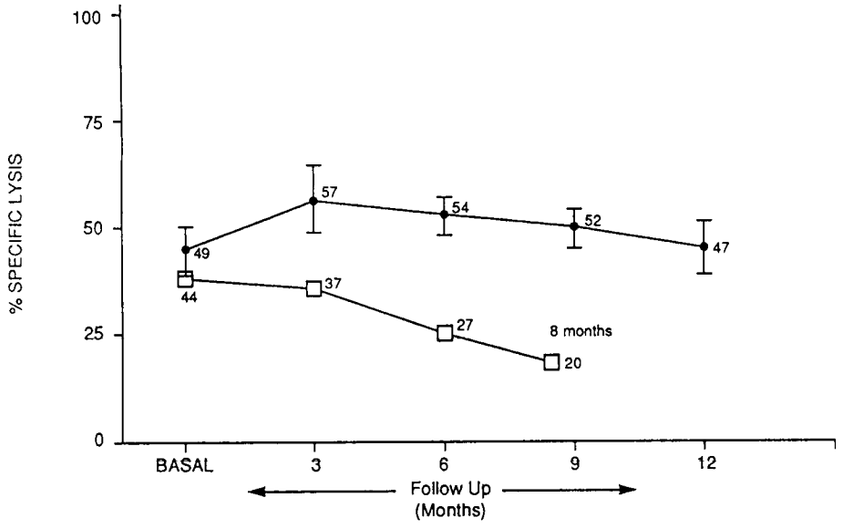

Figure 2. PBMNC from one patient with superficial TCC of the bladder, treated with complete transurethral resection of all visible tumors and intracavitary instillation with IFN-α2b and showing evidence of recurrence 8 months after treatment, were obtained at different times during follow up. PBMNC were used as effectors against ^{51}Cr-labeled K562 target cells in standard cytotoxic assays. Results represent the mean of the different triplicate cytotoxic assays performed at a 50:1 effector-to-target cell ratio at the indicated times.

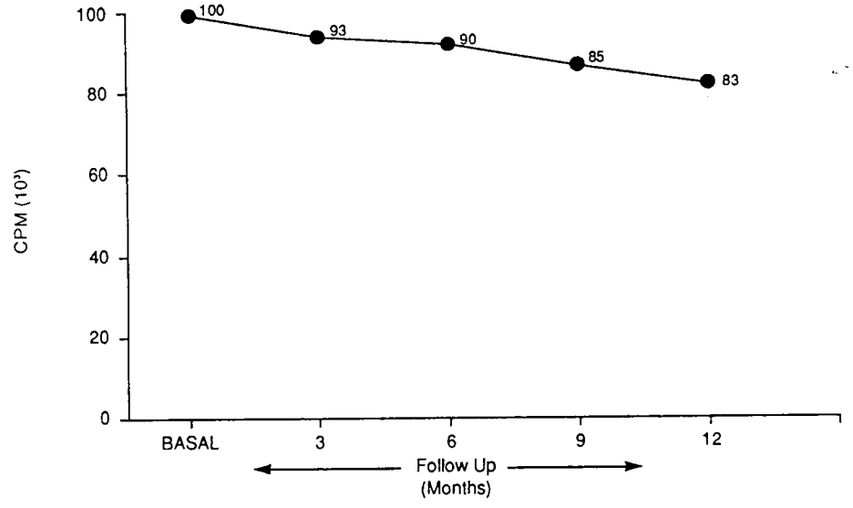

Figure 3. PBMNC from 11 patients with superficial TCC of the bladder, treated with complete transurethral resection of all visible tumors and intracavitary instillation with IFN-α2b, were obtained at different times during follow up. PBMNC (50 000 cells/well) were incubated in complete medium supplemented with 1% phytohemagglutinin for 5 days. The proliferative response of the lymphocytes was measured by the incorporation of pulsed [^{3}H]thymidine into the DNA. Results represent the mean counts per minute of the different triplicate assays performed in each patient at the indicated times.

Discussion

Although the results presented here can be considered to be preliminary, several comments should be made. Our immunologic data clearly demonstrate that therapy with intravesical IFN-α2b instillation is not only associated with a local immune response restricted to the bladder wall, but with a systemic effect in the immune system as well. These functional results might be attributed to the continuous recirculation of peripheral lymphocytes or to the complementary interaction among the different lymphocyte subsets.

It might be suggested that immunostimulation induced by IFN-α2b instillation could be involved in the clinical effectiveness of this therapy. In this respect, it is relevant that the patients with recurrence or progression of disease during the follow-up period are linked to the aforementioned immunologic effects of IFN-α2b.

In future studies, it may be possible to define the prognostic value of variations in these immunologic parameters in the intravesical IFN-α2b treatment of patients with TCC of the bladder. It is also necessary to establish whether prolonged intracavitary treatment with IFN-α2b is associated with a maintenance of the immune effects observed here, as well as with an enhancement of the clinical effectiveness of this therapeutic modality.

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