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Interferon-α conjugation to human osteogenic sarcoma monoclonal antibody 791T/36

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Summary. *Human lymphoblastoid interferon-α (IFN-α) has been coupled using N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) to a murine monoclonal antibody (791T/36) which reacts with antigens expressed on human osteogenic sarcomas. The purified conjugates retain antibody activity as defined by their capacity to compete with binding of fluorescein isothiocyanate-labelled 791T/36 antibody to 791T cells. IFN-α-791T/36 antibody conjugates synthesized with ^{125}I-trace-labelled IFN-α and ^{131}I-trace-labelled antibody also bound to 791T cells, but not to bladder carcinoma T24 cells. The conjugates also retain the capacity of free IFN to activate natural killer cells in human peripheral blood lymphocytes and show specific localization in human osteogenic sarcoma xenografts developing in immunodeprived mice. These findings establish that conjugates containing IFN linked to a monoclonal antibody reacting with osteogenic sarcoma-associated antigens have potential for targeted immunotherapy and in related investigations with antibody has been shown by gamma camera imaging of patients following infusion of ^{131}I-labelled antibody to localize in primary osteogenic sarcomas.*

Introduction

The development of monoclonal antibodies with potential for producing antibody-drug and antibody-toxin conjugates of high specificity has revived interest in the use of antibodies for targeting anti-tumor agents [2, 23]. The use of monoclonal antibodies as carriers for so-called 'biological response modifiers' has so far received little attention, but this approach may be potentially valuable in immunotherapy as a method of focussing host immune responses in tumours, particularly metastatic deposits. This requirement has been well documented in studies showing that bacterial agents such as BCG, as well as subcellular fractions, exert a more pronounced anti-tumour effect when administered so as to localize in tumour deposits [3, 4, 30]. The concept of targeting immunomodulating agents has been developed further through the use of liposome-encapsulated muramyl dipeptide for localization in, and activation of, lung macrophages in the immunotherapy of pulmonary metastases [16].

Interferons which may act both directly as anti-proliferative agents and indirectly via the augmentation of pre-existing cellular immune mechanisms represent an important class of biological response-modifying agent. There are now numerous studies showing that systemic administration of IFN leads to augmentation of natural killer cells in peripheral blood [18, 22, 25] and the studies reported here were developed to provide methods with potential for targeting IFN to tumour cells for NK cell activation.

The monoclonal antibody selected for these studies, a mouse IgG2b designated 791T/36, was raised against human osteogenic sarcoma cells [11]. This antibody binds to osteogenic sarcoma cells in vitro, quantitative binding studies showing that at saturation, up to 2.3×10^6 immunoglobulin molecules/cell were bound [26]. In vivo distribution studies with ^{125}I- and ^{131}I-labelled antibody have also demonstrated that the antibody localizes specifically in human osteogenic sarcoma xenografts in immunodeprived mice [5, 26], and ^{131}I-labelled antibody has been used to detect primary osteogenic sarcoma in patients by external imaging [15].

Materials and Methods

Interferon. Interferon-α (IFN-α) derived from Namalwa cells (Batches 830/3 and 872/3) was provided by Wellcome Research Laboratories, Beckenham, England. The specific activities of these preparations were 1.6×10^8 and 2.3×10^8 U/mg protein.

Anti-Osteogenic Sarcoma Monoclonal Antibody 791T/36. Supernatants from in vitro-cultured cells of hybridoma 791T/36, clone 3 [11] provided the source of antibody. Antibody was isolated from supernatants by passage through Sepharose-Protein A (Pharmacia, Uppsala, Sweden). The column was extensively washed in phosphate-buffered saline, pH 7.2 (PBS) and antibody eluted with 3 *M* NaSCN. Eluted protein was separated from NaSCN on a Sephadex G25 column and concentrated by positive pressure membrane filtration (Amicon P10, High Wycombe, Bucks., England). Immunodiffusion tests with mouse immunoglobulin typing antisera (Miles Laboratories, Stoke Poges, Bucks, England) identified 791T/36 antibody as IgG2b.

SPDP. N-Succinimidyl-3-(2-pyridyldithio) propionate (SPDP) was obtained from Pharmacia (Uppsala, Sweden).

Preparation of SPDP-Modified IFN. IFN (830/3) was applied to a Sephadex G25 PD-10 column (Pharmacia) equilibrated with PBS and protein eluted with PBS. The absorbance at 280 nm of 0.5-ml fractions was measured and peak fractions

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pooled. Protein concentration of the pool (800 μ/ml) was calculated assuming $E^{1\%}_{1\,cm} = 10$.

