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Concomitant treatment of MBT-2 bladder tumour by tumour necrosis factor alpha and interferon alpha in conjunction with delayed type hypersensitivity immunotherapy

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Summary. In our previous study [9], we reported the anti-tumour effect of TNF on mouse bladder tumour (MBT-2) both in vivo and in vitro. Inoculation of a single dose of TNF alone caused significant but transient tumour growth inhibition. Subsequent repeated doses of TNF did not sustain or augment the anti-tumour effect. The current experiments were undertaken to assess the anti-tumour activity of (i)-concomitant treatment of TNF-A and IFN-A against MBT-2 bladder tumour and (ii)-concomitant TNF + IFN-A treatment in conjunction with T-DTH (delayed-type hypersensitivity) immunotherapy. Systemic administration of multiple doses of TNF + IFN-A in vivo caused initial partial tumour regression followed by tumour growth inhibition up to 14 days following treatment. This combined treatment showed an enhanced anti-tumour effect compared to TNF-A treatment alone. Immunotherapy of MBT-2 tumour-bearing mice with T-DTH "immune" effector cells alone did not cause significant tumour growth inhibition. In contrast, concomitant administration of both T-DTH effector cells and TNF + IFN-A in MBT-2 tumour-bearing mice resulted in significant tumour growth inhibition for up to 16 days. The immune effector cells conferring immunotherapy were isolated from the spleens of tumour-immunized, "DTH-primed" animals and were characterized as Lyt 1^+2^- helper/DTH T cells ($CD4^+$ phenotype). These cells mediate both DTH response to MBT-2 tumour antigens as well as anti-MBT-2 tumour protection. In vitro treatment of the "immune" cells with TNF-A resulted predominantly in the proliferation of Lyt 1^+ T cells versus Lyt 2^+ cells. The anti-tumour effect of TNF + IFN-A can be augmented by immunotherapy possibly via the immune capacity of tumour sensitized T-DTH effector cells.

Key words: Tumour necrosis factor – Interferon alpha – Bladder tumour – Delayed type hypersensitivity response – Immunotherapy

Recent developments in the area of biological response modifiers (BRM) have provided a focus for the formulation of new strategies in cancer immunotherapy. Some BRM's have been shown to have both direct cytotoxic effects on tumour cells and indirect effects mediated through the immune system. Tumour Necrosis Factor alpha (TNF-A) is a potent inducer of necrosis and regression of certain animal and human tumours [1, 7, 23]. It also has a wide range of indirect proliferative [12, 13, 25, 31] and immunoregulatory [20, 21, 27] activities including stimulation of activated T cells [24] and enhancement of surface antigen expression on vascular endothelial cells [4]. Interferon alpha (IFN-A) possibly has an anti-tumour effect against superficial bladder carcinomas [16, 28]. It has also been shown to modulate the expression of murine tumour [6] and major histocompatibility antigens [29]. Synergistic anti-tumour effect of TNF-A and IFN-A has been demonstrated on human breast [2], colon [14, 15] and renal cell carcinoma cells [3]. However, such combined treatment has not been evaluated in superficial bladder cancer. Numerous reports demonstrated that the T cell-mediated delayed-type hypersensitivity (T-DTH) reaction has potent anti-tumour capabilities [5, 17, 19]. Prompted by this evidence we attempted to investigate whether T cells which mediate a DTH reaction to MBT-2 tumour transplantation antigens (TTAs) could accentuate growth inhibition of MBT-2 when used as a form of immunotherapy in conjunction with TNF + IFN-A. This therapeutic regimen was chosen on the basis of the positive synergistic effect between TNF-A and IFN-A and their dual mode of action directly on cancer cells and indirectly through the "immune" effector cells.

