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Original Articles

Influence of Interferon Alfa-2c on the Kinetics of Spontaneous Cell-Mediated Cytotoxicity in Urothelial Carcinoma in Vivo and in Vitro

J. W. Grups, T. Stock, and H. G. W. Frohmüller

Department of Urology, University of Würzburg, Medical School, Würzburg, Federal Republic of Germany

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Summary. It was demonstrated that patients with superficial bladder cancer displayed a raised spontaneous cell-mediated cytotoxicity (SCMC) compared to patients with advanced bladder cancer and healthy control subjects. By use of recombinant interferon alfa-2c, the activity of the spontaneous cell-mediated cytotoxicity at the level of the individual cell could be increased both in vitro and in vivo. In vitro, this was the case in patients with superficial bladder cancer as well as in patients with advanced bladder carcinoma, and in healthy control subjects. The kinetics of cytolysis were not affected by recombinant human interferon (rHu IFN) alfa-2c. After in-vivo application of rHu IFN, there was an elevation of the target binding cells (TBC) and the number of active natural killer (NK) cells within 24 h, but this was only detected for a brief period of time.

Key words: Interferon – Cytotoxicity – Urothelial carcinoma

Introduction

Natural killer cells (NK cells) are regarded as the effector cells of the spontaneous cell-mediated cytotoxicity (SCMC). SCMC plays an important role in tumor defence. Studies have been published which showed that a reduction of SCMC occurred in advanced malignancies [2, 3, 6].

The aim of this study was to explore the processes responsible for the lowered SCMC. Furthermore, it was to be established whether stimulation of SCMC can be obtained with rHu IFN alfa-2c in patients with urothelial bladder carcinoma. Investigations were carried out in patients with different tumor stages to enable further differentiation.

Material and Methods

Peripheral mononuclear cells (PMC) were isolated via a Ficoll density gradient from the peripheral venous blood of 20 patients with urothelial bladder carcinoma. The mononuclear cells obtained in this way were placed in plastic tissue culture dishes and were adjusted to a concentration of 10^6/ml with RPMI + 10% FCS. Afterwards, these cells were stimulated with 1.000 IU/ml rHu IFN alfa-2c for 12 h at 37 °C and 5% CO_2 gasing in the incubator. Single cell binding tests were then performed in analogy to the method of Grimm and Bonavida [1]. 9×10^5 mononuclear cells as well as 9×10^5 target cells of the permanent cell line K562 were mixed and incubated for 15 min at 37 °C and 5% CO_2 gasing. The number of effector-target cell conjugates was counted under the microscope. Afterwards the cells were mixed in 0.5% agarose (Bacto-Agar, Difco, Detroit, USA), incubated at 37 °C and 5% CO_2 and afterwards stained with trypan blue. The cytolytically active cells were determined on the basis of the stained target cell conjugates. The percentage of spontaneously lysed target cells was demonstrated by means of control tests performed in parallel. To check the kinetics of cytolysis, the investigations were performed after 30, 60 and 180 min of incubation. To demonstrate the effect of IFN, parallel investigations were carried out without IFN stimulation. The cytolysis was stopped by addition of formaldehyde solution. The calculation of the maximum cytotoxicity (Vmax) was made in accordance with the method of Ullberg and Jondal [5] with a ^{51}Cr-releasing test. It is assumed that the kinetics of cytolytically active cells as well as the corresponding target cells are exactly the same as those of an enzyme-substrate mixture. The Vmax was determined by means of a regression analysis calculated from the six cytolysis values determined in the ^{51}Cr-releasing test.

The determination of the cell-mediated cytotoxicity after in-vivo stimulation was carried out on the PMC of seven patients treated with rHu IFN alfa-2c i.m. The lymphocytes were isolated from the peripheral venous blood. The statistical analysis was carried out with the Student-*t*-test.

All 20 patients suffered from histologically verified urothelial carcinoma of the bladder. Stages pTA–pT1 were present in 10 patients and stages pT2–pT4 in a further 10 patients. Furthermore, 10 healthy subjects of the same age were tested as a control group.

rHu IFN alfa-2c (Berofor®) provided by Boehringer, Ingelheim, Germany was used both in the in-vitro testing and in the in-vivo administration.