An aliquot of the pool (125 μg/ml) was labelled with ^{125}I (500 μCi) using Iodogen [26]. Briefly, Iodogen was dissolved in methylene chloride to give a final concentration of 40 μg/ml, and 0.3 ml of this solution was added to a conical polypropylene tube and the tube dried using nitrogen.

The sample to be iodinated was added to the tube followed by ^{125}I-Na (for protein iodination, Amersham International, Amersham, England) and the reaction allowed to proceed for 15 min at room temperature. Free ^{125}I-iodine was removed by applying the iodinated sample to a PD-10 column equilibrated with PBS and the labelled protein was eluted in 0.5-ml fractions with PBS. Radioactivity was measured in aliquots of each fraction and peak tubes were pooled. The two peak tubes of ^{125}I-labelled IFN were pooled and 0.8 ml of this pool (303,580 cpm/5 μl) was added to the remaining 2.4 ml of unlabelled IFN. SPDP (112 μl : 0.28 mg) freshly dissolved in ethanol was added to ^{125}I-trace-labelled IFN (3 ml : 1.8 mg : 77,020 cpm/5 μl) with vigourous mixing. After 30 min incubation at room temperature, the solution was applied to a PD-10 column equilibrated with PBS. The SPDP-modified protein was eluted with PBS and 0.5-ml fractions collected. Radioactivity was measured in a 5-μl aliquot of each fraction and peak fractions were pooled to give a 3-ml pool (53,100 cpm/5 μl) of SPDP-modified IFN.

Preparation of Reduced SPDP-Modified 791T/36 Monoclonal Antibody. An aliquot of 791T/36 monoclonal antibody (150 μl : 210 μg) was labelled with ^{131}I (200 μCi) using the Iodogen method [26].

The two peak tubes of ^{131}I-labelled 791T/36 antibody eluting from a PD-10 column were pooled and 0.8 ml of this pool (281,220 cpm/5 μl) was added to 0.8 ml (1.1 mg) of unlabelled antibody.

To the ^{131}I-trace-labelled antibody preparation (1.2 mg : 141,890 cpm/5 μl) was added, with vigourous stirring, a 12-fold molar excess of SPDP freshly dissolved in ethanol. After 30 min incubation at room temperature, the solution was applied to a PD-10 column equilibrated with 0.1 *M* sodium acetate buffer, pH 4.5, containing 0.1 *M* sodium chloride. The SPDP-modified antibody was eluted with this buffer and 0.5-ml fractions collected. Radioactivity was measured in 5-μl aliquots of each fraction and peak fractions were pooled to give a 1.5-ml sample (70,800 cpm/5 μl) of SPDP-modified antibody.

The 2-pyridyl disulphide groups which had been introduced into the antibody by SPDP treatment were converted to free thiol groups by reduction with dithiothreitol (DTT). To the 1.5-ml pool of SPDP-modified antibody was added 170 μl 0.5*M* DTT to give a final concentration of 50 m*M*. The solution was incubated at room temperature for 10 min and the increase in absorbance at 343 nm monitored. After incubation, the reduced SPDP-modified antibody was applied to a PD-10 column equilibrated with PBS, the reduced antibody eluted with PBS, and 0.5-ml fractions collected. Radioactivity was measured in a 5-μl aliquot of each fraction and peak fractions were pooled to give a 2-ml sample (31,690 cpm/μl) of reduced antibody. From the increase in absorbance at 343 nm, it was calculated that there was an average of five 2-pyridyl disulphide residues per antibody molecule.

Reaction of SPDP-Modified IFN with Reduced SPDP-Modified 791T/36 Monoclonal Antibody. Antibody with free thiol groups (1.3 ml : 0.35 mg) was added to SPDP-modified IFN (1 ml : 0.41 mg). The mixture was incubated for approximately 3–5 h at room temperature and then a further 18 h at 4° C. The mixture was then applied to a calibrated Sephacryl S-200 column (2.6 cm × 78 cm) equilibrated with PBS. The column was eluted with PBS with an ascending flow rate of 15 ml/h and 2.1-ml fractions were collected. The column was calibrated under the same conditions with the following standards: dextran blue, catalase, huma γ-globulin, BSA, ovalbumin, trypsin inhibitor, and cytochrome c.

