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Immunomodulatory effect of interferon-α2b on natural killer cells and T lymphocytes from patients with transitional cell carcinoma of the bladder

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Incubation of peripheral blood mononuclear cells (PBMNC) from patients with transitional cell carcinoma of the bladder, with interferon (IFN-α2b) causes dose-dependent enhancement of natural killer (NK) and cytotoxic activity. Increased lytic activity was also observed against both NK-sensitive and NK-resistant target cells and T lymphocytes from such patients showed altered activity upon incubation with IFN-α2b. Furthermore, intracavitary instillation of IFN-α2b is associated with infiltration of the bladder wall by NK cells and T lymphocytes. Future work will be aimed at investigating the possible prognostic value of these results as related to the therapeutic effect of intracavitary instillation of IFN-α2b.

Key words: Bladder cancer, cytotoxicity, interferon-α2b, mononuclear cells, natural killer cells, T lymphocytes

Introduction

Interferon (IFN) was first described in 1957 by Isaacs and Lindenmann, who were working at that time on the phenomenon of viral interference.[1] They discovered that a protein induced by virus infection inhibited the growth of another virus. They called this factor 'interferon'. Although the IFNs have extensive biologic activity, for many years they were considered to be the special province of virologists. A considerable period of time elapsed before the effects on the immune system and regulation of cell growth were studied intensively. At present, the IFNs are also used clinically as antitumor agents.[2,3]

Initially it was thought that a single type of IFN existed in each animal species, but subsequent investigation showed conclusively that numerous types of IFN may exist in a given species. The initial classification of an IFN was based on the cells that produced it. Presently, IFNs are typed on the basis of the primary structure of their genes, although the immunologically-defined terms—α, β, and γ—have been maintained.[4,5]

Alpha IFNs α-IFNs are produced by macrophages, lymphoblastoid cells and fibroblasts. Their genes have been cloned and α-IFNs are divided into two general classes. The 20 different human α-IFNs genes described are all located in a cluster on chromosome g. The human class I α-IFNs consist of 165–166 amino acids, whereas those of class II have 172 amino acids. Disulfide bonds exist between amino acids 1 and 98 and between 29 and 138. Only the latter is necessary for biologic activity.[6,7]

In order to be biologically active, α-IFNs have to bind to cell surface receptors. The gene for this receptor is on chromosome 21. After α-IFNs bind to their receptor, the complex is internalized and degraded. Limited information is available about the steps that occur between α-IFNs binding and the regulation of specific genes that cause their biologic effects.

It has been clearly shown that α-IFNs have a marked antiviral effect, but they are also capable of inhibiting the growth of both normal and transformed cells. α-IFNs reverse the mitogenic actions of different growth-stimulatory factors and regulate the expression of genes associated with neoplastic transformation.[8–10]

Regulatory effects of α-IFNs on different cells of the immune system have also been shown.[11] α-IFNs can modulate the activation and proliferation of macrophages, natural killer (NK) cells, and T and

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B lymphocytes.[12,13] There is increasing evidence that the immune system is involved in defense against the growth and dissemination of tumor cells.[14,15] NK cells and T lymphocytes play a pivotal role in the immunologic response to neoplastic cells.[16–19] NK cells are large granular lymphocytes than can spontaneously lyse tumor cells without prior sensitization in a non-major histocompatibility-restricted fashion.[20] NK cell cytotoxic activity is enhanced by different cytokines including α-IFNs and interleukin 2.[21] Cytotoxic T lymphocytes exert their lytic activity against the target cells in a major histocompatibility-restricted fashion. The lymphokines produced by helper T lymphocytes regulate the activation of effector cells such as NK cells, cytotoxic T lymphocytes and macrophages. The cytokines can also modulate the differentiation of B lymphocytes to antibody secreting plasma cells.[22] The deficiencies in the recognition and/or response of the immune cells to tumoral cells appear to be implicated in the local growth and systemic dissemination of the neoplasia.

