ENHANCED EXPRESSION OF CELLULAR RECEPTORS FOR HUMAN INTERFERON α ON PERIPHERAL LYMPHOCYTES FROM PATIENTS WITH DOWN'S SYNDROME

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1. Introduction

There is good evidence that chromosome 21 carries the gene(s) that determines the sensitivity of human fibroblasts to the antiviral activities of interferon α, β [1–12] and γ [8]. Furthermore, it has also been shown that the lymphocytes of patients with Down's syndrome (trisomy for chromosome 21) were more sensitive to inhibition by interferon of lymphoblastogenesis after stimulation with phytohemagglutinin or allogenic cells, than lymphocytes disomic for chromosome 21 [13,14], and that cultured peripheral blood monocytes from these patients also showed an enhanced sensitivity to the maturation-inhibiting effect of leucocyte interferon [15]. The use of antibodies raised against cells carrying chromosome 21 to block interferon action [7] and the increased recovery of interferon from interferon-treated human fibroblasts trisomic for chromosome 21 [9], suggested that the gene product responsible for increased interferon sensitivity might be a specific cell-surface receptor.

With purified ^{125}I-labelled human interferon (Hu IFNα), we have demonstrated the presence of high-affinity receptor sites for Hu IFNα on a wide variety of lymphoid cells [16]. Herein, we show that lymphocytes from patients with Down's syndrome show an increased specific binding of ^{125}I-Hu IFNα compared to lymphocytes from normal donors. This study also provides norms for the affinity of binding and the interferon receptor concentration that may prove useful in investigating the interferon sensitivity of peripheral blood lymphocytes from patients with other diseases.

2. Materials and methods

2.1. ^{125}I-Labelled interferon

The IFN was prepared [17], purified [18] and radiolabelled [16] as described. Two preparations of labelled interferon were used. The biological activity, protein content, radioactive incorporation, radioactive decay and the purity were controlled for each preparation. The Hu IFNα (derived from Namalwa cells) has been well characterized [19,20] and this allows us to make a conversion from cpm to mol interferon. Details of the calculation and the estimation of error has been described in [16]. Briefly, for 20 000 M_r and a specific biological activity of 2×10^8 units/mg protein, a radioactive counting efficiency of 80% and a mean incorporation of 1 ^{125}I/atom interferon molecule we obtain a value of 400×10^{16} cpm/mol interferon (2.193×10^6 Ci/mol I; 1.8×10^{12} cpm/Ci). Thus 400 cpm are equivalent to 10^{-16} mol interferon: and 1000 cpm to 1 unit of interferon for 100% incorporation of ^{125}I. We currently estimate the error at ⩽25%.

2.2. Lymphocytes and interferon binding

Heparinized peripheral blood samples were obtained from 8 normal donors and 6 patients with Down's syndrome (ages 11–18 years). All these patients were shown to be trisomic for chromosome 21. Lymphocytes were separated on a Ficoll-Hypaque gradient. Depending upon the yield, lymphocytes were resuspended at $0.5–2.0 \times 10^6$ cells/ml and incubated with different concentrations of radiolabelled interferon at 37°C for 90 min, equilibrium being attained within 1 h [16]. Washing and counting was done at 4°C as in [16].

Direct binding and Scatchard plots [21] were cal-

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culated from the free interferon concentration and the specifically bound interferon, as in fig.1. The regression of the Scatchard plot was taken as linear, the intercept on the abscissa giving the maximum cpm bound (equivalent to the total number of receptor sites), and the reciprocal of the slope giving the free interferon concentration when half the sites are occupied (equivalent to the dissociation constant, K_d for the interferon–receptor complex).

3. Results and discussion

Fig.1 shows the direct binding and Scatchard plots from one normal donor and one patient with Down's syndrome and table 1 gives the mean values of the binding constants for the two groups. Lymphocytes from patients with Down's syndrome have 3-times as many binding sites/cell compared to lymphocytes from normal donors. Furthermore, the binding sites on the lymphocytes from patients with Down's syndrome showed a higher affinity for Hu IFNα than did the binding sites on lymphocytes from normal donors.