Materials and methods

Bladder tumour model

FANFT-induced MBT-2 transitional cell carcinoma of the bladder (originally derived by Dr. M. Soloway [26]), was maintained in vivo as a solid subcutaneously (s.c.) growing tumour by weekly serial

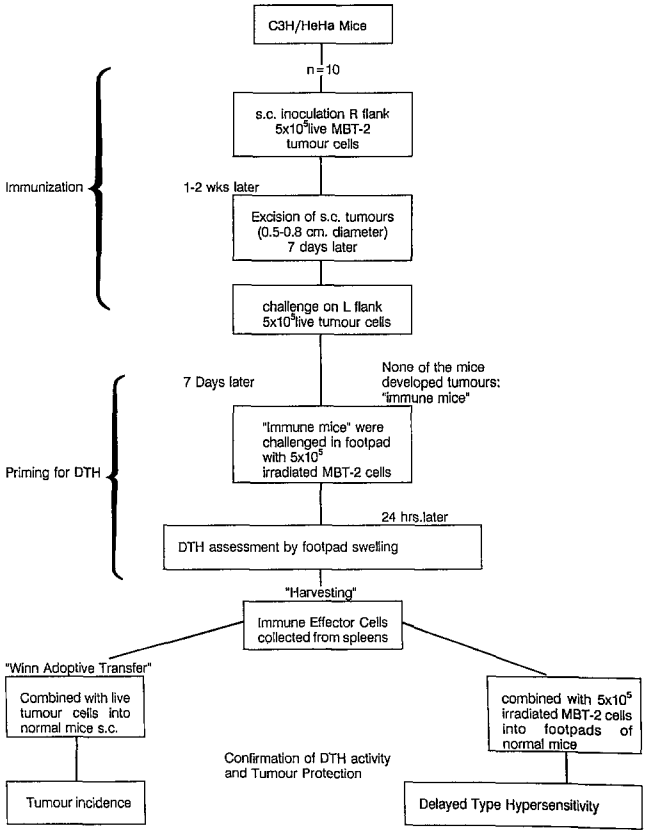

Fig. 1. Summary of procedures for tumour immunization, tumour protection and generation of MBT-2 tumour-specific "immune" effector cells

transplantation of 5×10^5 tumour cell in syngeneic C3H/HeHa female mice. Approximately 90–95% of all the inoculated animals develop s.c. tumours all of which represented solid mass with minimum or no necrosis at the early stage of growth but with increasing necrosis as tumours progress in size. The MBT-2 cell line was also maintained in vitro in RPMI 1640 plus 10% fetal calf serum [TD-50 (tumourigenicity dose 50) is approximately 1×10^2 cells]. Single tumour cell suspensions were prepared by enzymatic digestion of the minced tumour with a mixture of collagenase (1 mg/ml) (collagenase type II from Sigma Chemical Co.), proteinase K (0.01 mg/ml) and DNA-ase (0.01 mg/ml) (from Sigma) (for 20 min at 37°C with gentle stirring). Determination of tumour cell viability was done by trypan blue exclusion.

Biological response modifiers

Recombinant human TNF-A was obtained from Amgen Biologicals, Canada Inc. (specific activity 1×10^6 units/mg protein). Biological assay of TNF-A was carried out by cytolysis of mouse L-929 cells incubated with TNF-A for 48 h in the presence of actinomycin D. The concentration required to yield 50% lysis of L-929 cells was defined as one TNF-A Lytic Unit (L.U.). Recombinant human IFN-A (Intron A) was obtained from Schering Canada Inc. [specific activity 3×10^6 Interferon Units (I.U.)/ml].

