Urol Res (1992) 20:229–232

Urological Research


# Expression of interferon-γ receptors on bladder cancer cells: does it correlate with biological response?

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Accepted: November 1, 1991

**Summary.** Previously we have shown a differential biological response of three human bladder cancer cell lines (RT4, RT112 and MGH-U1) to gamma interferon (IFN-γ). The present study examines the relationship between the biological response and the expression of the interferon-γ receptor on the tumour cell surface. Using a competitive radioligand binding assay and Scatchard analysis, we measured the number and affinity of the IFN-γ receptors on each of the above cell lines. Individual cells from each line expressed large numbers (29,100–41,800) of high-affinity receptors ($k_d = 2.4–3.9 \times 10^{10}$M). There was no statistically significant difference in either of these parameters between the three lines. We therefore conclude that the biological response of these bladder lines to IFN-γ does not relate to the number or affinity of its receptor on the plasma membrane of these tumour cells.

**Key words:** Interferon-γ – Receptors – Bladder cancer

Intravesical gamma interferon (IFN-γ) has begun to be evaluated in the treatment of superficial bladder cancer [3]. IFN-γ is also found in high levels in the urine of patients with this disease who are treated with intravesical BCG and may contribute to the overall success of this form of immunotherapy [10]. Both these treatment regimens would benefit from a measurement that could predict the response to therapy.

Previously we have shown that IFN-γ has inhibitory effects on the growth of three different bladder cancer cell lines, in addition to enhancing or inducing the expression of class II antigens on their cell membranes [5, 6]. Although each cell line responded to these in vitro actions of IFN-γ, there were large differences in susceptibility, RT4>RT112>MGH-U1. In this study we have examined the relationship between the biological response to IFN-γ treatment and the expression of the IFN-γ receptor on the cell membrane of three different bladder cancer cell lines.

## Materials and methods

### *Cell lines*

Three human transitional cell carcinoma lines, namely RT4, RT112 and MGH-U1, were obtained from Dr. J. Masters, Institute of Urology, London. These were originally derived from primary bladder tumours of histopathological grades 1, 2, and 3, respectively. All three lines were routinely grown as monolayers in RPMI 1640 medium, supplemented with 5% fetal calf serum, sodium pyruvate (5 mM), and the antibiotics penicillin (50 U $ml^{-1}$), streptamycin (50 μg $ml^{-1}$) and amphoteracin B (2.5 μg $ml^{-1}$).

### *Interferon-γ*

Recombinant human IFN-γ ($25 \times 10^6$ U $mg^{-1}$) was purchased from Boehriner Mannheim (FRG). The (3-[$^{125}$I]iodotyrosyl) IFN-γ (specific activity 730 Ci $mmol^{-1}$) was purchased from Amersham Laboratories (Amersham, s., Buck UK).

### *$^{125}$I-IFN-binding assay*

A competitive radioligand-binding assay was performed on each cell line in triplicate. The optimum number of cells for use in the assay was determined for each line by measuring the specific binding of the appropriate amount of $^{125}$-I-IFN-γ with increasing numbers of cells.

Cell monolayers were dispersed using 0.02% EDTA, washed in complete medium and resuspended at $10^6$ $ml^{-1}$. A fixed amount of $^{125}$I-IFN-γ (in 100 μl) was added to $2 \times 10^5$ cells, and its binding determined in the presence of increasing doses of unlabelled IFN-γ (0.1–30 ng). The amount of radiolabelled IFN-γ varied slightly between individual experiments (0.2–0.3 ng), but was kept constant within each assay. The non-specific binding of $^{125}$I-IFN-γ was determined in the presence of a 200-fold excess of unlabelled IFN-γ. The final reaction volume was 500 μl, and the incubation took place in plastic test tubes at 4°C for 2 h. The cells were then washed twice in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA), and the remaining bound activity in the cell pellet measured on a gamma counter (LKB, Wallac, Finland). Data were analysed using a Scatchard plot from which the affinity and number of receptors per cell were calculated [12]. It has been shown that active IFN-γ exists in both the monomeric (molecular weight 17,200) and dimeric forms (molecular weight 34,000) [8]. For the purpose of the calculations we have presumed that the dimeric form