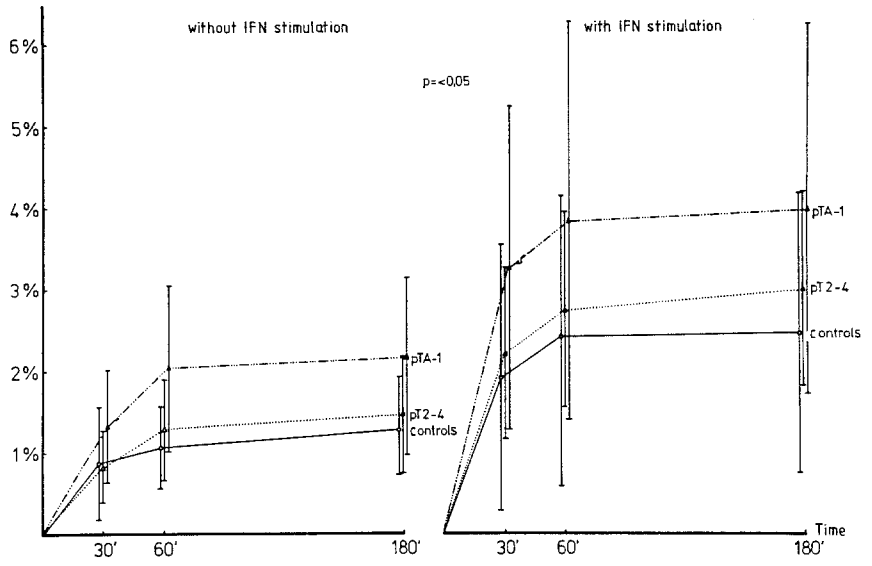

Fig. 1. Percentage of active NK cells in PMC in patients with bladder cancer and healthy control subjects without (left half of the figure) and with (right half of the figure) IFN stimulation

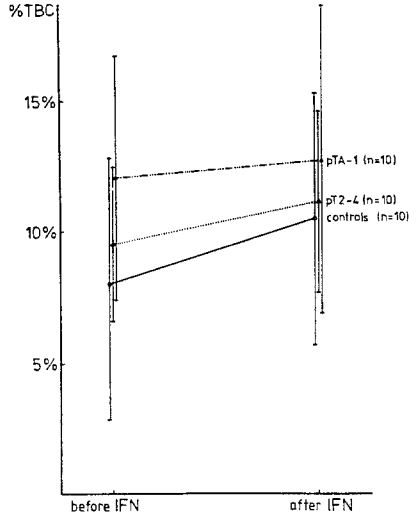

Fig. 2. Mean levels of the percentage of lymphocytes binding to tumor cells

Results

Terminology is listed below.

TBC (Target Binding Cells)

Peripheral mononuclear cells which bind to target cells and mainly comprises NK cells and a small proportion of monocytes.

Vmax

Theoretical maximum number of tumor cells which can be lysed in three hours by 10^5 mononuclear cells (PMC).

Active NK Cells

Percentage of Lymphocytes Amongst the PMC Which bind to Tumor Cells and Lyse These Within Three Hours. In 10 patients with superficial urothelial carcinoma (stage pTA to pT1), a raised number of active NK cells could be demonstrated in vitro following 60 and 180 min after IFN stimulation compared to healthy control subjects and compared to patients in whom infiltrating urothelial carcinomas were present (stage pT2–pT4) (see Fig. 1, left). Owing to the in-vitro stimulation with rHu IFN alfa-2c, the proportion of active NK cells could be raised further in all three groups (see Fig. 1, right). These differences were statistically significant ($P < 0.05$). The kinetics of cytolysis did not differ with or without IFN. A plateau had been reached in both tests after 60 min.

In comparison of the percentage of mononuclear cells (TBC) of patients with urothelial carcinomas which bind to tumor cells and that of healthy control subjects, an elevation of TBC could be demonstrated in the group of carcinoma patients. This finding was more pronounced in those patients with superficial carcinomas than in the patients with infiltrating tumors. However, after in-vitro stimulation with IFN alfa-2c, a significant rise of TBC could not be measured in any group (see Fig. 2).

In the carcinoma patients investigated, the calculated level of Vmax (maximum number of tumor cells which can be lysed in three hours by 10^5 lymphocytes) was raised compared to the control group. This elevation of Vmax could be further increased in vitro by stimulation with rHu IFN alfa-2c. This effect of IFN was statistically significant in those patients in whom urothelial carcinoma was present ($P < 0.05$) (see Fig. 3).

When the relative numbers of TBC after administration of rHu IFN alfa-2c in vivo and in vitro are compared, a markedly more pronounced rise can be demonstrated after in-vivo stimulation (see Fig. 4).