TNF + IFN-A and DTH immunotherapy treatment

Animals were inoculated s.c. with 5×10^5 MBT-2 tumour cells. Approximately fourteen days later when tumours became palpable, tumour-bearing mice were divided randomly into study and control groups. In each group each MBT-2 tumour-bearing mouse was tagged and monitored individually for treatment response starting from day 0, the starting point of treatment. Due to a large number of individually monitored animals in the study and control groups three separate experiments were performed: (i) effect of TNF-A therapy alone; (ii) effect of TNF-A therapy in combination with IFN-A and (iii) effect of combined TNF + IFN-A with AIT (adoptive immunotherapy). In the first experiment Group 1 mice (15/group) were injected intravenously (i.v.), once a week for 3 weeks with TNF-A alone (30,000 L.U.), and Group 2 i.v. with 0.2 ml saline alone. In the second experiment the mice (15/group) were divided randomly into 3 groups (Groups 3–5). Group 3 animals were injected intravenously (i.v.), once a week for 3 weeks with TNF + IFN-A (30,000 L.U. and 10,000 I.U. respectively); Group 4 i.v. with IFN-A alone (10,000 I.U.) and Groups 5, control untreated were inoculated i.v. with 0.2 ml saline alone. Optimal doses of TNF-A and IFN-A for the i.v. inoculations were chosen on the basis of dose response curves in MBT-2 tumour-bearing mice. These doses represent maximum tolerable dose of TNF-A and IFN-A corresponding to 5×10^5 L.U./kg body weight and 1×10^5 I.U./kg body weight, respectively. In the third experiment ($n = 10$ mice/group) a total of 2×10^7 T-DTH "immune" effector cells (see later for source of cells) plus TNF-A (30,000 L.U.) and IFN-A (10,000 I.U.) or "immune" cells alone (Groups 6 and 7, respectively) were injected intravenously (i.v.) via the tail vein into MBT-2 tumour bearing mice once a week for 3 weeks. Group 8, control untreated mice were injected i.v. with 0.2 ml saline only. The dose of "immune" effector lymphocytes was chosen on the basis of our previous dose response assessment of T-DTH cells in the SV_{40}-induced (mKSA) and methylcholanthrene-induced (MCA) sarcoma tumour models [8]. For the i.v. inoculations a 30 gauge needle was used and blood return was ensured prior to injections to avoid extravasation of injected material into the interstitium. Tumours of all groups were measured 3 times weekly for 3 weeks along the longest axis (a) and the perpendicular shortest axis (b). Tumour sizes were estimated by calculating the mean increase in tumour volumes (T.V. ± S.E.) using the formula $T.V. = 0.4 \times ab^2$ [9] that takes into account 3 orthogonal directions of an ellipsoid-shaped tumour. The statistical significance between tumour sizes and tumour incidence of all groups of individual experiments was estimated using Student's *t*-test or CHI-square test with significance determined at $p = < 0.05$.

Generation of DTH effector cells

Details of the conditions for tumour immunization, DTH "priming" and assessment of DTH activity and tumour protection against MBT-2 tumour are summarized in Fig. 1.

Source of DTH effector cells

C3H/HeHa mice were immunized by inoculation with 5×10^5 live MBT-2 tumour cells in the flanks followed by excision of s.c. small tumours (0.5–0.8 cm in diameter) 1–2 weeks later. The mice were then challenged in the contralateral flank area 7 days later with 5×10^5 live tumour cells. The mice that did not develop tumours (95% of all mice challenged) in the new inoculation site were designated "immune" mice. These "immune" mice were then primed for DTH reaction by s.c. footpad challenge with 5×10^5 gamma-irradiated (5,000 rads) MBT-2 tumour cells and the DTH reaction assessed 24 h later by measurement of footpad swelling. One DTH unit measured by Oditest callipers was defined as 1×10^{-2} mm. Spleen cells from these DTH-primed mice were obtained by splenectomy. By separating the spleen cell homogenate on Lymphocyte-M density separation gradient (Cedarlane Lab., Canada), "immune" effector cells were derived for use in combination with TNF-A and IFN-A treatment.