Transitional cell carcinoma (TCC) of the bladder is associated with several phenotypical and functional alterations in the immune system. We have demonstrated that in patients with bladder carcinoma, there is a negative correlation between the levels of NK activity in peripheral blood mononuclear cells (PBMNC) and the clinical evolution and pathologic stages of the disease.[18]

The optimal treatment of patients with superficial TCC of the bladder has not been fully established. The marked tendency of these tumors to recur after surgical resection has been partially obviated by the adjuvant intracavitary use of cytostatic agents and immunomodulators. There is increasing evidence that the intracavitary use of α-IFNs may be an efficient therapeutic approach in these patients.[23,24]

As mentioned above, the mechanism of action by which α-IFNs prevent the recurrence of the TCC tumors appears to be complex. A direct growth-inhibiting effect of IFN-α on the tumor cells may be involved in this therapeutic effect. The immunomodulatory effect of IFN-α also appears to be implicated in the clinical results found in patients with superficial TCC of the bladder. In this respect, we have found that IFN-α2b can significantly enhance ($p < 0.05$), in a dose-dependent fashion, the NK activity of PBMNC from patients with superficial TCC of the bladder after 18 h of *in vitro* incubation (Figure 1).[25] It is also relevant that the IFN-α2b-incubated PBMNC from this patient population have increased cytotoxic activity when compared with that found in the absence of the cyto-

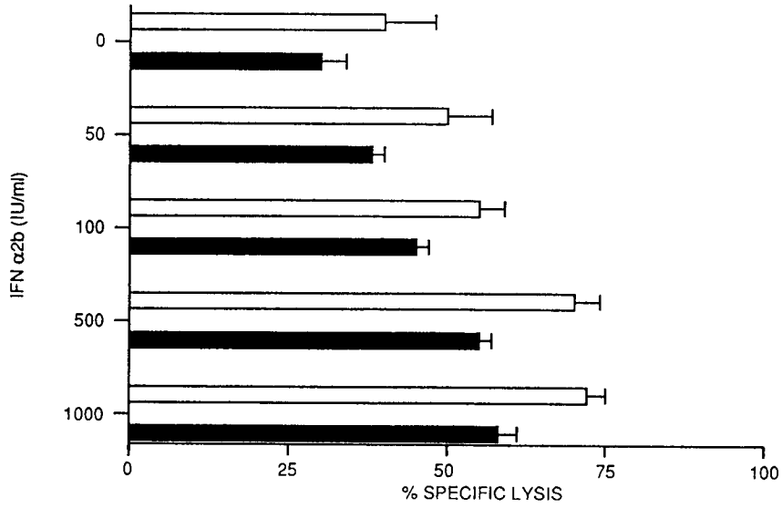

Figure 1. IFN-α2b increases the NK cytotoxic activity of PBMNC from patients with superficial TCC of the bladder. PBMNC from eight patients with superficial TCC of the bladder were incubated in complete medium in the presence or absence of different concentrations of IFN-α2b for 18 h. These cultured cells were used as effectors against ^{51}Cr-labeled K562 target cells. Results represent the mean plus the standard deviation of the specific lysis of the different triplicate cytotoxic assays performed in each patient at 50:1 (□) and 25:1 (■) effector-to-target cell ratios.