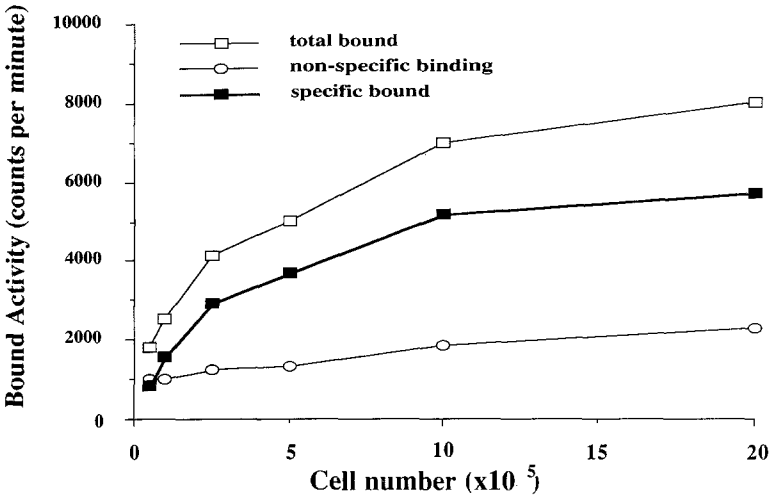



**Fig. 1.** Effect of cell numbers on the amount of gamma interferon (IFN-γ) bound. A fixed amount of $^{125}$I-IFN was added to increasing numbers of cells, and incubated at 4°C for 2 h. Non-specific binding was determined for each concentration of cells in the presence of a 200-fold excess of IFN-γ. The plotted points represent the means of triplicate values. Specific counts bound were calculated by subracting the non-specific binding from the total. For subsequent assays, the number of cells that bound 50% of the IFN-γ was used, i.e. $2\times10^5$

binds to the receptor. Normal human erythrocytes were used as a negative control. Assays were repeated five times for each cell line over a period of several weeks.

### *Statistics*

Linear regression equations were calculated for each Scatchard plot using an Apple Macintosh computer and the dedicated Cricket Graph program.

The differences in the receptor number and affinity between each possible pair of lines were analysed using the nonparametric Wilcoxon rank sum test.

## Results

The optimum number of cells for the assay was determined for each cell line by adding a fixed amount of $^{125}$I-IFN (0.2–0.3 ng) to increasing numbers of cells (Fig. 1). The number of cells that bound approximately 50% of the ligand was chosen. The number of cells used was the same for each cell line ($2\times10^5$).

Human erythrocytes did not shown any specific competable binding, and were considered without IFN-γ receptors. The three human bladder tumour lines, on the other hand, all expressed high-affinity receptors in large numbers. Table 1 summarizes the results. The means are of five different assays, each performed in triplicate. The correlation coefficients for the Scatchard plots were calculated, and the *P*-values ranged from $<0.05$ to $<0.001$, making them significant to at least the 5% level. The non-specific binding of $^{125}$I-IFN-γ ranged between 9% and 34% of the total bound, and tended to be higher in the RT4 cell line. Examples of the competing out of $^{125}$I-IFN-γ with unlabelled IFN-γ and the resulting Scatchard plots are shown for each line in Figs. 2–4.

Each cell line shows a wide variation in both affinities and receptor numbers, and although there are small differences in the means of these parameters, they do not reach statistical significance ($P>0.2$). Daudi cells (a B lymphoblastoid cell line) were used as a positive control in our assay. They expressed significantly lower numbers of receptors per cell (mean of 4,200) with higher affinities (mean $K_d$ of $0.6\times10^{-10}$ M) than the bladder cancer cells.

**Table 1.** Binding of gamma interferon (IFN-γ) to human bladder cancer cell lines, erythrocytes and Daudi cells

| Cell line | $K_d$ ($\times10^{-10}$M) | | Receptor number | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| RT4 | 2.4 | 0.9–4.3 | 32,000 | 11,900–48,800 |
| RT112 | 2.6 | 0.8–4.2 | 29,000 | 10,500–39,800 |
| MGH-U1 | 3.9 | 0.9–5.9 | 41,800 | 14,300–70,500 |
| RBCs | 0 | | 0 | |
| Daudi | 0.6 | 0.5–0.8 | 4,200 | 2,800– 6,100 |

## Discussion

These results indicate that high affinity IFN-γ receptors are present in large numbers on the surface of the three bladder cancer cell lines. However, there is no statistical difference between the concentration of the receptors in the three lines, and therefore the differential biological response of these lines to IFN-γ [5, 6] cannot be accounted for by the level of receptor expression.