The effluent was continuously monitored for absorbance at 280 nm and the radioactivity was measured in a 0.5-ml aliquot of each fraction. The indicated fractions (Table 1) were pooled, concentrated and dialysed overnight against PBS. Samples were aliquoted into small volumes and stored at −70° C to use.

Antibody-Binding Assays. Target cells used for antibody binding tests were human osteogenic sarcoma 791T and bladder carcinoma T24 cells. These cell lines were grown as monolayers in Eagle's minimum essential medium supplemented with 10% fetal calf serum. Trypsin-harvested target cells were diluted in 1% (w/v) bovine serum albumin in Hank's balanced salt solution (Hank's BSA), aliquoted at 10^5 cells/well in round bottomed Sterilin M24A microtest plates and sedimented by centrifugation. Supernatants were removed from wells and cells resuspended in 100 μl of antibody or IFN conjugate solution (100 μl) labelled with ^{131}I and also ^{125}I for IFN. After incubation for 1 h on ice, cells were again sedimented by centrifugation, washed four times in Hank's BSA and dried. Plates were then sprayed with a plastic film to fix the cells and the wells cut up and radioactivity counted.

The reactivity of fluorescein isothiocyanate (FITC)-labelled 791T/36 antibody (FITC : IgG ratio 4.6 : 1) with tumour cells was analysed by flow cytofluorimetry [26]. Briefly, 791T target cells (10^5) were incubated with saturating amounts of FITC-791T/36 antibody (500 μg) and analysed in a FACS IV flow cytofluorimeter (Becton Dickinson, Sunnyvale, CA, USA). The specificity of the reaction of FITC-791T/36 antibody with 791T cells was assessed by a competitive bindung assay with unlabelled 791T/36 antibody. This competitive binding of 791T/36 antibody with target cells was then used to evaluate the reactivity of IFN-antibody conjugates.

NK Cell-Mediated Cytotoxicity Assay. Details of this assay have been described [9]. ^{51}Cr-labelled target cells (human erythroleukaemic cell line K562) were aliquoted into Falcon

Table 1. Composition of interferon-791T/36 antibody conjugate

Pool[a]	791T/36 antibody[b] μg	Interferon[c] μg	Molar ratio IFN : Antibody
1 (71–79)	15.6	12.5	6.0
2 (80–84)	12.5	4.6	2.7
3 (85–89)	6.7	3.1	1.0

[a] Figures in parenthesis indicate the fractions pooled to yield each fraction (Fig. 2a)

[b] Antibody content determined by radioactivity assay of ^{131}I from trace-labelled ^{131}I-791T/36 antibody

[c] IFN content determined by assay of ^{125}I from trace-labelled ^{125}I-IFN (see *Materials and Methods*)

3034 flat-bottom microtiter plates at 5×10^3 cells/well. Appropriate numbers of peripheral blood lymphocytes isolated by the Ficoll-Hypaque technique were added to give effector : target cell ratios ranging from 50 :1 to 1.5 : 1, three to four ratios being used in each test. Cytotoxicity measured after 4 h at 37° C by release of ^{51}Cr into the supernatant was expressed as the slope of the linear regression line fitted to the plot of cytotoxicity versus effector cell number. Lymphocytes were incubated with IFN or IFN-antibody conjugates for 1 h at 37° C and augmentation of NK cytotoxicity determined as the ratio of

$$\frac{\text{slope treated-lymphocyte cytotoxicty}}{\text{slope untreated lymphocyte cytotoxicity}} \times 100 .$$

In Vivo Organ Distribution of IFN-791T/36 Antibody Conjugate in Osteogenic Sarcoma 791T Xenografts. Osteogenic sarcoma 791T xenografts were established in CBA mice (Bantin and Kingman, Hull, UK) immunodeprived by thymectomy at 3–4 weeks of age, whole-body γ-irradiation from a ^{60}Co source (9 Gray), and IP treatment with cytosine arabinoside (200 mg/kg) to prevent the lethal effect of irradiation [26, 29]. Groups of three to four mice bearing 791T xenografts were inoculated IP with ^{125}I-labelled 791T/36 antibody alone or conjugated to IFN. Drinking water was supplemented with NaI (0.1% v/v) throughout the experiment to minimize thyroid uptake of radioiodine. Mice were killed 2 days after antibody infusion and organs dissected. Results are expressed as a ratio of injected radioactivity/g tissue to the radioactivity/g blood (tissue : blood ratio).