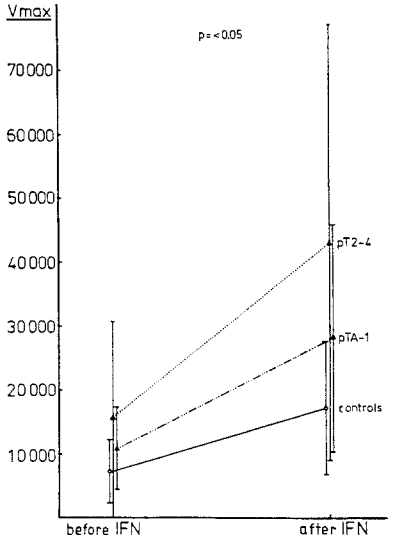

Fig. 3. Theoretical maximum number of tumor cells which could be lysed in three hours by 100.000 mononuclear cells (PMC) before and after IFN stimulation in patients with bladder cancer

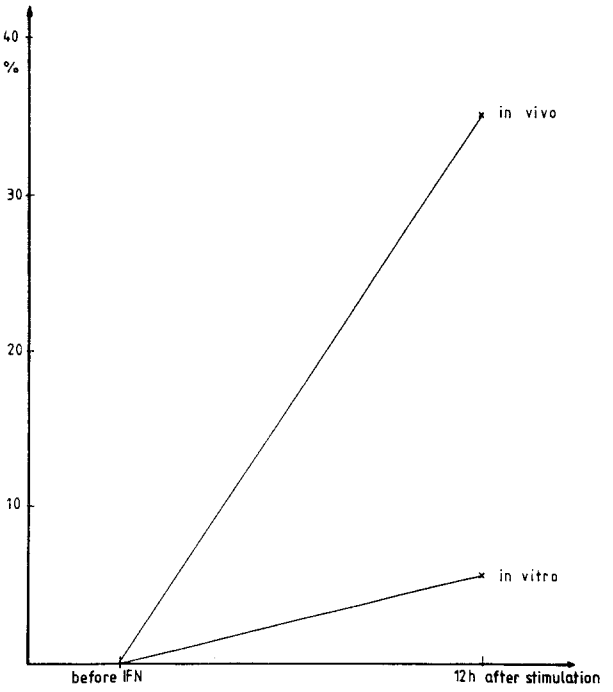

Fig. 4. Comparison of the relative percentage of TBC after in-vivo and in-vitro stimulation with rHu IFN alfa-2c

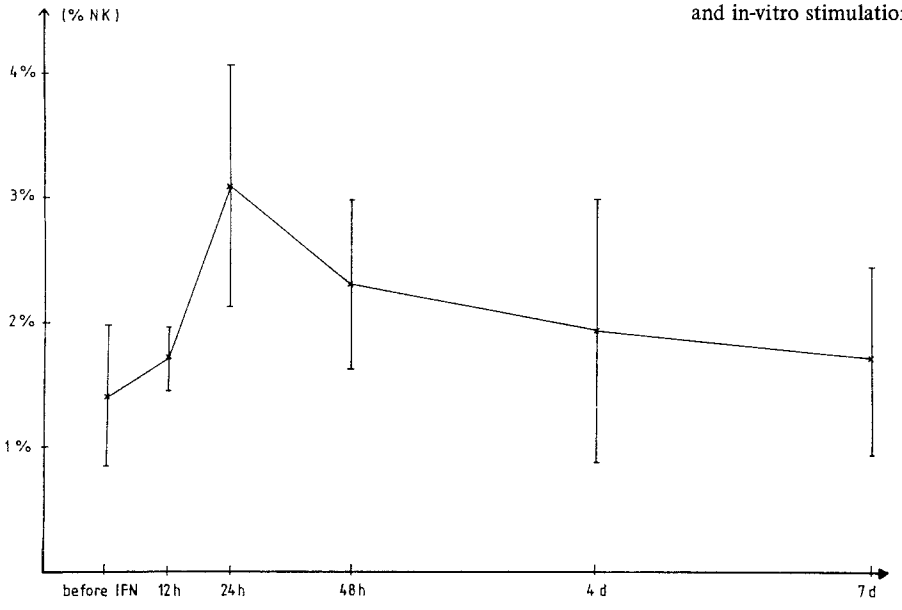

Fig. 5. Curve of the percentage of active NK cells after in-vivo stimulation with rHu IFN alfa-2c in patients with superficial bladder cancers

However, in those patients suffering from a superficial urothelial carcinoma, only a brief rise within the first 24 h can be demonstrated for the percentage of active NK cells and TBC after systemic in-vivo administration of rHu IFN alfa-2c. During the further course of treatment, the initial pretherapeutic levels were reached again within one week (see Figs. 5 and 6).

