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Binding of Human Interferon α to Cells of Different Sensitivities: Studies with Internally Radiolabeled Interferon Retaining Full Biological Activity

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The characteristics of interferon binding to various cells with different interferon sensitivity were studied by using [^{3}H]leucine-labeled, pure human interferon α from Namalwa cells. Scatchard analysis of the binding data on cells sensitive to interferon α (human FL and fibroblasts and bovine MDBK) indicated the presence of two kinds of binding sites with high and low affinities. The binding constants of the high-affinity sites in these cells were similar (4×10^{10} to 11×10^{10} M^{-1}). Cells insensitive to human interferon α (human HEC-1 and mouse L cells) were shown to have only low-affinity sites, suggesting that high-affinity binding sites are indispensable for interferon sensitivity and represent interferon receptors. However, the number of sites in three human diploid fibroblast strains and one strain trisomic for chromosome 21 were not proportionally correlated to the interferon sensitivity of the cells. The high-affinity binding to human cells was completely inhibited by both nonradioactive human interferons α and β in a similar manner, but binding to bovine MDBK cells, on which human interferon β is practically inactive, was inhibited effectively only by interferon α and not by β. These results suggest that the receptor for human interferon α is common to human interferon β in human cells, whereas the receptor on bovine cells binds only human interferon α.

Interferon binding to receptors on the plasma membrane is the important first step in exerting its biological activity in target cells (5, 23). Recently, cellular binding of interferon was analyzed by using mouse and human interferons labeled with radioactive iodine (1–3, 16), and high-affinity binding of interferon to specific cell surface receptors was reported (1, 6, 16). In these reports, the receptor for interferon α was suggested to be the same as that for interferon β, but the receptor for interferon γ was different (2, 6).

Recently, we obtained [^{3}H]leucine-labeled human interferon α from Namalwa cells in an electrophoretically pure form retaining full biological activity (25). This prompted us to study its binding to cells, since the metabolic labeling obviates the possibility of alterations in molecular properties inherent in radioiodination or any other chemical labeling. In this report, we describe the binding of the labeled human interferon α to various cell types differing in susceptibility to its antiviral action.

Time course of interferon binding to human FL cells. The time course of binding of radioactive interferon to FL cells at three temperatures is shown in Fig. 1. The quantity of interferon bound to the cell surface was determined as the radioactivity released by trypsin treatment, as described in the legend to Fig. 1. When cells were incubated at 37°C, the bound radioactivity was half maximal after 15 min and maximal after 1 h. Thereafter, it began to decrease and dropped to one half of the maximum after 5 h. On the other hand, binding at 21°C was maximal after 2 h of incubation and did not decrease even after prolonged incubation. At 2°C, the interferon binding increased slowly during a 5-h period. These patterns were essentially unchanged for various interferon concentrations. At the time of maximum binding at 21°C (2.5 h), more than 90% of the radioactivity associated with the cells was released by trypsin treatment, indicating that the interferon bound at 21°C remained on the cell surface. However, at the time of maximum binding at 37°C (1 h), about 20% of the radioactivity was not released by trypsin treatment. This may indicate internalization of some of the bound interferon at this temperature. Thereafter, the binding of labeled interferon to various cells was studied at 21°C to avoid possible complexities due to internalization.

Binding of human interferon α to sensitive and insensitive cells. Maximum binding of interferon

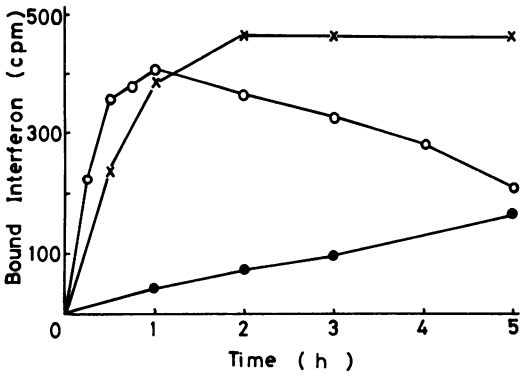

FIG. 1. Time course of binding of radioactive human interferon α to FL cells. FL cells (1.3×10^6 per 3.5-cm plastic dish) were incubated with 1 ml of growth medium containing [^{3}H]leucine-labeled human interferon α (160 IU/ml and 4.8×10^3 cpm/ml) for the indicated times at 37 (○), 21 (X), or 4°C (●). Cells were then washed twice quickly at 4°C with 0.5 ml of the medium without serum and incubated with 0.5 ml of Dulbecco phosphate-buffered saline containing 0.5% trypsin (Difco Laboratories, Detroit, Mich.) and 0.02% EDTA for 15 min at 37°C. The radioactivity in the supernatant after centrifugation was measured with ACS II (Amersham Corp., Arlington Heights, Ill.). The radioactive interferon preparation (30 cpm/IU) contained all of the four electrophoretic components of Namalwa cell interferon (25). All experiments were performed in triplicate, and standard deviations were less than 7%.