Results

Interferon Conjugation to Anti Osteogenic Sarcoma Antibody 791T/36

Conjugates prepared as described by reaction of reduced SPDP-791T/36 antibody with SPDP-modified interferon yielded two major peaks following chromatography on Sephacryl S200 (Fig. 1). The major peak migrated at a position equivalent to that of IgG in calibration studies, the other peak eluting before this. These components were further defined by the analysis for ^{131}I (to detect trace-labelled 791T/36 antibody) and ^{125}I (to detect trace-labelled IFN) (Fig. 2a). The major ^{131}I-labelled peak (II) eluted at a position corresponding to that of ^{131}I-labelled 791T/36 antibody in the calibration curve (Fig. 2b). IFN (^{125}I-labelled) eluted as a broad band after 791T/36 antibody in the calibration curve (Fig. 2b). In comparison, the major ^{125}I-containing product obtained following reaction of SPDP-modified IFN with 791T/36 antibody eluted in peak I in association with ^{131}I-labelled antibody (Fig. 2a).

Three fractions were obtained following Sephacryl S200 chromatography on the conjugates: Pool 1 (fractions 71–80), Pool 2 (fractions 80–84), and Pool 3 (fractions 85–89). Table 1 summarizes the composition of each pooled fraction as determined from assays of ^{131}I-labelled 791T/36 antibody and ^{125}I-labelled IFN.

Antibody Reactivity of Conjugates

The antibody reactivities of the pooled fractions obtained following conjugation of IFN to 791T/36 antibody were assayed by determining their capacity to compete with the binding of fluorescein isothiocyanate-linked 791T/36 antibody (FITC-791T/36) to 791T target cells, this being detected by flow cytofluorimetry. The distribution of fluorescence intensities of 791T target cells reacted with FITC-791T/36 antibody is illustrated in Fig. 3. In competitive binding tests, the fluorescence/cell was reduced when tumour cells were reacted with FITC-791T/36 antibody (500 ng) mixed with unlabelled 791T/36 antibody (500 ng). Figure 3 illustrates tests showing that IFN-791T/36 (Pool 1) sample (500 ng) produces a similar

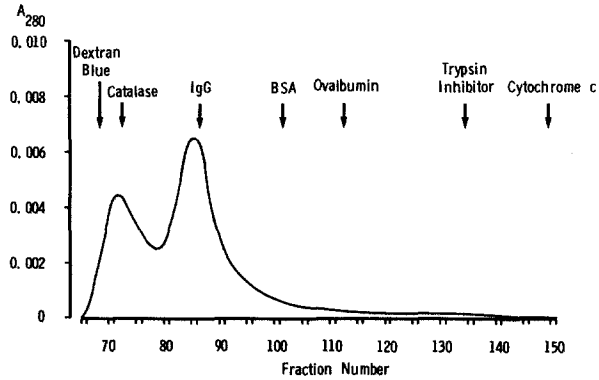
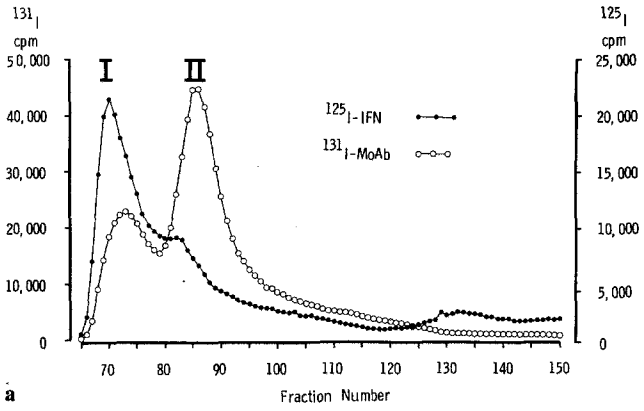


Fig. 1. Sephacryl S200 chromatography of IFN-α-791T/36 antibody conjugate



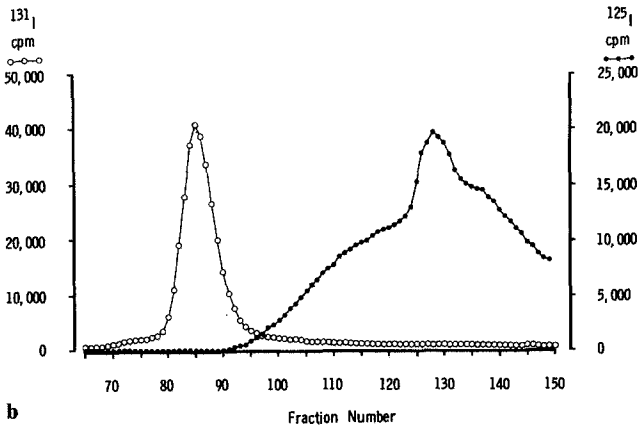


Fig. 2. (a) Sephacryl S200 chromatography of conjugate prepared using IFN-α-trace-labelled with ^{125}I and 791T/36 antibody trace-labelled with ^{131}I; **(b)** Sephacryl S200 chromatography of a mixture of ^{125}I-labelled IFN-α and ^{131}I-labelled 791T/36 antibody