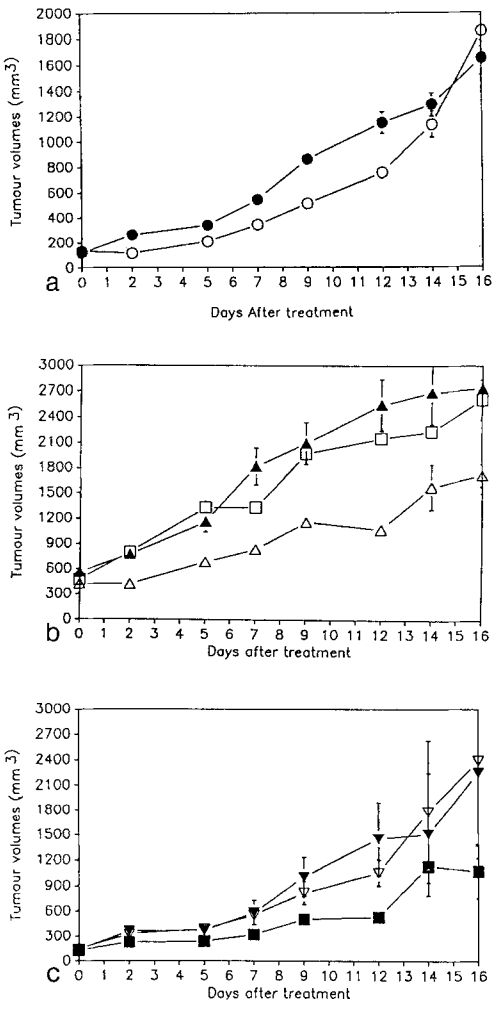

Fig. 2a–c. Tumor sizes following TNF-A treatment of MBT-2 tumours. **a** ○—○ = Gr 1, TNF-A treated; ●—● = Gr 2, untreated. $P < 0.05$ for Gr 1 vs Gr 2 from day 2 till day 12. **b** △—△ = Gr 3, TNF + IFN-A treated; □—□ = Gr 4, IFN-A treated; ▲—▲ = Gr 5, untreated. $P < 0.05$ and $P < 0.01$ for Gr 3 vs Gr 5 from day 2 till day 14. **c** ■—■ = Gr 6, TNF + IFN-A and AIT treated; ▽—▽ = Gr 7, AIT treated; ▼—▼ = Gr 8, untreated. $P < 0.05$ and $P < 0.01$ for Gr 6 and Gr 8

Confirmation of DTH reaction and tumour protection

The ability of "immune" effector cells (from DTH-primed mice) to mediated DTH response against MBT-2 tumour antigens was assessed by transferring 1×10^7 spleen cells from "immune" mice with specific antigen (5×10^5 irradiated MBT-2 tumour cells) s.c. into footpads of normal recipients ($n = 5$ mice per group) and measurement of DTH reaction 24 h later. For comparison, spleen cells from tumour-bearing mice (tumours not excised) and from untreated (normal) mice were transferred with antigen for DTH response. The control groups included "immune" spleen cells alone without antigen and tumour cells only. The statistical significance of DTH reactivities of all groups was estimated by comparison with control group of mice transferred with "immune" cells alone using Student's *t*-test.

Tumour protection against MBT-2 tumour was assessed by Winn adoptive transfer [30] by inoculation of admixture of 1×10^7 "immune" cells plus 5×10^5 live MBT-2 tumour cells (effector to target cell ratio E:T = 20:1) s.c. in the flanks of normal mice. For comparison, spleen cells from tumour-bearing (non-immune) mice and from untreated mice were injected s.c. along with live MBT-2 tumour cells (E:T = 20:1). Control mice were injected s.c. with tumour cells only. Tumour incidence was recorded at Day 21 after transfer. The statistical significance of tumour incidence of all groups was estimated by comparison with control group of mice with tumour cells alone using Chi-square test.

Phenotypic characterization of DTH cells

The phenotype of "immune" effector lymphocytes conferring both the DTH response to MBT-2 tumour antigens and the anti-tumour protection was determined by selective depletion of various T cell subsets by complement-dependent cytotoxicity with anti-lymphocyte monoclonal antibodies plus complement (Cedarlane Lab., Canada). Three groups of the "immune" cells ($n = 5$ spleen equivalent per group) were treated at 1/20 dilution with anti-Thy 1.1, anti-Lyt 1.1 and anti-2.1 antibody respectively plus rabbit "low-tox" complement at 1/10 dilution (Groups i, ii and iii in Table 2). DTH response and tumour incidence were then assessed in the manner described in the previous section with transfer of irradiated and live MBT-2 cells respectively. For comparison, untreated "immune" spleen cells (Group iv), complement treated cells alone (without antibodies) (Group v) and normal spleen cells (Group vi) were also assessed for DTH response and tumour incidence. Control groups included normal spleen cells only, immune spleen cells only and MBT-2 cells only (Groups vii, viii and ix). The statistical significance of DTH reactivities of mice transferred with "immune" cells treated with antibody plus complement or with untreated "immune" cells (Groups i, ii, iii and iv) was estimated by comparison with DTH of control group transferred with complement only treated "immune" cells (Group v) using Student's *t*-test. Significance between tumour incidence of all groups and control group transferred with tumour cells alone (Group ix) was estimated by Chi-square test.

In vitro assessment of lymphoproliferative activity

Lymphoproliferative activity of untreated "immune" cells and that of antibody and complement-treated (T-cell subsets depleted) "immune" cells was assessed by the standard [^{3}H]thymidine incorporation assay described elsewhere [9] in the presence of TNF-A at a concentration of 100 L.U./ml for 5 days in culture. Stimulation Index (S.I.) = Counts in TNF-A treated cells/Counts in control (untreated cells).

