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Interferon receptors on the surface of interferon-sensitive and interferon-resistant urothelial carcinomas*

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Summary. In previous investigations [3], it was demonstrated that interferons (IFN) have antiproliferative effects in human urothelial carcinomas. However, appreciable differences were found in the sensitivity of the individual tumors investigated. We therefore examined whether this might be due to a different receptor status of the cells. The IFN-sensitive cell lines RT4 and SD as well as the IFN-resistant cell line 639V were investigated with regard to their IFN receptor status. It was demonstrated that IFN receptors were present on the cell surface in all three urothelial carcinomas investigated. The number of IFN receptors calculated for the IFN-resistant cell line 639V was 4.661 per cell, whereas the IFN-sensitive cell line SD had 4.391 receptors and RT4 had 3.307 receptors. The IFN affinity of the three cell lines tested differed only slightly. Therefore IFN affinity is unlikely to account for their marked differences in IFN sensitivity.

Key words: Interferon alfa – Urothelial carcinomas – Receptors

A percondition for biological action of Interferon (IFN) is the binding to high-affinity receptors on cell surface. Such binding was demonstrated in studies using radioactive ligands [1]. Previous publications indicated that the determination of interferon receptors on the surface of urothelial carcinomas may have clinical significance. Subsequently Shortliffe et al. showed that the intravesical instillation treatment of IFN alfa-2, led to encouraging results in superficial bladder cancers [7].

In our previous investigations the antiproliferative effects of IFN were tested in vitro in a total of 17 different urothelial carcinoma cell lines. Each of the three classes of IFN known so far, had antiproliferative effects on these cells. Of the 17 long-term cell cultures investigated, eight were rated as IFN-sensitive, four as semisensitive and five showed no significant inhibitory effect of IFN on growth [3]. The objective of the present study was therefore to determine the receptor status, i. e. the number of receptors per cell and the IFN affinity for the receptor. Any differences present might provide a possible explanation for the divergent IFN sensitivity of the different cell lines.

Materials and methods

The IFN-sensitive cell lines RT4 and SD as well as the IFN-resistant cell line 639V were investigated with regard to their IFN receptor status. All three cell lines were established monolayer cultures from urothelial carcinomas. The cell lines were grown in DMEM plus 15% calf serum (FCS) at 37°C in 5% CO_2. 10^5 IU penicillin G, 128 mg streptomycine, 2 mg amphothericin B and 5 mg L-glutamine were added per liter of culture medium. The interferon used was recombinant human alfa-2c (rHu IFN alfa-2c) from Boehringer/Mannheim. The radioactive labelling of the IFN alfa-2c with ^{125}J was kindly carried out by Dr. P. Scheurich, Clinical Study Group of Max Planck Society, Göttingen, FRG. The bladder tumor cells were detached from the cell culture dishes with a $Ca^{2+}Mg^{2+}$-free HBSS-trypsin-EDTA solution. The solution contained 0.025% Trypsin and 0.01 M EDTA. The cells were afterwards washed three times in HBSS containing $Ca^{2+}Mg^{2+}$. The proportion of dead cells was determined by eosin stain exclusion. The cell concentration was adjusted to 2×10^6 cells per 100 µl 199 medium. One liter of the medium contained 20 ml 1 M Tris-HCL pH 7.4 + 2.2 g $NaHCO_3$ + 1% bovine serum albumin.

The actual receptor determination was then carried out on 96-well-plates with removable cups. Five different IFN concentrations were used. The dissociation constant, i. e. the concentration at which 50% of the receptors are occupied by IFN was assumed to be 3×10^{-10} mol/l for the test analogy to studies published earlier [5, 9]. Accordingly, 1.6×10^{-10} M to 1.6×10^{-9} M was chosen as test range.

Since the ^{125}J-labelling reduced the binding capacity of IFN to 44%, 20 ng (3.6×10^{-9} mol/l) per well was used as the highest IFN concentration and 3.6×10^{-10} mol/l per well was used in the lowest concentration. The final reaction volume was 300 µl per well; 100 µl containing 2×10^6 viable cells suspended in 199 medium, 100 µl ^{125}J-labelled IFN at the desired concentration and 100 µl of a 200-fold access of cold IFN. The cells were incubated at 4°C (in order to prevent downregulations of the receptors during the test) for 90 minutes with ^{125}J-IFN [8]. After the first 45 minutes, the cells were mixed. For saturation of nonspecific binding, a 200-fold excess of

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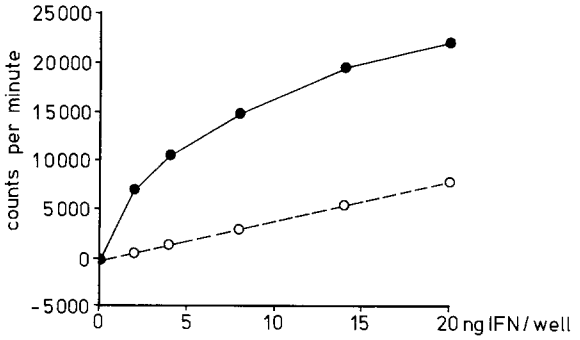

