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RESPONSIVENESS OF HUMAN CELLS TRISOMIC FOR CHROMOSOME 21 TO THE ANTIVIRAL ACTION OF HUMAN IMMUNE INTERFERON

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Seven human diploid cell strains (three disomic and four trisomic for chromosome 21) were tested for sensitivity to preparations of the three types of human interferon (IFN). Relative to the disomic control strains, the trisomic-21 cell strains were found to be more sensitive to leukocyte (HuIFN-α) and to fibroblast IFN (HuIFN-β). This chromosome 21-controlled increase in sensitivity to HuIFN-α and -β, was not always accompanied by a parallel increase in sensitivity to HuIFN-γ. In fact, in some D21/T21 comparisons the sensitivity to HuIFN-γ and that to HuIFN-α and -β diverged, suggesting that the cellular receptor site(s) for the different IFN types may be different. The presence of a high concentration (> 2%) of bovine serum in the culture medium inhibited the responsiveness of all cells to HuIFN-γ but not to HuIFN-α or HuIFN-β.

chromosome 21 immune interferon trisomic-21 cells

Human cells trisomic for chromosome 21 (T21 cells) are known to be more sensitive to the antiviral effect of interferon (IFN) than normal disomic cells (D21 cells), which in turn are more sensitive than cells monosomic for chromosome 21 (M21 cells) [11, 13]. These differences have been interpreted in terms of the hypothesis that human chromosome 21 contains a gene that controls the synthesis of a (membrane) receptor for IFN [4, 10, 15] or that regulates intracellular processes involved in the establishment of the antiviral effect [11]. The difference in sensitivity between T21 and D21 cells is well documented for both IFN preparations of the leukocyte (α) and fibroblast (β) type (HuIFN-α and HuIFN-β) [5, 15]. It is less well documented in the case of the immune (γ) type human interferon (HuIFN-γ) [7]: Epstein et al. [8] compared the sensitivity of genetically matched D21/T21 pairs of cell strains and presented evidence suggesting that T21 cells are indeed more sensitive than D21 cells to HuIFN-γ. Since we found that crude preparations of immune-induced IFN are heterogeneous in that they may contain HuIFN-β [6], we decided to investigate the relative sensitivity of T21 cells using a preparation of HuIFN-γ that had been purified extensively.

The IFN preparations used were: 1) leukocyte IFN prepared by induction of fresh human leukocytes with Sendai virus and partially purified as described by Cantell et al. [2] ($10^{3.5}$ international units (I.U.)/ml, specific activity $10^{6.0}$ units/mg protein); 2)

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fibroblast IFN induced by superinduction of diploid human cells [1] with poly(I:C), actinomycin D and cycloheximide (unpurified, $10^{4.7}$ I.U./ml, specific activity $10^{4.0}$ units/mg protein; and 3) immune IFN induced by concanavalin A (Con A) in fresh human leukocytes and purified by a two-step procedure [6]: adsorption on controlled pore glass at neutral pH and desorption by 50% ethylene glycol in 1.5 M NaCl followed by gel filtration and collection of the biologically active fractions forming a peak at ~45,000 dalton (45 kilodalton fraction quoted in ref. 6, having a specific activity around ~ $10^{6.0}$ units/mg protein). The sample contained $10^{3.3}$ arbitrary units/ml as estimated from the average end-point in a large number of titrations on human diploid cells.

The cell strains used for the determination of IFN sensitivity were: VGS, E_1SM and E_6SM (D21-diploid human fibroblast strains derived from the skin and muscle of human embryos); GM-2504 (a T21-cell strain described in the literature under code name GM258) [12]; LR, RL and Gritti (T21-cell strains derived from skin biopsies of patients with Down's syndrome at the Center for Human Genetics (Dr. J.J. Cassiman), University of Leuven, Belgium). The cells were propagated in Eagle's minimum essential medium with Earle's salts, supplemented with non-essential amino acids and 10% fetal bovine serum (FBS).

Interferon was titrated on monolayers grown to confluency in 1.6 cm diameter polystyrene culture vials (Costar, Cambridge, MA). Serial 0.5 $\log_{10}$ dilutions were prepared, and 0.1 ml aliquots were added to the culture vials to which 1 ml of medium, containing the appropriate concentration of FBS, had already been added. After 24 h incubation the supernatant fluids were replaced by 1 ml of fresh medium containing the appropriate concentration of FBS and $10^{6.0}$ plaque-forming units (p.f.u.) of vesicular stomatitis virus. The plates were then reincubated for 24 h. The cytopathic effect was estimated by measurement of neutral red uptake as described by Finter [9]. For each sample titrated, neutral red uptake (absorbance reading) was plotted against $\log_{10}$ of final dilution applied, and the titration end-point was calculated as the dilution corresponding to a neutral red uptake of 50% of the uninfected cell control.

The three preparations of IFN (HuIFN-α, and HuIFN-β and HuIFN-γ) were titrated on the seven cell strains mentioned, in the presence of four different concentrations (1%, 2%, 5% and 10%) of FBS. The titration end-points were plotted against serum concentration in Fig. 1. Panel A shows that all T21 cells examined were more sensitive than the disomic E_6SM strain to the preparation of HuIFN-α. However, with higher serum concentrations the difference became less apparent and finally disappeared, as a result of the fact that E_6SM cells became more sensitive at higher serum concentration. Panel B shows the results obtained with the HuIFN-β preparation: the T21 cells were not consistently more or less sensitive than E_6SM. Panel C shows the titration end-points obtained with the HuIFN-γ preparation. Surprisingly, the T21 strains were less sensitive than the disomic E_6SM cells, and this difference became even more pronounced at high serum concentrations, to an extent such that no activity could be detected. The inhibitory effect of serum was also seen in titrations of HuIFN-γ on the disomic E_6SM cells but was relatively less pronounced.

