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Cytotoxic effects of alpha- and gamma-interferon and tumor necrosis factor in human bladder tumor cell lines

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Abstract We investigated the activity of alpha-interferon (α-IFN), gamma-interferon (γ-IFN) and tumor necrosis factor-alpha (TNF-α) in a panel of ten human bladder tumor cell lines. All cytokines were tested at concentrations of 100-10000 U/ml in a clonogenic assay system. We found that α-IFN was active against five of the ten lines while γ-IFN was only active against one line. TNF was active against five of the ten lines. Maximum synergisms were obtained between the α-IFN and TNF, occurring in nine of the ten cell lines. We conclude that α-IFN and TNF are active as single agents and synergistic when used together in vitro in human bladder tumor cell lines.

Key words Biological response modifiers · Bladder cancer · Alpha-interferon · Gamma-interferon · Tumor necrosis factor-alpha

Superficial bladder cancer is currently treated with endoscopic resection followed by adjuvant intravesical chemotherapy or immunotherapy with thiotepa, doxorubicin, mitomycin C or bacillus Calmette-Guérin (BCG) [24]. Owing to the high response rates and prolonged tumor control, particularly in high-grade lesions, many investigators advocate the use of BCG as the primary form of adjuvant therapy for superficial bladder tumors [22].

Although the exact mechanisms of action for BCG therapy are largely unknown, much evidence supports the role of the immune system in its antitumor effect. Shortly after the institution of BCG therapy, mononuclear infiltrates develop within the urothelium which are primarily helper-induced T cells and macrophages [2]. BCG therapy has also been shown to result in the liberation in the urine of a variety of cytokines [17, 20]. Recently, a number of these cytokines have been shown to have single-agent activity in bladder cancer both in vitro and in vivo [7]. Further elucidation on the activity of individual cytokines in bladder cancer could provide more specific intravesical immunotherapies.

Interferons have been shown to have activity in cancer by both direct cytotoxic and immunostimulatory mechanisms. Interferons have a direct antiproliferative effects on tumor cells causing an extension of all phases of the cell cycle and prolongation of the cell generation time [13, 19, 26]. This cytostatic effect probably plays a major role in the anticancer effects of these agents. Interferons also modulate the immune system by recruitment and enhancement of the cytotoxic activity of NK cells [10]. The immunostimulatory effects of gamma interferons (γ-IFN) involve increasing the number and density of Fc receptors with subsequent increased antigen-presenting function resulting in enhanced phagocytosis by macrophages [19, 26].

Tumor necrosis factor (TNF produces an antitumor effect by a variety of mechanisms. A direct cytotoxicity effect is apparently mediated by specific binding to high-affinity surface receptors. These cytotoxic effects appear to be cell cycle specific. TNF causes accumulation of tumor cells in the G_2 phase and cytolysis in the late stages of mitosis [3, 28]. TNF may also mediate the cytostatic properties of NK cells and macrophages [23, 31].

In the present study, we investigated the in vitro cytotoxic activity of three cytokines (α-IFN, γ-IFN and TNF) against a panel of human bladder tumor cell lines. We also attempted to determine whether the cytotoxic activities of these cytokines are synergistic in vitro.

Materials and methods

Human bladder tumor cell lines (HBTCLs)

The HBTCLs used in this study were purchased from the American Type Culture Collection (Rockville, Md., USA). The panel of cell

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Table 1 Single-agent in vitro activity of α-IFN, γ-IFN and TNF. Values represent the percentage mean colony survivals compared with control plates using α-IFN, γ-IFN and TNF at 10000 U/ml. Less than 25% of colony survivals scored as a sensitive cell line

Cell line	α-INF	γINF	TNF
CUB-2	7.0 ± 6	54.0 ± 15	1.0 ± 1
T24	38.0 ± 4	57.0 ± 5	81.0 ± 8
RT4	59.0 ± 5	2.0 ± 1	7.0 ± 7
J82	43.0 ± 12	81.0 ± 7	93.0 ± 13
HT1197	36.0 ± 16	61.0 ± 11	59.0 ± 13
5637	14.0 ± 7	66.0 ± 18	13.0 ± 3
HT1376	12.0 ± 7	26.0 ± 10	11.0 ± 9
SCaBER	10.0 ± 12	29.0 ± 5	43.0 ± 11
TCCSUP	7.0 ± 4	96.0 ± 6	8.0 ± 10
UM-UC-3	31.0 ± 7	94.0 ± 9	86.0 ± 14