(2.5 h at 21°C) to human FL cells was determined as a function of its concentration. The data were analyzed by the Scatchard plot (19), and a nonlinear curve with upward concavity was observed (Fig. 2A). The simplest explanation for this is the presence of two types of binding sites, one with high affinity and low capacity and the other with low affinity and high capacity, as has been proposed for mouse and human interferons (1, 16) and for some peptide hormones (15, 17, 20), although other interpretations, such as negatively cooperative site-site interaction (9, 10) and mobile receptors (13), cannot be excluded. Binding to high-affinity sites was completely inhibited by the presence of excess unlabeled pure human interferon α from Namalwa cells (26), as well as by purified recombinant human interferon α_2 (kindly supplied by S. Nagata, Institute of Medical Science of the University of Tokyo) (data not shown). However, high-affinity binding was not inhibited by large amounts of mouse interferon, which is barely active on FL cells. On the other hand, the binding corresponding to the low-affinity portion of the curve was not inhibited by excess unlabeled human interferon α.

Interestingly, human HEC-1 cells, which are entirely insensitive to interferon (7, 24) (kindly supplied by T. Kuwata, School of Medicine, Chiba University) did not have high-affinity binding sites for human interferon α, but only low-affinity sites (Fig. 2B). The same results were obtained with mouse L cells, which are practically insensitive to human interferon (Fig.

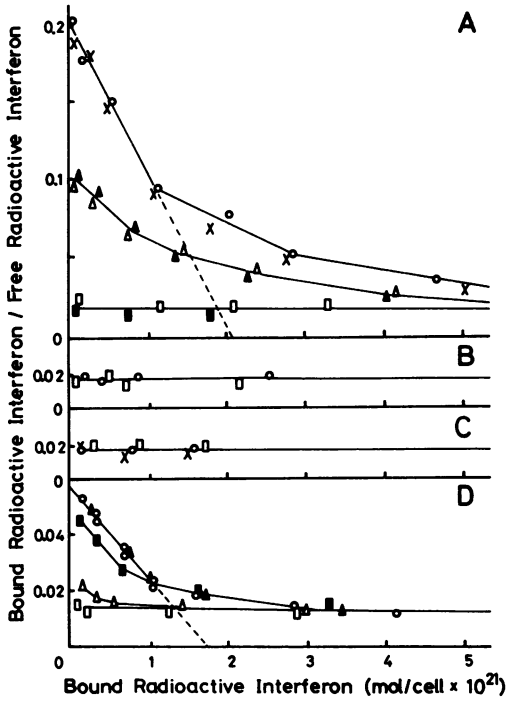

FIG. 2. Scatchard plot of interferon binding to (A) human FL cells (8×10^5 per dish), (B) human HEC-1 cells (8×10^5 per dish), (C) mouse L cells (8×10^5 per dish), and (D) bovine MDBK cells (5×10^5 per dish). Cells were incubated with 1 ml of radioactive human interferon α (30 cpm/IU) at various concentrations for 2.5 h at 21°C. The radioactivity of bound interferon was measured as described in the legend to Fig. 1 without unlabeled interferon (○) or with the following unlabeled interferons: 3,000 IU of mouse interferon per ml (X), 300 IU of human interferon α (△) or β (▲) per ml, or 3,000 IU of human interferon α (□) or β (■). Unlabeled interferons used were mouse (α, β) interferon from L cells (10^6 IU/mg of protein, kindly supplied by Y. Kawade, Kyoto University); pure human interferon α from Namalwa cells (7.6×10^8 IU/mg of protein) (26); and human interferon β from diploid fibroblasts (10^7 IU/mg of protein, kindly supplied by S. Kobayashi, Basic Research Laboratory, Toray Industrial Inc.). One mole of interferon (30 cpm/IU) corresponded to 4.1×10^{17} cpm on the assumption that the specific activity of pure interferon α is 7.5×10^8 IU/mg of protein (26) and the mean molecular weight of human interferon α is 18,000.