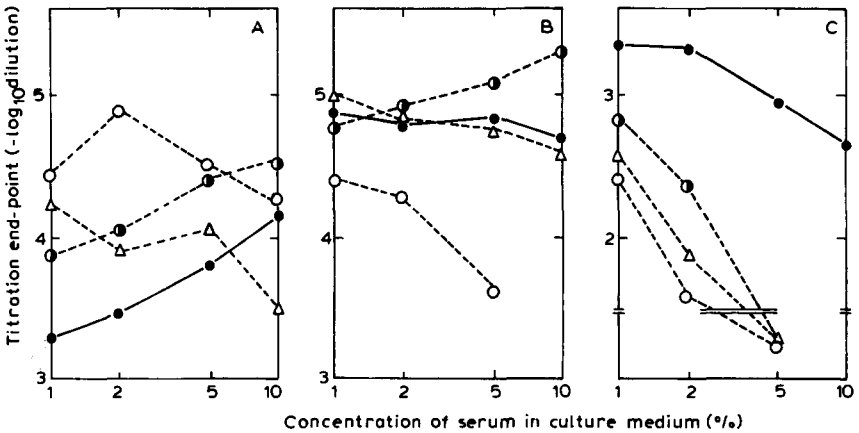

Fig. 1. Antiviral effect of preparations of HuIFN-α (A), HuIFN-β (B) and HuIFN-γ (C) on human cells disomic or trisomic for chromosome 21, in the presence of increasing concentrations of serum: ●, E_6SM; ◑, GM-2504; ○, RL; △, LR.

A practical implication of our finding is that assays for HuIFN-γ, as opposed to those for HuIFN-α and -β, may sometimes lose rather than gain in sensitivity by the use of T21 cells.

The results of this experiment diverge from those reported by Epstein and Epstein [7, 8] who found that the higher reactivity of T21 cells to HuIFN-α and -β, was accompanied by a parallel increase in sensitivity to HuIFN-γ. Since in our experiment only a single D21-cell strain was used as a reference, it seemed appropriate to expand the observation by testing additional cell strains. Table 1 shows such a comparison of four T21 with three D21-cell strains. The average sensitivity of the T21, as compared to D21 cells was increased in the case of HuIFN-α and -β, but unchanged in the case of HuIFN-γ. It is clear that one could select D21/T21 pairs (e.g. all combinations of T21 with VGS) which behave as described by Epstein and Epstein [7], i.e. concordant higher sensitivity of T21 to the three IFN types. However, certain D21/T21 pairs selectable from Table 1, display a higher sensitivity of the T21 strain to HuIFN-α and -β, accompanied by unchanged or even decreased sensitivity to HuIFN-γ.

Two possible explanations may be provided to account for this observation. The sensitivity of cells to IFN-γ might entirely be governed by factors independent from chromosome 21, a possibility which seems to be refuted by the examination of genetically matched D21/T21 pairs [8]. The second possibility is that chromosome 21 does have a parallel dosage effect on the sensitivity to all IFNs, including IFN-γ, but that other cellular factors, when present, reduce this effect on the sensitivity to IFN-γ to zero or even revert it, without affecting the chromosome 21 effect on sensitivity to IFN-α or -β.

Both possibilities imply that the chain of biochemical events triggered by IFN-α and -β, on the one hand, and IFN-γ on the other hand, are in some stages divergent from each other. The most straightforward proposition would be that cells possess different

TABLE 1

Comparison of sensitivities to different IFN types of disomic and trisomic cell strains

IFN preparation[a]	IFN titration end-points[b] (log_{10} end-point dilution) on								
	Disomic-21 cell strains				Trisomic-21 cell strains				
	VGS	E_1 SM	E_6 SM	Mean value	GM-2504	Gritti	RL	LR	Mean value
Leukocyte	3.00 (−; 1)[c]	4.11 (0.21; 5)	3.73 (0.12; 6)	3.61 (0.33; 3)[d]	4.05 (−; 1)	4.56 (0.02; 3)	4.58 (0.13; 5)	3.86 (0.06; 3)	4.26 (0.18; 4)
Fibroblast	4.18 (−; 1)	4.76 (0.15; 5)	4.82 (0.13; 6)	4.58 (0.20; 3)	4.91 (−; 1)	5.37 (0.03; 3)	5.20 (0.28; 5)	5.38 (0.33; 3)	5.22 (0.11; 4)
Immune	1.87 (−; 1)	2.83 (0.28; 5)	2.97 (0.12; 6)	2.56 (0.35; 3)	2.36 (−; 1)	2.40 (0.23; 3)	2.65 (0.27; 5)	3.10 (0.61; 3)	2.62 (0.17; 4)

[a] Leukocyte IFN at $10^{3.5}$ I.U./ml; fibroblast IFN at $10^{4.7}$ I.U./ml; immune IFN (45 kilodalton component) at $10^{3.3}$ arbitrary units/ml.

[b] Assays done in the presence of 2% FBS.

[c] (S.E. means; No. of assays done).

[d] (S.E. means; No. of cell strains tested).

receptors for different IFNs. Chany [3] has proposed an IFN-receptor model consisting of a loose, membrane-associated complex of an aspecific binding site and a species specific triggering site. The results of our experiments would be compatible with a triggering site that is identical for all IFNs and binding sites that are different for the various IFN types. This model would also allow to explain the differential effect of serum concentration on the sensitivity of cells to IFN-α and -β on the one hand and IFN-γ on the other.

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