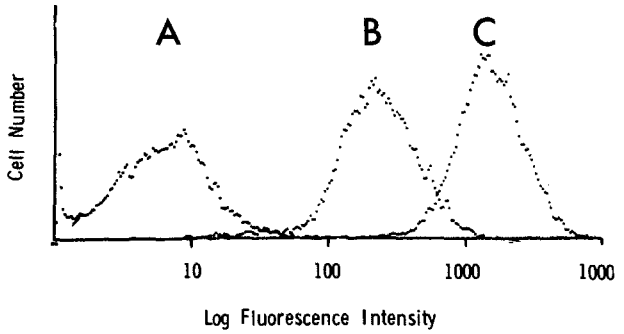


Fig. 3. Flow cytofluorimetric analysis of osteosarcoma 791T cells. Treated with medium (A), FITC-labelled 791T/36 antibody + IFN-791T/36 conjugate (B), or FITC-791T/36 antibody (C)

Table 2. Competitive binding of IFN-791T/36 antibody and 791T/36 antibody with osteogenic sarcoma 791T cells

Competing[a] antibody	Amount (ng)	Mean fluorescence/ cell (channel no.)	Reduction in uptake of FITC-791T/36 antibody (%)
791T/36	1,500	343	85
	500	757	68
	167	1,310	44
IFN-791T/36 (Pool 1)	1,500	244	90
	500	694	70
	167	1,370	41
IFN-791T/36 (Pool 2)	1,500	173	93
	500	638	72
	167	1,198	49
IFN-791T/36 (Pool 3)	1,500	279	88
	500	610	74

[a] IFN-791T/36 antibody conjugates were tested for their capacity to compete with FITC-791T/36 antibody uptake and this was compared with the competition shown by unconjugated 791T/36 antibody. (See *Materials and Methods*)

reduction in FITC-791T/36 antibody binding to tumour cells. The results derived from tests on the competitive binding of antibody fractions from the IFN conjugation (Pools 1–3) in comparison with unconjugated 791T/36 antibody are summarized in Table 2. Each of the three fractions retained antibody and the conjugation procedure did not lead to any loss of activity.

Binding of antibody fractions to 791T cells was also determined by radioimmunoassay using IFN-791T/36 conjugates containing trace-labelled antibody (^{131}I) and IFN (^{125}I) (Fig. 4). Pool-1 fraction reacted with 791T cells as assessed both by binding of ^{125}I-IFN and ^{131}I-antibody. It should also be noted that the degree of binding of IF (^{125}I) and antibody (^{131}I) was proportional at the two doses of antibody conjugate tested. In controls, no binding of either ^{131}I or ^{125}I was observed with bladder carcinoma T24 cells which do not express the 791T/36 antibody-defined antigen.

Antibody in Pool-2 and Pool-3 fractions also bound to 791T cells as determined from the uptake of ^{131}I, and again these fractions did not bind to T24 cells (Fig. 5). However, there was no concomitant binding of ^{125}I-IFN with either of these fractions, indicating that they do not contain significant amounts of IFN conjugated to antibody.