lines included CUB-2, T24, RT4, J82, SCaBER, TCCSUP, UM-UC-3, HT-1376, HT-1197 and 5637 [4, 30]. All tumor cell lines were maintained in monolayer culture grown in 100-mm plastic Petri dishes incubated at 37°C in 6% CO_2 and 100% humidified atmosphere with the recommended media. Tumor cells were harvested from monolayer culture by incubation for 10 min at 37°C in 0.25% typsin-ethylenediaminetetra-acetic acid (EDTA) solution.

Drug assay methods

Biologicals were evaluated for activity using a modification of the Hamburger and Salmon clonogenic assay [8, 18]. Briefly, the underlayer consisted of 1 ml RPMI medium with 0.5% agar and 10% horse serum plated on 35-mm scored Petri dishes (LUX, Miles Laboratories, Naperville, Ill., USA). Tumor cells were suspended in the upper layer in 0.3% agar in RPMI medium, 10% horse serum, the biological being studied and tumor cells at 1×10^5 to 3×10^5 cells/plate. Cultures were incubated at 37°C in 6% CO_2 and 100% humidified atmosphere. Cultures were examined with an Olympus CK inverted microscope at ×40 and ×100. Final colony counts were made from 14 to 21 days after plating. Aggregates of 50 or more cells were scored as colonies. Each percentage colony survival was an average of the colony counts from four plates at each treatment and control level. Greater than 100 colonies/plate were required for an adequate study. The plating efficiencies for these HBTCLs ranged from 0.5 to 3%.

Drug preparation

Intron A (recombinant IFN-α 26) was purchased through Schering Corporation (Kenilworth). α-IFN was reconstitued in bacteriostatic water and then diluted to the desired concentration in media. γ-IFN [CR-human γ-IFN (recombinant)] was purchased through Collaborative Research Inc. (Two Oak Park, Bedford, Mass., USA). The material was mixed in 82.3 mM $Na_2HPO_4\cdot7H_2O$/51.7 mM $NaH_2PO_4\cdot H_2O$/79 mM sucrose 2.7 g% human serum albumin and stored at −70°C. The preparation had a specific activity of 2.9×10^7 U/mg protein. TNF, human (rDNA) (Genetech, South San Francisco, Calif., USA) was provided at a concentration of 40000 U/ml and was reconstituted in 1 ml sterile distilled water and stored at 2–8°C for up to 1 month prior to use.

Methods of analysis

The level of drug sensitivity was determined in the clonogenic assay using a cutoff for activity of 25% or less colony survival. The statistical methods of Momparler [16] were used for determining synergism between the biologicals. The formula $SF_{A+B}/(SF_A)(SF_B)$ was used to define in vitro synergism. For simplicity, the ratios identified from the formula were graded as 0 or less = 0; 1 − 2 = 1+; >2 − 4 = 2+; >4 − 10 = 3+; and >10 = 4+.

Results

Drug assays using each of the three biologicals were performed on the panel of ten HBTCLs. Each cytokine was tested in the range of 100–10000 U/ml and compared with control plates. Table 1 lists the level of colony survival of the ten HBTCLs with each cytokine. Five cell lines were sensitive to α-IFN at the highest dose (10000 U/ml). Only SCaBER was sensitive at 1000 U/ml α-IFN. γ-IFN was only active against RT4. This HBTCL was sensitive to γ-IFN at levels as low as 100 U/ml cytokine. Five cell lines were sensitive to TNF-α at 10000 U/ml. Two lines, CUB-2 and TCCSUP, were also sensitive at 1000 U/ml cytokine. Four lines were sensitive to both α-IFN and TNF. SCaBER was sensitive to α-IFN and resistant to TNF while RT4 was sensitive to both and TNF and resistant to α-IFN.

In order to determine whether synergisms existed between the cytokines, combinations were compared simultaneously with each cytokine used alone. Clear synergisms were demonstrated between each pair of biologicals tested. Figure 1 depicts synergistic drug assays with each combination of biologicals. Table 2 lists the relative degree of synergism found with each drug pair. Overall, α- and γ-INF were synergistic in six of the ten cell lines. TNF and γ-INF were synergistic in seven of the lines tested while TNF and α-INF produced synergisms in all but one of the lines tested.