Discussion

The results indicated that there were immunological interactions between the tumor and the immune system in patients with urothelial bladder carcinoma. These interactions were intensified by rHu IFN alfa-2c. This was evident because both the number of lymphocytes binding tumor cells and the percentage of active NK cells were raised in patients with superficial urothelial carcinoma (pTA–pT1). This finding correlates clinically with a markedly better prognosis of such patients as compared to patients with advanced bladder cancers. However, it has not been explained why the evaluation of these parameters of SCMC occurs. The elevated percentage of active NK cells already present can be further raised by in-vitro stimulation with IFN alfa-2c. However, the number of TBC, which was raised in patients with bladder cancers

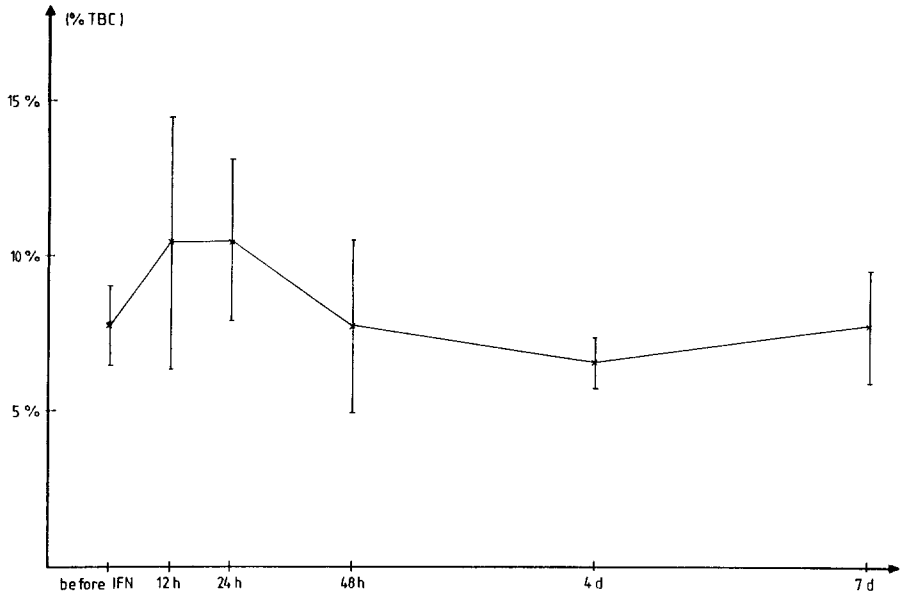

Fig. 6. Curve of the percentage of TBC after in-vivo stimulation with rHu IFN alpha-2c in patients with superficial bladder cancers

compared to healthy control subjects, could not be appreciably increased in vitro by IFN stimulation. On the other hand, after systemic in-vivo administration of IFN, a marked rise of TBC could be measured 12 h following the first IFN application.

In accordance with the therapeutic model [4], this result might be interpreted in the following terms: owing to the influence of IFN in vivo, mature NK cells are first of all formed from immature NK precursor cells which then bind to tumor cells (TBC). In a second step, they are possibly converted into active NK cells with cytolytic activity [7]. On the other hand, only this second step of activation takes place in vitro, whereas an increase in the number of mature NK cells by conversion from precursor cells is not possible in vitro. A reason for this might be that the pool of NK precursor cells is not present in the population of PMC, or that other cell populations and their mediator substances are lacking in vitro. The determination of the number of active NK cells as well as the number of TBC showed only a brief transient rise, so that a long-term treatment with IFN for immunomodulation does not appear useful in solid tumors.

In clinical terms, the increase of the calculated level of Vmax after rHu alfa-2c stimulation in vitro might mean that an immunomodulating treatment of IFN in vivo would intensify the antitumoral effect. Since no appreciable alterations of the cytolytic kinetics could be attained by rHu IFN alfa-2c, it can be assumed that the intensified NK cell activity is due to an increased secretion of cytolytic factors by the individual NK cell [7].

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Dr. J. W. Grups
Urologische Klinik der
Universität Würzburg
Josef-Schneider-Strasse 2
D-8700 Würzburg
Federal Republic of Germany