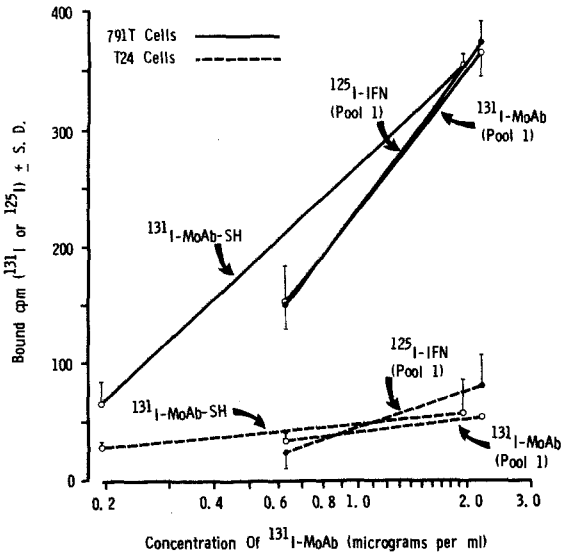


Fig. 4. Radioimmunoassay of binding of IFN-α-791T/36 antibody conjugate (Pool 1 fraction) to osteogenic 791T cells and bladder carcinoma T24 cells. Conjugate prepared using ^{125}I-labelled IFN and ^{131}I-791T/36 antibody (MoAb). ^{125}I-IFN (Pool 1) ^{125}I detects cell binding of IFN in conjugate. ^{131}I detects cell binding of 791T/36 antibody moiety in conjugate. ^{131}I-MoAb-Sh indicates target cell binding of 791T/36 antibody treated with SPDP and reduced with dithiothreitol

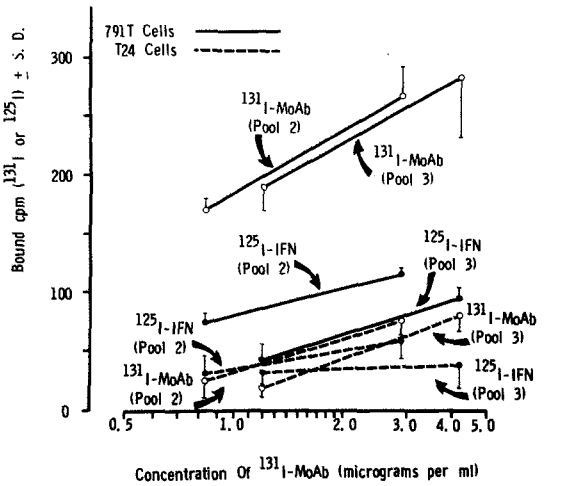


Fig. 5. Radioimmunoassay of binding of IFN-791T/36 antibody conjugates (Pool-2 and Pool-3 fractions) to osteogenic sarcoma 791T cells and bladder carcinoma T24 cells. ^{131}I-MoAb (Pool 2) and ^{131}I-MoAb-Pool 3) detect binding of antibody moiety in fractions; ^{125}I-IFN (Pool 2) and ^{125}I-IFN (pool 3) detect binding of IFN in fractions

Augmentation of NK Cell Activity by IFN-791T/36 Antibody Conjugates

Figure 6 shows the effect of pre-incubatin PBL with IFN-791T/36 conjugate prior to testing for NK-cell activity against the erythroleukaemic cell line K562. Cytotoxic activity, expressed as the slope of the regression line fitted to the plot of

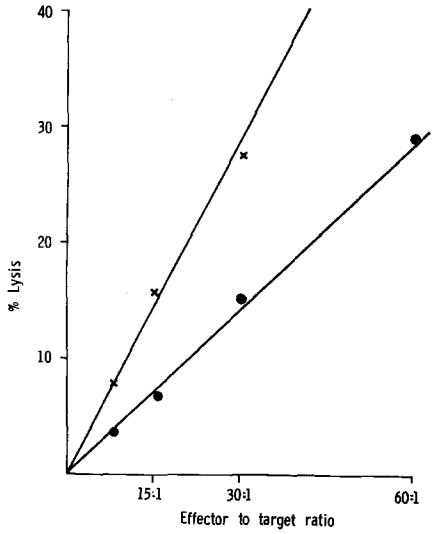


Fig. 6. Augmentation of NK-cell cytotoxicity of human peripheral blood lymphocytes from a normal donor following treatment with interferon (●———●) or IFN-α-791T/36 antibody conjugates (×———×)

Table 3. Augmentation of NK cell-mediated lysis of K562 by human peripheral blood lymphocytes with IFN-α-791T/36 antibody conjugates

Expt	IFN-α-791T/36 antibody conjugate	PBL pre-incubated with[a]	Cytotoxicity[b] (slope ± SD)	Augmentation[c] (%)
1	B1	medium	12.82 ± 0.11	–
		IFN-α	36.11 ± 1.66	281.6*
		IFN-791T/36	25.06 ± 2.03	195.4*
2	B1	medium	15.80 ± 0.03	–
		IFN-α	25.07 ± 0.13	158.8***
		IFN-791T/36	26.35 ± 0.32	166.9***
3	B2	medium	13.17 ± 0.59	–
		IFN-α	30.86 ± 1.37	234.4***
		IFN-791T/36	23.04 ± 1.19	175.0**
4	B2	medium	12.82 ± 0.60	–
		IFN-α	22.63 ± 0.40	176.5***
		IFN-791T/36	24.96 ± 1.29	194.7***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

[a] Human PBL pre-incubated 1 h at 37° C with medium, IFN-α (100 IU) or IFN-791T/36 (80 ng) washed × 3 and tested at four effector to target ratios.