Fig. 1. Curve of total and nonspecific IFN alfa-2c binding in the cell-line RT4

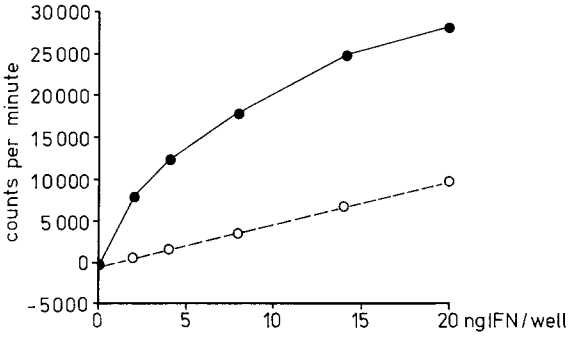

Fig. 2. Curve of total and nonspecific IFN alfa-2c binding in the cell-line SD

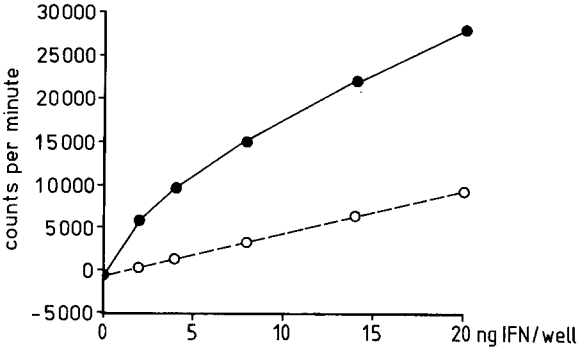

Fig. 3. Curve of total and nonspecific IFN alfa-2c binding in the cell-line 639V

unlabelled IFN was added. Duplicate assays were carried out for each test. The bound radioactivity was determined by means of a gamma counter.

Results

The share of nonspecific IFN binding in total IFN binding was between 35% and 40% in the highest IFN concentration (20 ng) tested (Figs. 1–3). At the concentration of 10 ng IFN alfa-2c, the percentage of nonspecific binding was between 20% and 25% of the total binding.

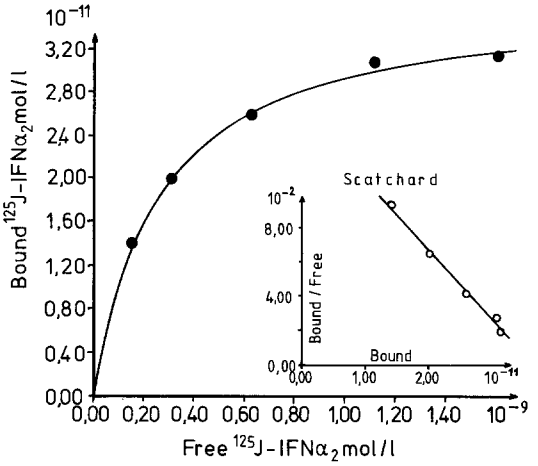

Fig. 4. Curve of specific IFN binding with Scatchard analysis in the cell-line RT4

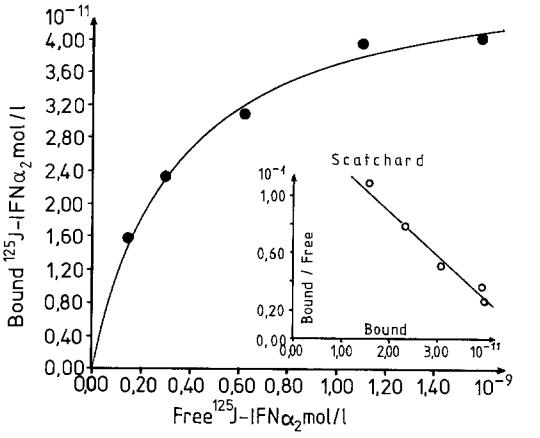

Fig. 5. Curve of specific IFN binding with Scatchard analysis in the cell-line SD

Figures 1–3 show that the IFN binding rose with increased IFN concentration both in the IFN-sensitive and in the IFN-resistent cell line. The marked discrepancy between the total and the nonspecific IFN abinding shows that the rHu-IFN alfa-2c is bound with high affinity to the cell surface and thus receptors are present on cell surface. The specific IFN binding was determined by subtraction of the nonspecific from the total IFN binding. With increasing IFN concentration, the total binding curve was largely parallel to nonspecific binding curve (i. e. the specific binding reaches a saturation range). The binding curve of the cell line 639V (IFN-resistent) is least parallel to the nonspecific binding, whereas the cell line RT4 (IFN-sensitive) most closely approximates to a parallel line. The receptors have a corresponding distance from their IFN saturation range.