These results provide further evidence of a relationship between chromosome 21 and the cellular receptor for Hu IFNα, suggesting that there may be a direct causal relationship between enhanced sensitivity to interferon shown by 21-trisomic lymphocytes [13,14] and the enhanced values for the receptor binding constants.

The values we have found for the binding constants of the normal lymphocytes correspond well

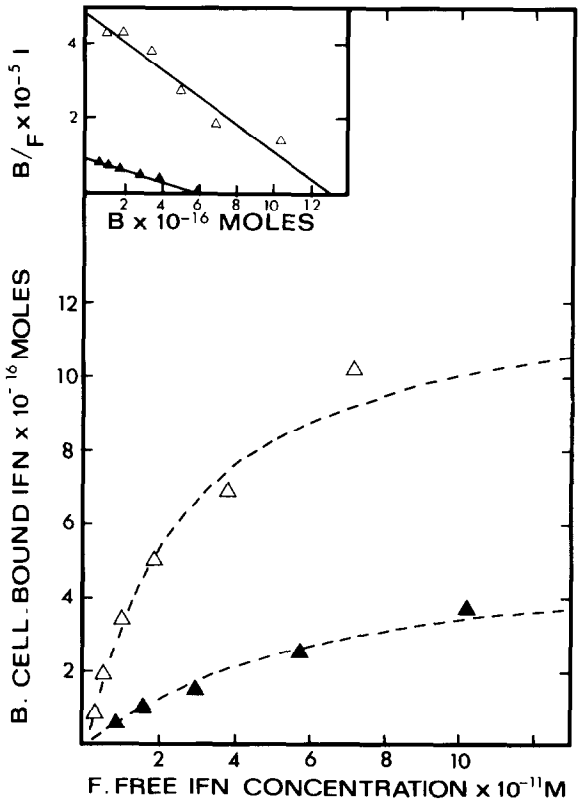

Fig.1. Direct binding graph and Scatchard representation (inset) for binding data from one normal donor (▲——▲) and one patient with Down's syndrome (○——○). Bound Hu IFN (B) was calculated from radioactive counts bound to 10^6 cells (total cpm bound – cpm bound in presence of 100-fold excess of unlabelled interferon). Free IFN concentration (F) calculated from radioactivity added (total cpm/ml added – cpm bound/ml culture). The symbols indicate the actual experimental values, and the curves were calculated from the linear regression of the Scatchard plots.

Table 1
A comparison of binding constants (37°C) for binding of Hu IFNα to peripheral lymphocytes from normal donors and patients with Down's syndrome[a]
(Standard deviations in parenthesis)

Donors	No. of donors	Mean K_d[b] ($\times 10^{-11}$ M)	Mean receptor density[c] ($\times 10^{-16}$ mol/10^6 cells)
Normal	8	5.20 (0.87)	4.80 (0.99)
Down's	6	3.36 (1.27)	14.50 (1.79)
Significance (t-test) for difference of means		$p \simeq 0.01$	$p < 0.001$

[a] Lymphocytes were incubated at 0.5–2.0 × 10^6 cells/ml depending upon the yield from blood. We found no correlation between lymphocyte yield and the values for the binding constants
[b] From the reciprocal of slope of Scatchard plot
[c] From intercept on the abscissa of Scatchard plot

with the estimates in [16]. Similar K_d-values were obtained from competition experiments with unlabelled interferon (unpublished) suggesting that the labelling procedure has not altered the affinity of binding. A mean K_d of 5.0×10^{-11} M (table 1) for binding to normal lymphocytes (at $\sim 10^6$ cells/ml) means that the half receptor sites are occupied at an interferon concentration of ~200 units/ml and that saturation is approached at >1000 units/ml. With 1 site/molecule of Hu IFNα bound, there would be a mean of 300 receptors/lymphocyte.

While it was not practical to use age-matched donors, we have tested occasional 21-disomic donors that fall within the Down's age group, and found binding in the range for normal donors. A value for receptor density on human cord lymphocytes also fell within the normal range. We feel that possible age or sex-related differences in receptor expression by normal 21-disomic lymphocytes would only be revealed among a much larger number of donors than has sufficed to point out the difference in this study.

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