[b] Cytotoxic activity expressed as the slope of the regression line fittet to the plot of cytotoxicity vs effector cell number (see text)

[c] Augmentation calculated as $\frac{\text{slope treated}}{\text{slope untreated}} \times 100$

cytotoxicity vs effector cell number [9], is increased 2-fold by preincubation with conjugate at 80 ng protein/5 × 10^6 cells. In a further series of such experiments, similar augmentation of NK-cell killing was observed, ranging from 1.5- to 2-fold increases. Four of these experiments are detailed in Table 3 and these indicate the augmentation resulting from the conjugate bound IFN compared with a standard dose (100 U) of free IFN. We have previously found (data not shown) that preincubation of PBL with 100 U IFN evokes optimal

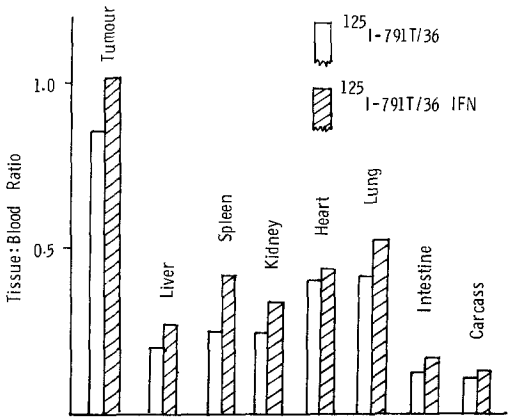


Fig. 7. Distribution of ^{125}I-791T/36 and ^{125}I-791T/36-IFN-α conjugate (2 μg of each) in groups of mice with subcutaneous osteogenic sarcoma 791T xenografts. Three to four mice/group, mean tumour weight 92 mg. Mean % of injected doese of radiolabelled/g of tumour tissue 11.8% (free antibody) and 14.1% (conjugate), analysed 2 days after injection

augmentation against K562 and other human targets, and these data indicate that 80 ng of IFN-791T/36 conjugate is able to provide similar levels of NK-cell activation.

Distribution of ^{125}I-Labelled 791T/36 Antibody and IFN Conjugates in Tumour Xenografts

Figure 7 compares the organ distribution of ^{125}I-labelled 791T/36 antibody and ^{125}I-791T/36-IFN conjugates in immunodeprived mice bearing osteogenic sarcoma xenografts. These organ distribution studies, performed 2 days after antibody infusion, demonstrated comparable uptake of free antibody (whole-body survival of radioactivity 35.7% ± 2.36%) with that of IFN conjugate (whole-body survival of radioactivity 41.3% ± 4.0%). With both free antibody and IFN conjugate the highest levels of radioactivity in non-tumour tissue were in the lung, but compared with this the tumour : non-tumour ratio of radiocativity was 2 : 1 with both preparations.

Discussion

Agents which augment natural killer cells and/or activate macrophages are being developed for tumour immunotherapy. But for these effector cells to exert anti-tumour effects, they either have to be stimulated within tumours, or following peripheral activation, traffic to and localize within tumours. This requirement has been well established in numerous trials with bacterial vaccines or subcellular fractions, such as cell wall preparations where deposition of agents in tumours often provokes a marked anti-tumour response [3, 4]. In contrast, systemic treatment is ineffective, or at best, induces a weak anti-tumour response. This is illustrated by the use of liposomes containing muramyl dipeptide or muramyl tripeptide to activate alveolar macrophages, so causing significant destruction of murine B16 melanoma lung metastases [20].

These considerations have led to the proposal that monoclonal antibodies reacting with human tumour-associated antigens may be used to target to tumour immunomodulating agents like interferons and lymphokines which modulate a range of host responses. This forms the basis of the present

studies to develop conjugates of IFN-α with monoclonal antibody 791T/36. This antibody, prepared against an antigen associated with human osteogenic sarcoma 791T, localizes specifically in human osteogenic sarcomas developing as xenografts in immunodeprived mice [5, 26]. Radiolabelled 791T/36 antibody also localizes in primary human osteogenic sarcoma, gamma-camera imaging indicating a tumour : non-tumour ratio of radioactivity of 5 : 1 [7, 15].