Results

Assessment of TNF + IFN-A treatment plus T-DTH immunotherapy

A marked variation in tumour sizes at day 0 (the starting point of treatment) was observed between each experiment. However, tumours of all study and control groups at day 0 within each experiment were of comparable sizes, thus a valid statistical evaluation of treatment response was possible.

Results illustrating the effects of TNF-A, IFN-A and TNF + IFN-A treatments alone on tumour growth of MBT-2 tumour are shown in Fig. 2.

Inoculation of 2 doses of TNF-A alone (Fig. 2a) into tumour-bearing mice at (days 0 and 7) resulted in a significantly ($p < 0.05$) slowed tumour growth only from day 2 till day 12 following treatment with accelerated tumour growth thereafter. The third dose of TNF-A given

Table 1. Confirmation of DTH reaction and tumour protection

	Mean DTH ± S. E.	Tumour incidence
Spleen cells source		
"Immune" mice	74.8 ± 7.6[a]	0/5[b]
Tumour-bearers	13.2 ± 2.9	5/5
Untreated mice	21.8 ± 2.7	5/5
Controls		
"Immune" spleen cells alone	19.2 ± 4.0	n.a.
Tumour cells alone	14.2 ± 1.9	5/5

[a] $P < 0.01$ by student's t-test
[b] $P < 0.05$ by Chi-square test
n.a. = not applicable

Table 2. Phenotypic characterization of effector cells

	Mean DTH ± S.E.	Tumour incidence
Groups		
i. "Immune" spleen cells + anti-Thy 1.2 + Co.	28.8 ± 10.7[a]	5/5
ii. "Immune" spleen cells + anti-Lyt 1.1 + Co.	38.4 ± 17.8[a]	4/5
iii. "Immune" spleen cells + anti-Lyt 2.1 + Co.	78.2 ± 20.2	1/5[b]
iv. Untreated "Immune" spleen cells	98.0 ± 7.2	0/5[b]
v. Co. alone	70.2 ± 12.2	1/5[b]
vi. Normal spleen cells	33.6 ± 7.7	5/5
Controls		
vii. Normal spleen cells alone	12.5 ± 5.4	n.a.
viii. "Immune" spleen cells alone	13.2 ± 4.3	n.a.
ix Tumour cells alone	25.4 ± 3.2	5/5

[a] $P < 0.01$ by student's t-test
[b] $P < 0.05$ by Chi-square test
n.a. = not applicable

at day 14 had no additional anti-tumour effect. Inoculation of IFN-A alone (Fig. 2b) had no anti-tumour effect and in fact tumour growth enhancement (statistically insignificant) was noted at some time points.

Inoculation of 3 doses of a combination of TNF + IFN-A (Fig. 2b) at days 0, 7 and 14 resulted in a significant ($P < 0.05$) retardation of tumour growth (from day 2 to day 14). At day 16 and onwards difference in tumour sizes between control group was not statistically significant.

Results demonstrating the effect of combined TNF + IFN-A with T-DTH cell adoptive immunotherapy (AIT) are shown in Fig. 2c. Concomitant treatment of tumour-bearing mice with 2 inoculations of admixtures of "immune" T-DTH effector cells and TNF + IFN-A was most effective in slowing tumour growth with the statistical significance ($P < 0.01$) extending over the period of 16 days. Inoculation of a 3rd dose of T-DTH cells and TNF + IFN-A was less effective in retarding tumour growth ($P < 0.05$). Immunotherapy with T-DTH cells alone had no significant delay on tumour growth, when compared to controls. After the interval of measurements (from day 0 to day 16) tumours in all the groups including the ones responding to combined therapy continued with a geometric growth pattern until day 21, at which time point the experiment was terminated.

Confirmation of DTH reaction and MBT-2 tumour protection

Transfer of "immune" spleen cells from DTH-primed mice with antigen (irradiated MBT-2 cells) showed a much stronger DTH reactivity compared to all other "non-immune" groups ($P < 0.01$ by Student's t-test) (Table 1). Similarly, transfer of "immune" spleen cells with live MBT-2 tumour cells demonstrated complete tumour protection against tumour challenge (T.I. = 0/5). In contrast, none of the other groups exhibited tumour protection ($P < 0.05$ by Chi-square test) (Table 1).