The actual concentration of IFN capable of binding is plotted in mols per liter on the x-axis for the individual cell lines (Figs. 4–6). The biological activity of IFN was 44%. In addition, the Scatchard analysis is entered for each of the individual binding curves. The specific binding rises

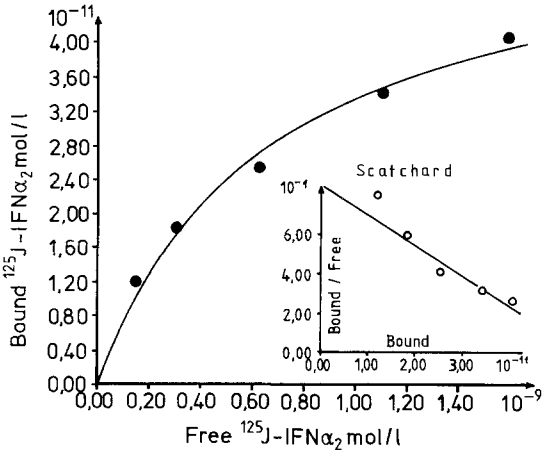

Fig. 6. Curve of specific IFN binding with Scatchard analysis in the cell-line 639V

over the entire concentration in all three cell lines. The binding curve showes that the steepest course in the IFN-sensitive RT4 cells. The slope is lowest in the IFN-resistant 636V cells. Since the same concentration range (1.6×10^{-10} mol to 1.6×10^{-9}mol) was chosen for all three cell lines, the similar curves show that all three cell lines have similar dissociation constants. The Scatchard analysis confirms this. The ratio of bound to free IFN alfa-2c is 10% in the lower concentration range and 2% in the upper concentration range. With exploration up to the intersection with the x-axis, the maximum specific binding capacity is obtained. Assuming that one IFN molecule is bound per receptor, the following density per cell was calculated taking into consideration the molecular weight of the IFN alfa-2c used:

Cell line RT4: Kd = 2.4×10^{-10} M (linear regression: 0.99). 3,307 receptors per cell.

Cell line 639V: Kd = 5.3×10^{-10} M (linear regression: 0.93). 4,661 receptors per cell.

Cell line SD: Kd = 3.1×10^{-10} M (linear regression: 0.97. 4,391 receptors per cell.

The cells of the line RT4 (IFN-sensitive) have the highest affinity and the lowest receptor density. On the other hand, the interferon-resistant cell line 639V has most receptors on the cell surface, but the lowest affinity for IFN. The cell line SD, which is also sensitive to IFN, has an intermediate position.

Discussion

In our own previous investigations, it was demonstrated that the urothelial carcinomas have different IFN sensitivity [3]. We examined the possibility that this was due to receptor density on the cells.

In 1980, AGUET described IFN receptors on IFN-sensitive cells, whereas no receptors could be detected on a resistant mutant of the same cell line [1]. In 1984, Hannigan et al. [4] described that differences may be present with regard to the receptor affinity between IFN-resistant and IFN-sensitive cell lines and one IFN-resistant cell line were simultaneously analysed with regard to their receptor status. It was demonstrated that all three cell lines display IFN receptors on their cell surface. The dissociation constant for the cell line RT4 was 2.4×10^{-10} M, and 3.1×10^{-10} M for 639V.

These relatively small differences appear too small to explain differences with regard to IFN sensitivity. Pfeffer et al. [6] demonstrate dissociation constants for IFN of 2.0×10^{-11} and 5.2×10^{-11} in a 1987 study. Although these are an order of magnitude lower than the values determined here, the relative difference between the individual Kd's corresponds to the differences measured in the three bladder carcinoma cell lines investigated here. The number of receptors in the study published by Pfeffer et al. [6] also had a similar magnitude: 2,300 and 3,700. These values were appraised as "a similar number of high-affinity receptors in all three cell lines". They considered that these differences did not account for the different IFN sensitivity.

In 1984, Hannigan et al. [4] were able to demonstrate a low-affinity and a high-affinity receptor on Daudi cells which differed from each other by two powers of ten in its Kd. Such dissimilar affinities could not measured here.

According to these findings our results seems to reflect a similar number of high-affinity receptors for all three cell lines.

By definition, the Kd is the concentration in mols per liter at which 50% of the receptors are occupied by ligands. It was determined arithmetically how many units of IFN per ml are necessary in order to attain a degree of receptor saturation of 50%. The following concentrations were necessars for this at a molecular weight of 18.000:

Cell line RT4: 4.3×10^{-9} g/ml = 1,075 IU/ml
Cell line SD: 5.6×10^{-9} g/ml = 1,400 IU/ml
Cell line 639V: 9.1×10^{-9} g/ml = 2,275 IU/ml

For the clinical application of IFN, it can be concluded from the present study that the IFN levels measured in serum during systemic IFN alfa-2c treatment [2] are unlikely in order to attain an adequate saturation of IFN receptors. However it remains to be investigated whether the Kd alters at a physiological temperature (37°C) and the degree of receptor saturation which requires a full antiproliferative effect. In contrast to this, local intravesical IFN treatment might bring about such a high concentration at the cell surface that the IFN receptors are sufficiently saturated.

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