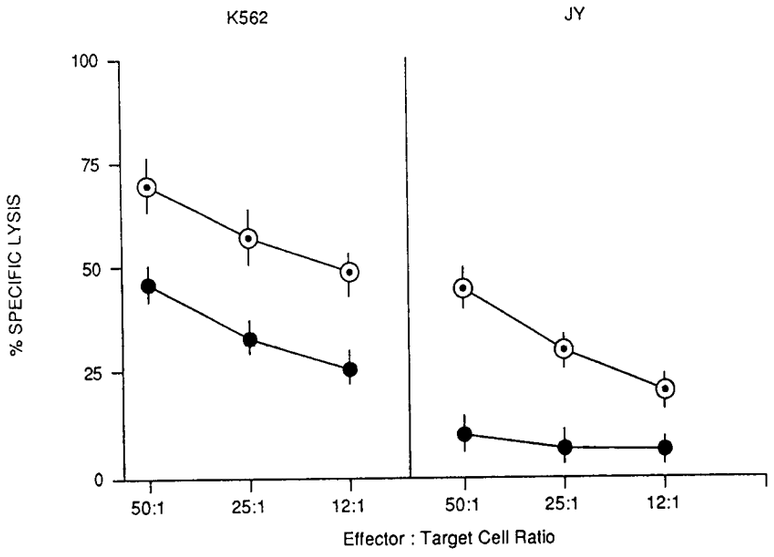

Figure 2. The IFN-α2b-induced cytotoxic activity in PBMNC from patients with superficial TCC of the bladder is directed against NK-sensitive and NK-resistant target cells. PBMNC from five patients with superficial TCC of the bladder were radiolabeled in complete medium in the presence (○) or absence (●) of 500 IU/ml of IFN-α2b for 18 h. These cultured cells were used as effectors against ^{51}Cr-labeled K562 and JY target cells. Results represent the mean plus the standard deviation of the specific lysis of the different triplicate cytotoxic assays performed in each patient at 50:1, 25:1 and 12:1 effector-to-target cell ratios.

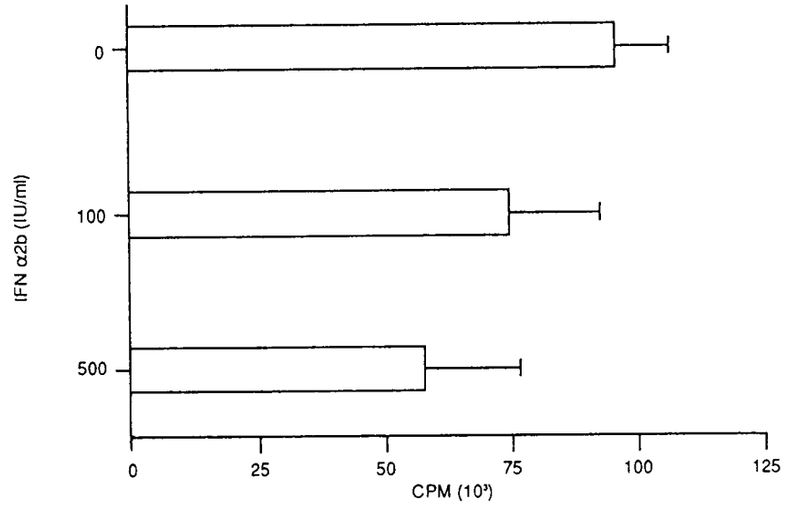

Figure 3. IFN-α2b inhibits the proliferative response of T lymphocytes to plant lectins. T lymphocytes from PBMNC from eight patients with superficial TCC of the bladder were purified by double rosetting with sheep red blood cells. The PBMNC (50 000 cells/well) were incubated in complete medium supplemented with 1% phytohemagglutinin in the presence or absence of different concentrations of IFN-α2b for 5 days. The proliferative response of the T cells was measured by the incorporation of pulsed [^{3}H]thymidine into the DNA. Results represent the mean plus the standard deviation of the counts per minute (cpm) of the different triplicate assays performed in each patient.

kine, and show lytic activity against NK-sensitive and NK-resistant target cells (Figure 2). Taking into account that NK cells are involved in immunosurveillance against neoplasia, it could be suggested that this cytotoxicity-enhancing effect of IFN-α might be related to the therapeutic action found in these subjects.

We have also seen that IFN-α is capable of modulating T lymphocytes from patients with superficial TCC of the bladder (Figure 3). The regulatory effect of IFN-α on T lymphocytes may be another factor leading to the clinical results obtained in those who receive intracavitary treatment with these cytokines.

These findings demonstrate that IFN-α show a clear modulatory effect on the cytotoxic effector cells of patients with superficial TCC of the bladder. Furthermore, our results show that the intracavitary instillation of IFN-α2b in these subjects is associated with an infiltration of T lymphocytes and NK cells into the bladder wall. In the near future, it will be necessary to define the possible prognostic value of the *in vitro* immunomodulatory effect of IFN-α on the NK cells and T lymphocytes from peripheral blood of patients with superficial TCC of the bladder and the therapeutic effect of intracavitary instillation with these cytokines.

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