Phenotypic characterization of effector cells

Pretreatment with anti-Thy 1.2 or anti-Lyt 1.1 monoclonal antibodies and complement partially abrogated the DTH reactivity of "immune" cells transferred with MBT-2 tumour antigen (Table 2). This was statistically significant when compared with the DTH of "immune" cells treated with complement only (Gr 5) ($P < 0.01$ by Student's t-test). The depletion of the Lyt 1.1^+ subset did not totally abolish the efficacy of the immune population to mediate DTH response (DTH reactivity (38.4 units), and the depletion of Lyt 2.1^+ cells had some effect towards decreasing their DTH efficacy (78.2 units) compared to the DTH level of "immune" spleen cells alone (13.2 units). This indicates that the majority of the DTH effector cells express a Thy 1.2^+, Lyt 1.1^+ ($CD4^+$) phenotype and some express Lyt 2.1^+ ($CD8^+$) phenotype. When inoculated in vivo in admixture with live MBT-2 tumour cells in the Winn adoptive transfer assay, these cells were similarly sensitive to treatment with anti-Thy 1.2 + Co. (T.I. = 5/5) or anti-Lyt 1.1 +Co. (T.I. = 4/5) but not anti-Lyt 2.1 + Co. (T.I. = 1/5), ($P < 0.05$) (Table 2). Transfer of complement alone treated "immune" spleen cells with antigen (irradiated MBT-2 cells) showed both a strong DTH reactivity (70.2 units) and a strong tumour protection against tumour challenge (T.I. = 1/5). This indicates that complement treatment did not abrogate the functional capacity of the "immune" cells. However, the conferred tumour protection was not complete (T.I. upon challenge 1/5 instead of 0/5). This could be due to the fact that a small number of "immune" cells (approx. 5–10% of the total cell population) were non-specifically lysed by complement treatment, thus resulting in a slight decrease of their ability to mediate tumour protection.

These findings indicate that the Lyt 1^+ ($CD4^+$) T-DTH effector cells operating against MBT-2 bladder carcinoma are capable of mediating both DTH reaction to tumour antigens and in vivo tumour protection.

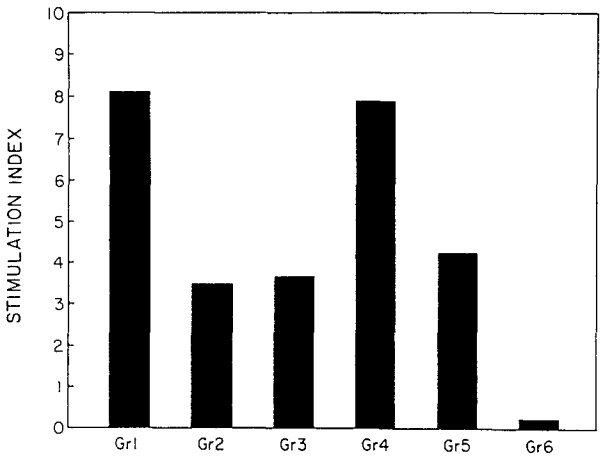

Fig. 3. Lymphoproliferative activity of "immune" cells. Gr 1 = Untreated "immune" spleen cells; Gr 2 = Anti-Thy 1.2 + complement treated; Gr 3 = Anti-Lyt 1.1 + complement treated; Gr 4 = Anti-Lyt 2.1 + complement treated; Gr 5 = Complement treated alone; Gr 6 = Untreated normal cells. $P < 0.001$ by Student's *t*-test (Gr 2 and Gr 3 vs Gr 1)

In vitro assessment of lymphoproliferative activity

In vitro lymphoproliferative response of the "immune" effector cells to stimulation with TNF-A was further assessed by [^{3}H]thymidine incorporation of "immune" cells treated with antiserum and complement in the presence of TNF-A in the culture medium. "Immune" spleen cells treated with anti-Thy 1.2 and anti-Lyt 1.1 plus complement showed a significant decrease in the levels of lymphoproliferative activity ($P < 0.05$) in comparison to that of untreated or anti-Lyt 2.1 and complement treated TNF-A stimulated cells (Fig. 3).

Normal "non-immune" lymphocytes and lymphocytes obtained from spleens of tumour-bearing mice did not show significant levels of proliferation to in vitro TNF-A stimulation.