Discussion

We demonstrated the activity of three cytokines against a panel of HBTCLs. Throughout these experiments we used continuous exposure in vitro to high doses of the cytokines. These concentrations would be unlikely to be achievable systemically without producing significant toxicity for the patient. Intravesical chemotherapy, however, allows for exposure to extremely high doses of anticancer agents or immunotherapy locally to the tumor with minimal absorption of the agent if conditions are optimal. α-IFN has been used intravesically in high doses with minimal or not toxicity. Several investigators have shown that α-IFN can be given in doses of 50 to 1000 MU intravesically with minimal local and systemic toxicity observed [11]. The doses of α-IFN that we used ($C\times T = 10000\ \text{U}\times24\ \text{h}\times10\ \text{days} = 2.4\ \text{MU}$) are well within these intravesical levels.

We found that α-IFN significantly inhibited the clonal growth of 50% of the HBTCLs tested. The clinical activity of α-IFN in bladder cancer has been demonstrated by several recent reports. Using a variety of schedules, α-IFN has been shown to produce response rates between 25 and 63% in patients with superficial bladder cancer [6, 14, 27].

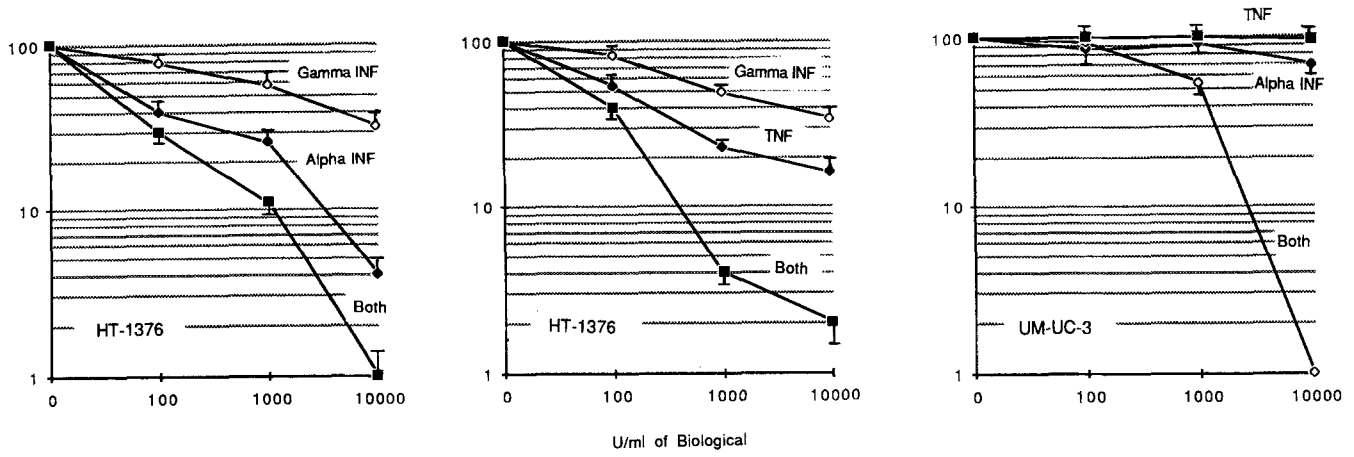

Fig. 1 Colony survival curves produced using combinations of the cytokines. Each cytokine was tested at ranges between 100 and 100000 U/ml either alone or in combination using continuous drug exposure. Each point is the mean and SE of four colony counts

Table 2 Combination in vitro activity of α-IFN, γ-IFN and TNF. The formula by Momparler, $SF_{A+B}/(SF_A)(SF_B)$, was used to define in vitro synergism. SF is the surviving fraction of colonies in vitro. For simplicity, ratios from this formula were rated as 0; 1 – 2(1+); >2 – 4(2+); >4 – 10(3+); >10(4+)

Cell line	α- + γ-IFN	TNF + γ-IFN	TNF + α-IFN
CUB-2	4+	0	1+
T24	4+	2+	1+
RT4	1+	3+	4+
J82	0	1+	0
HT1197	0	0	2+
5637	3+	3+	2+
HT1376	1+	2+	1+
SCaBER	0	2+	1+
TCCSUP	0	0	1+
UM-UC-3	3+	2+	4+

α-IFN had significant activity with little or no toxicity when used intravesically.