It is commonly accepted that IFN-γ acts by initially binding to a specific receptor on the plasma membrane [15]. There is a single, high-affinity, human IFN-γ receptor [13], which does not bind either IFN-α or IFN-β [9]. Ücer et al. have studied the expression of IFN-γ receptors on a range of different tumour cell types, including both cell lines and fresh tumour samples [14]. Only 6 of 77 types failed to express the receptor, and all of these negative tumours were either of leukaemic or lymphoid origin. It seems from this and other similar studies [1] that the majority of cells in the body express IFN-γ receptors, and that at least amongst the lymphoid and haematological cells, receptor expression is lower in normal than in malignant or transformed cells [13]. Expression of IFN-γ receptors on cells that are both responsive and resistant to the biological actions of IFN-γ clearly indicate that although the possession of the receptor is a prerequisite, it is not sufficient to confer sensitivity [13]. Following

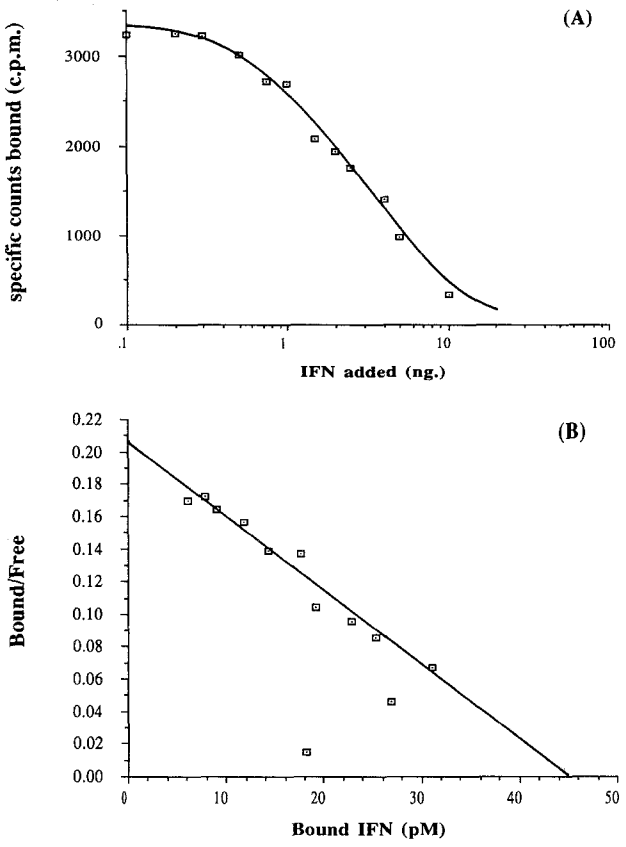


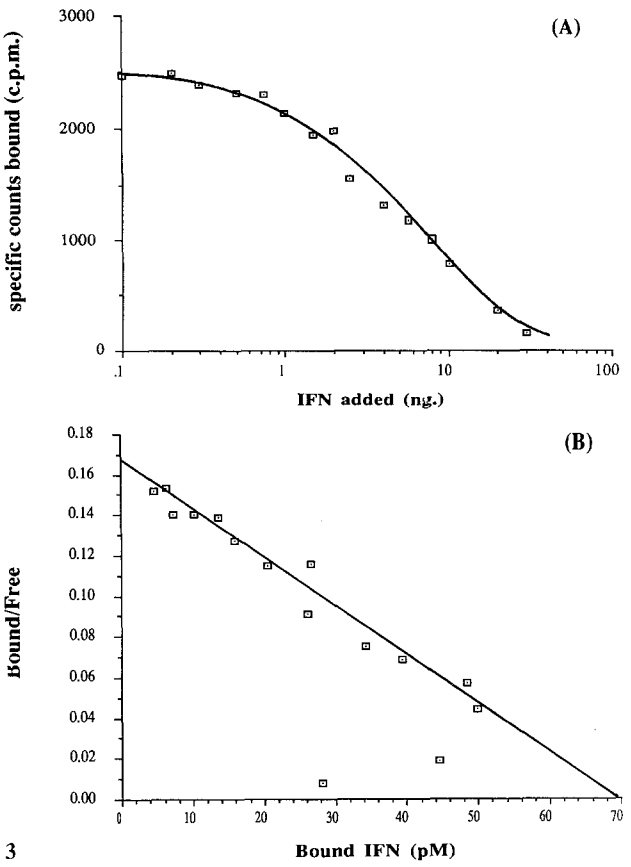

**Fig. 2.** A The amount of $^{125}$I-IFN-γ (in counts per minute) that specifically bound to $2 \times 10^5$ RT4 cells in the presence of increasing amounts of unlabelled IFN-γ. The reaction was allowed to continue for 120 min at 4°C, before the cells were washed and the radioactivity measurement using a gamma counter. Each *point* represents the mean of triplicate samples. B Scatchard plot of the values derived from the assay in A. The *lowest two plotted points* (bound/free values of 0.046 and 0.018) show the tailback phenomenon, caused by overestimation of non-specific binding. The dissociation constant, $k_d$, and the number of receptors per cell were calculated from the gradient of the line, and the *x*-axis intercept ($B_{max}$). The number of receptors per cell =

$$B_{max}\ (\text{mol}) \times \frac{\text{Avogadro's number}}{\text{Number of cells}}$$