2C). Low-affinity binding to these cells was not inhibited by excess unlabeled human interferon α, as was the case with FL cells (Fig. 2). These observations suggest that the presence of high-affinity binding sites is indispensable for interferon action, although their presence does not seem to be a sufficient condition for cell sensitivity, as shown by the data on Raji cells (16).

The association constant of interferon to the high-affinity sites on FL cells was estimated to be 1.1×10^{11} M^{-1}, a value much higher than those given for some peptide hormones (4, 11, 14, 20). The number of high-affinity sites was estimated to be 1.3×10^3 per cell. These results agree well with those of Aguet and Blanchard (3) on mouse interferon and Mogensen et al. (16) on human interferon α.

With human FL cells, which are sensitive to both interferons α and β, high-affinity binding was inhibited not only by unlabeled interferon α, but also by interferon β (Fig. 2A). Essentially the same results were obtained with human fibroblast cell strains GM-258, TM-6, TM-8, and FS-7 and with human lymphoblastoid cell lines Namalwa and Daudi (data not shown). These results indicate that the high-affinity binding sites on these human cells are common to interferons α and β, as was also suggested by Branca and Baglioni (6) and Aguet and Blanchard (3).

Relation between cellular binding and interferon sensitivity. Interferon binding was investigated on four human fibroblast cell strains with different interferon sensitivities, three diploid (TM-6 and TM-8, obtained in this laboratory, and FS-7 from J. Vilček, New York University) and one trisomic for chromosome 21 (GM-258, kindly provided by T. Taniguchi, Cancer Institute, Tokyo). They showed binding characteristics similar to those of FL cells. The association constants of the high-affinity sites were similar (3×10^{10} to 4×10^{10} M^{-1}), slightly lower than those of FL cells (Table 1). Also, the number of high-affinity sites in the three diploid cell strains was similar, both to each other and to FL cells. GM-258 cells were found to have measurably more sites, about 1.5 times as many as the diploid strains had (Table 1). This result is consistent with the idea that chromosome 21 codes for the interferon receptor (8, 10, 21, 22). However, the amount of interferon required to obtain the same biological effects on the four fibroblast strains examined differed markedly (Table 1). Interferon sensitivity and the number of high-affinity binding sites were not related in direct proportion, suggesting that some intracellular reaction other than receptor occupancy is important for cellular sensitivity to interferon.

Binding to bovine MDBK cells. Bovine MDBK cells are known to be sensitive to human interferon α, but not to human interferon β (12). In our system, 0.5 IU of human interferon α per ml inhibited the growth of vesicular stomatitis virus (viral RNA synthesis) to half that of the control, whereas more than 100 IU of human interferon β per ml was required for the same effect. The binding of radioactive human interferon α to MDBK cells had a similar time course as in FL cells, reaching a maximum after 2.5 h at 21°C (data not shown). The Scatchard plot (Fig. 2D) again gave a nonlinear curve with upward concavity. The high-affinity portion of the curve showed an estimated association constant of 6.6×10^{10} M^{-1} and a capacity of 10^3 sites per cell, which are thus similar to human cells. The high-affinity binding was competitively inhibited by nonradioactive interferon α, fairly strongly at 300 IU/ml and completely at 3,000 IU/ml. In contrast to FL cells, however, human interferon β was much less effective in competitive binding; the high-affinity binding was not inhibited by 300 IU/ml and only slightly inhibited by 3,000 IU/ml. These results suggest that the binding sites for human interferon α on MDBK cells do not bind human interferon β. That is, the receptors for human interferon α on MDBK cells are not identical to those on human cells in their properties.

Further studies on interferon binding and internalization are in progress with interferon-sensitive and -insensitive cells.

TABLE 1. Comparison of four human fibroblast cell strains for interferon binding parameters and interferon sensitivity

Cell strain	No. of receptors per cell	Binding constant (M^{-1})	Interferon sensitivity[a] (IU/ml)
TM-6	1.2×10^3	4×10^{10}	0.8
TM-8	1.5×10^3	3×10^{10}	2.0
FS-7	1.4×10^3	4×10^{10}	0.3
GM-258	2.0×10^3	4×10^{10}	0.1

[a] Interferon concentration necessary to reduce [^{3}H]uridine incorporation into vesicular stomatitis virus RNA by 50%.

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