Our studies did not find γ-IFN to be particularly active as a single agent. Only one cell line, RT4, was found to be sensitive to γ-IFN. Hawkyard et al. [9] has also demonstrated the sensitivity of RT4 to γ-IFN after a 24-h incubation with the cytokine. Riggs et al. [21] demonstrated in the transplantable murine bladder tumor model MBT-2 that γ-IFN as a single agent was ineffective in reducing tumor incidence, reducing tumor volume and prolonging survival. In a clinical trial of 13 patients with superficial bladder cancer [5], γ-IFN was reported to produce responses in less thant 10% of patients. These data together support our findings that γ-IFN as a single agent is unlikely to have significant activity in superficial bladder cancer.

Our data suggest that TNF as a single agent may have significant activity in superficial bladder cancer, with 50% of HBTCLs found to be sensitive to the biological at 10000 U/ml. Both laboratory and clinical evidence of activity of TNF in bladder cancer now exists in the literature. Kadhim and Chin [12] found partial tumor regressions that were transient using MBT-2. Lee et al. [15] showed that this antitumor effect was dose dependent, with 3700 U significantly suppressing the 7-day tumor growth when given intravesically in MBT-2. Van Moorselaar et al. [29] showed that the TNF significantly inhibited the growth of an established rat bladder tumor, RBT232, when injected peritumorally. The results of early clinical trials using TNF in superficial bladder cancer have identified significant intravesical activity [25]. More clinical data are needed to determine the true clinical activity of this single in bladder cancer.

In biological systems, cytokines work in synergy to direct the activity of effector cells in vivo. In a wide variety of tumor systems, cytokines have been found to exhibit synergistic antitumor activity. The demonstration of elevated urinary levels of interleukin-1, interleukin-2, TNF [2] and γ-INF [20] in patients receiving BCG therapy for superficial bladder cancer suggests that combinations of cytokines may be responsible for the immunotherapeutic effect seen in these patients. Combinations of cytokines have been shown to be synergistic in a variety of bladder tumor models. TNF plus γ-INF has been shown to be synergistic in the MBT-2 [1] and RBT323 rat bladder tumor model [26]. Combinations of α-INF and γ-INF [21] and interleukin-2 with either α-INF [21] or γ-INF [21, 25] have also been shown to be synergistic in MBT-2. We have demonstrated that the α-INF, γ-INF and TNF exhibit synergisms when paired in combination therapy in vitro. The degree of synergism seen varied with the HBTCL tested. TNF and α-INF displayed the most frequent synergistic combination in this panel of HBTCLs.

We demonstrated that a panel of HBTCLs displays a differential sensitivity to single-agent cytokines and a combination of these cytokines frequently acts synergistically in their antitumor effect. Combination of cytokines may optimize our immunotherapeutic approaches to superficial bladder cancer.