Assuming that IFN-γ is bound in its dimeric form this value is divided by 2

binding of IFN-γ to its receptor, the second message events leading to effects at the DNA level remain unclear. It appears that differences in the biological responses of individual tumours to IFN-γ are determined downstream of the receptor-ligand interaction. We are currently examining such possible events.

In the present study, we have used a competitive binding assay. The results show a wide range of affinities and surface receptor numbers for the individual cell lines. This has also been the experience of investigators who have used non-competitive assay systems [13, 14]. The same investigators have found a difference of a factor of

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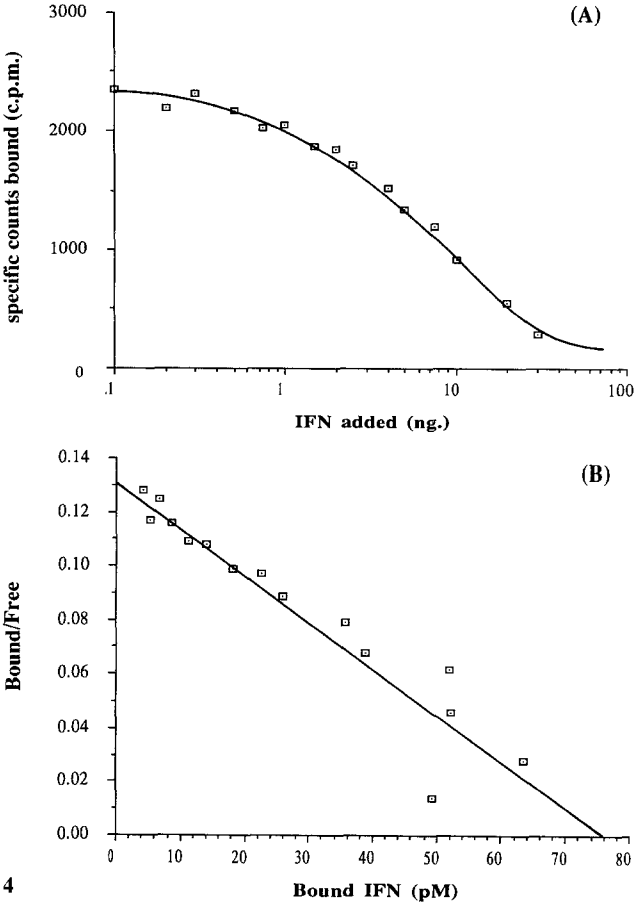


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**Fig. 3. A, B.** A representative assay for RT112

**Fig. 4. A, B.** A representative assay for MGH-U1 ▶

10 in the affinities of the same cell lines at different times. Such differences have several possible explanations, both experimental and biological. The source and specific activities of the labelled IFN-γ, as well as the labelling methods and the types of binding assays performed, are important factors. The possible biological explanations for such variability include tumour heterogeneity, alterations in receptor expression with cell cycle, and the presence of known modulating cytokines such as tumour necrosis factor-α [11] and granulocyte-macrophage colony stimulating factor [2].

Grups and Bange [4] have examined the expression of IFN-OC receptors on three human bladder cancer cell lines (RT4, SD and 637V) and found no correlation between affinity and sensitivity to this cytokine. Jakse et al. [7] have measured the expression of IFN-γ receptors on two other human bladder cancer cell lines, 647V and J82, which show different sensitivities to the growth inhibitory effects of IFN-γ. They used a non-competitive binding assay, and failed to show a relationship between receptor expression and biological response. Their calculated affinities, $0.6\text{–}1.1 \times 10^{-10}$ M, were of the same order of magnitude as ours, but receptor numbers were an order of magnitude lower (870–3,000 receptors per cell). Our use of a different assay method not only supports their findings, but counters arguments that the lack of correlation may be methodological in origin.

It seems from our present study and that of Jakse et al. [7] that the expression of IFN-γ receptors is not a useful parameter for predicting the response of bladder tumour cell lines to this cytokine. It remains to be shown whether this is also true in fresh tumour samples.

*Acknowledgements.* We are grateful to Dr. John Masters at the Institute of Urology for providing the bladder cancer cell lines.

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