Discussion

In our earlier studies we had demonstrated that TNF therapy alone had limited activity on MBT-2 murine bladder tumour with emergence of resistance to TNF [9, 10]. Parental MBT-2 (MBT-2P) and the in vitro derived TNF-A resistant (MBT-2R) cells were compared in terms of in vitro sensitivity to TNF-A, DNA profile, karyotype and in vitro growth kinetics. The acquisition of resistance to TNF-A was due to cell cycle derangement and differences in in vitro growth characteristics. DNA indices and karyotype of MBT-2R cells were not altered, indicating the anti-tumour action of TNF-A was not-mutagenic.

As a single agent TNF-A appears to have limited effects against most solid tumours [7]. Synergistic interaction of TNF-A and IFN-A has been demonstrated against human breast and colon cancers [2, 14, 15]. This may involve in part an overlapping direct tumour cell cytotoxicity and in part indirect immunomodulatory effects on the host. Our results show that systemic in vivo administration of multiple doses of admixtures of TNF-A and IFN-A resulted in growth retardation of MBT-2 tumour for a period of 14 days following inoculation, with acceleration of tumour growth thereafter despite an additional dose of TNF + IFN-A administered at Day 14. Such transient impedance in tumour growth indicates that MBT-2 tumours tend to acquire resistance to the exogenous cytokines.

We further explored the possibility of augmenting the anti-tumour effect by using TNF + IFN-A therapy as an adjunct to adoptive immunotherapy with T-DTH "immune" effector cells generated against MBT-2 tumour. A DTH inflammatory reaction may represent an independent anti-tumour mechanism conferring protection against tumours without involving other immune mechanisms [8, 11, 22]. However, the DTH reaction alone may be insufficient in achieving complete anti-tumour protection and supplementary mechanisms may be required. Our results suggest a favourable response of the MBT-2 tumours to a combination of TNF + IFN-A in conjunction with immunotherapy. In vivo administration of 2 doses of admixtures of tumour-specific DTH effector cells with TNF + IFN-A resulted in maximal tumour growth inhibition, thereafter animals showed geometric tumour progression and perhaps if followed for longer intervals (beyond day 21) their tumour sizes might have been comparable to that of controls. This implies that repeated inoculations of TNF + IFN-A and T-DTH cells are required to sustain constant tumour growth inhibition. However, once tumours progress and reach large sizes they tend to develop resistance to immunotherapy and subsequent administration of TNF + IFN-A alone or in combination with "immune" cells becomes ineffective. Immunotherapy with T-DTH cells alone without adjunctive TNF + IFN-A therapy had no effect on tumour growth inhibition. In support of this notion North et al. [18] demonstrated that adoptively transferred "immune" effector cells were incapable of mediating tumour protection in the recipient hosts since the host endogenous suppressor cells downregulated the anti-tumour immune response. We believe that the protective immunity elicited by concomitant administration of TNF + IFN-A with the "immune" cells may be attributed to the shift in the "suppressive balance" in the recipient so that by adding a combination of immunomodulating agents such as TNF-A and IFN-A, the T-DTH effector cells in the recipient are able to express protective activity. Our experiments show that the "immune" effector cells conferring both DTH response to MBT-2 TTAs and in vivo anti-tumour protection have Thy 1^+, Lyt 1^+, 2^+ ($CD4^+$) helper DTH phenotype.

To eliminate the possibility that TNF-A was altering T cell responsiveness indirectly through an action on another cell type and to demonstrate its capacity to amplify responses by Lyt 1^+ cells, subsets of "immune" effector cells were highly purified and depleted of contaminating spleen accessory cell populations. TNF-A appears to have a direct proliferative effect on T cells bearing Lyt 1^+ phenotype although it is still possible that the residual Lyt 2^+ cells and the splenic accessory cells played a role in the TNF-A mediated enhancement of T cell proliferation.

The mechanisms whereby TNF enhances proliferations of specifically sensitized T lymphocytes is not clear. It has been demonstrated that TNF-A can enhance Interlukin-2 (IL-2) production by enhancing high affinity receptor expression on activated T cells but not on resting T cells [24]. These results support the hypothesis that TNF-A enhances the expression of functional IL-2 receptors, thereby facilitating IL-2 dependent T cell growth and proliferation in the host. In addition TNF-A may cause expression of stimulation-dependent TNF-A receptors that could be directly involved in the regulation of T cell activity [24].

Our data indicate a potential role for TNF + IFN-A in the immunoregulation of anti-tumour immune response and demonstrate that concomitant immunotherapy with these cytokines could be useful as adjunctive treatment of murine bladder tumours.

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