References

1. Bahnson RR, Ratliff TL (1990) In vitro and in vivo anti-tumor activity or recombinant mouse tumor necrosis factor (TNF) in a mouse bladder tumor (MBT-2). J Urol 144:172
2. Bohle A, Nowc CH, Ulmer AJ, Musehold J, Gerdes J, Hofstetter AG, Flad LD (1990) Elevations of cytokines interleukin-1, interleukin-2 and tumor necrosis factor in the urine of patients after intravesical bacillus Calmette-Guerin immunotherapy. J Urol 144:59
3. Darzynkiewicz SB, Williamson B, Carswell E, Old LJ (1984) Cell cycle specific effects of tumor necrosis factor. Cancer Res 444:83
4. Fogh J (1978) Cultivation, characterization and identification of human tumor cells with emphasis on kidney, testis and bladder tumors. Natl Cancer Inst Monogr 49:5
5. Geboers ADH, Bergen TNL van, Oosterlinck W (1987) Gamma-interferon in the therapeutic and prophylactic management of superficial bladder cancer. J Urol 137:276
6. Glasen RW (1990) A randomized controlled study of intravesical alpha-2b interferon in carcinoma in situ or the bladder. J Urol 144:658
7. Goldstein D, Laszlo J (1986) Interferon therapy in cancer: From imaginon to interferon. Cancer Res 46:4315
8. Hamburger A, Salmon SE (1977) Primary bioassay of human myloma stem cells. J Clin Invest 60:846
9. Hawkyard SJ, Jackson AM, James K, Prescott S, Smyth JF, Chisholm GD (1992) The inhibitory effects of interferon gamma on the growth of bladder cancer cells. J Urol 147:1399
10. Herberman RB, Ortaldo JR (1981) Natural killer cells: their role in defenses against disease. Science 214:24
11. Horoszewicz JS, Murphy GP (1989) An assessment of the current use of human interferons in therapy of urological cancers. J Urol 142:1173
12. Kadhim SA, Chin JL (1988) Anti-tumor effect of tumor necrosis factor and its induction to tumor variant of MBT-2 transitional cell carcinoma of the bladder. J Urol 139:1091
13. Kirchner H (1984) Interferons, a group of multiple lymphokines. Semin Immunopathol 7:347
14. Kostakopoulos A, Deliveliotis Ch, Mavromanolakis E, Aravantinos G, Dimopoulos MA (1990) Intervesical interferon alpha-2b administration in the treatment of superficial bladder tumors. Eur Urol 18:201
15. Lee K-E, O'Donnell RW, Schoen S, Cockett ATK (1987) Effect of intravesical administration of tumor necrosis serum and human recombinant tumor necrosis on a murine bladder tumor. J Urol 138:430
16. Momparler RL (1980) In vitro systems for evaluation of combination chemotherapy. Pharmacol Ther 8:21
17. Morales A, Nickel JC (1992) Immunotherapy for superficial bladder cancer. Urol Clin North Am 19:549
18. Niell HB, Webster K, Rademacher D, Brausi M (1990) Clonal growth requirements of human bladder tumor cell lines. J Urol 143:1049
19. Paulnock DM, Borden EC (1985) Modulation of immune function by interferons. In: Reif E, Mitchell M (eds) Immunity to cancer. Academic, New York, p 545
20. Prescott S, James K, Hargreave TB, Chisholm GD, Smyth JF (1990) Radio-immunoassay detection of interferon-gamma in urine after intravesical Evans BCG therapy. J Urol 144:1248
21. Riggs DR, Tarry WF, DeHaven JI, Sosnowski J, Lamm DL (1992) Immunotherapy of murine transitional cell carcinoma of the bladder using alpha and gamma interferon in combination with other forms of immunotherapy. J Urol 147:212
22. Sargent ER, Williams RD (1992) Immunotherapeutic alternatives in superficial bladder cancer. Urol Clin North Am 19:581
23. Shalaby MR, Hirabayashi SE, Svedersky LP, Palladino MA (1986) Regulation of immune function in vitro by recombinant human tumor necrosis factor and lymphotoxin. Soluble Mediators Immunoregul 1:924
24. Shaw M, Mckeil CF, Ray V (1991) Characterization of cellular infiltrates in the rat urinary bladder following BCG and thiotepa intravesical therapy. J Surg Oncol 46:48
25. Sosnowski JT, DeHaven JI, Riggs DR, Lamm DL (1991) Treatment of murine transitional cell carcinoma with intralesional interleukin 2 and murine interferon gamma. J Urol 146:1164
26. Stewart WD, Blanchard DK (1985) Interferons, cytostatic and immunomodulatory effects. In: Reif E, Mitchell M (eds) Immunity to Cancer. Academic, New York, p 295
27. Torti FM, Shortliffe LD, Williams RD, Pitts WC, Kempson RL, Ross IC, Palmer J, Meyers F, Ferrari M, Hannigan J, Spiegel R, McWhirter K, Freiha F (1988) Alpha interferon in superficial bladder cancer : a Northern California Oncology Group study. J Clin Oncol 6:476
28. Tsujimoto M, Yip YK, Vilcek F (1985) Tumor necrosis factor: specific binding and internalization in sensitive and resistant cells. Proc Natl Acad Sci USA 82:7626
29. Van Moorselaar RJA, Hendriks BT, Borm G, Van Der Meide PH, Debruyne FMJ, Schalken JA (1992) Inhibition of rat bladder tumor (RBT232) grwoth by tumor necrosis factor alpha and interferon-gamma in vivo. J Urol 148:458
30. Williams RD (1980) Human urologic cancer cell lines. Invest Urol 17:359