Conjugation of IFN-α to 791T/36 antibody yielded a range of products, including IFN conjugate together with free antibody and IFN (Fig. 1). The use of trace-labelled ^{125}I-IFN and ^{131}I-antibody facilitated the development of separation techniques (Fig. 2a) which yielded IFN conjugates retaining antibody activity as determined by binding to target tumour cells. Moreover, determination of the capacity of IFN-791T/36 antibody conjugates to compete with the binding of FITC-labelled 791T/36 antibody to tumour cells indicated that conjugation to IFN caused little loss, if any, of antibody reactivity. This is consistent with related studies in which conjugation of the anti-mitotic agent vindesine to 791T/36 antibody produced no appreciable destruction of antibody reactivity [12, 28]. Also conjugates prepared by linking antibody to methotrexate bound to a human serum albumin spacer molecule retain antibody reactivity [17].

IFN-α-791T/36 antibody conjugates also retained the IFN capacity to augment NK activity of human peripheral blood lymphocytes (Table 3). Using the erythroleukaemic cell line K562 as target cell, optimal augmentation of NK activity was obtained following incubation of human PBL (5×10^6 cells) with 100 IU IFN (Flannery: unpublished findings). Under these conditions IFN treatment produced 2- to 3-fold increases in lysis of K562 cells. This is comparable to the effects on NK cell-mediated lysis produced by other IFN preparations, including recombinant human leukocyte IFN [19] and fibroblast IFN [24]. A direct comparison of IFN-791T/36 conjugate with free IFN is not possible because of the inherent difficulties in defining purity of IFN preparations derived from the human lymphoblastoid cell line Namalwa [1]. However, a comparable degree of augmentation of NK activity to that observed with IFN was obtained following incubation of PBL with IFN-791T/36 conjugate (80 ng antibody proteins/5×10^6 cells). This amount of antibody protein is well within the range which can be deposited in osteogenic sarcoma 791T xenografts [5, 26]. For example, it was found that following IP inoculation of ^{125}I-labelled 791T/36 antibody amounts of antibody ranging from 450 to 1,200 ng were deposited in 200-mg tumours. It has recently been established (Pimm and Baldwin, unpublished findings) that when large doses (up to 2 mg) of radiolabelled 791T/36 antibody is administered to 791T xenograft-bearing mice the maximum level of antibody deposited in the tumour is 70–80 μg/g tissue. Relative to these findings, comparable levels of antibody deposition in osteogenic sarcoma 791T xenografts were obtained when ^{125}I-labelled 791T/36 antibody and ^{125}I-labelled IFN-α-791T/36 conjugates are inoculated IP into tumour-bearing mice (Fig. 7). Further experiments are required to examine more precisely the distribution and biological half-life of IFN791T/36 antibody conjugate, since these might be expected to vary from those of free antibody due to the increased molecular weight of conjugate. In addition, it is necessary to define the degree of localization and peristence of the IFN moiety of the antibody conjugates, since this will be influenced by the in vivo stability of the linkage obtained using the heterobifunctional reagent SPDP [10]. Immunotoxins prepared using SPDP to link toxins to monoclonal antibodies have proved to be relatively stable in vivo as evidenced by their therapeutic effects against tumours [21]. Also, in vitro studies with IFN-α-791T/36 antibody have established that conjugates bound to 791T tumour cells retain the capacity to activate NK cells against K562 as a second-party target cell [6].

The results presented in this paper indicate that immunomodulating agents like interferon linked to anti-tumour monoclonal antibodies have potential for 'targeted immunotherapy', In this approach the deposition of immunomodulating agents will be dependent upon the tumour-localizing characteristics of the monoclonal antibody. In this context, there are now several reports of gamma-camera imaging of patients following infusion of radiolabelled antibodies showing specific, or more usually preferential, localization in human tumours [8, 13–15]. The next stage in developing targeted immunotherapy will be to select appropriate immunomodulating agents and design methods for antibody conjugation. These approaches are being developed to evaluate antibody targeting not only of interferon, but also interleukins, especially interleukin-2, in view of related studies [27] indicating that the latter factor is an important component in the pathway leading to specific rejection of tumours. Provided that problems possibly associated with clinical administration of heterologous antibody protein can be overcome this approach may have considerable potential for specific tumour targeting of biological response modifiers.

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