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Antiviral and Anticellular Activities of Human and Murine Type I and Type II Interferons in Human Cells Monosomic, Disomic, and Trisomic for Chromosome 21 (41405)

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Abstract. Two trisomic-21 (T-21), one monosomic-21 (M-21), and three diploid (D-21) human fibroblast cell strains were compared for their sensitivity to the antiviral and anticellular activities of human type I (HuIFN-β), human type II (HuIFN-γ), murine type I (MuIFN-β), and murine type II (MuIFN-γ) interferons. Antiviral activity was monitored by the reduction of vesicular stomatitis virus yield and anticellular activity was assessed by the inhibition of [*methyl*-^{3}H]thymidine uptake in proliferating cells. T-21 cells were more sensitive to the antiviral activities of HuIFN-β and MuIFN-β than either D-21 or M-21 cells. However, T-21 cells were not more sensitive to the antiviral activities of HuIFN-γ and MuIFN-γ. Neither did T-21 cells exhibit an increased sensitivity to the anticellular activity of either HuIFN-β or -γ, or MuIFN-β or -γ. A chromosome 21 dosage effect could not be observed under any of our test conditions.

Previous studies have established that human cells which are trisomic for chromosome 21 (T-21 cells) are more sensitive to the antiviral activity of human interferon [HuIFN-α, -β (1–9) or HuIFN-γ (10)] and mouse interferon [MuIFN-β (11)] than are normal diploid (D-21) cells or cells which are monosomic for chromosome 21 (M-21 cells). T-21 cells are also more susceptible to various other effects of interferon, i.e., its inhibitory action on cell growth (12), lymphoblastogenesis (13, 14), and monocyte maturation (15). This leads some authors (16) to infer that "all" interferon-mediated effects are specified by chromosome 21, and the assumed supersensitivity of T-21 cells to interferon's cell-growth inhibitory effect has even been held responsible for the developmental abnormalities associated with Down's syndrome (17).

The hypothesis has been advanced that chromosome 21 codes for an interferon receptor located at the outer cell membrane (4, 6, 11), from where all interferon-mediated effects would be generated. However, this unifying hypothesis is still open for debate, since T-21 cells are not invariably more sensitive to all interferon types and all interferon actions. For example, unlike the antiviral activity, some of the nonantiviral activities of HuIFN-α and HuIFN-β, viz., the priming and toxicity-enhancing activity, are not better expressed in T-21 than in D-21 or M-21 cells (5); T-21 lymphocytes stimulated by phytohemagglutinin are not more sensitive to the antiproliferative effect of HuIFN-α than are D-21 lymphocytes (18); and, in some conditions, especially in the presence of high serum concentrations, T-21 cells are even less responsive to the antiviral activity of HuIFN-γ than are D-21 cells (19).

In view of these disparate findings, we decided to compare in the same test systems different types of human interferon [type I (HuIFN-β) and type II (HuIFN-γ)] and murine interferon [type I (MuIFN-β) and type II (MuIFN-γ)] for their antiviral and anticellular activities in several human T-21, D-21, and M-21 cell cultures. Our results indicate that T-21 cells are indeed more sensitive than D-21 and M-21 cells to the antiviral activities of HuIFN-β and MuIFN-β, but not to the antiviral activities of HuIFN-γ or MuIFN-γ or anticellular activities of either β or γ interferon.

Materials and Methods. *Cells.* Six human fibroblast cell strains were used for

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determination of interferon activities: three diploid fibroblast (D-21) cell strains, derived from the skin-muscle of human embryos and designated E_1SM, E_3SM or VGS; two T-21 fibroblast cell strains, derived from skin biopsies of patients with Down's syndrome and designated LR (Center for Human Genetics, University of Leuven, Belgium; courtesy of Dr. J. J. Cassiman) or GM 2504A (Mammalian Genetic Mutant Cell Repository, Camden, N.J.); and one M-21 fibroblast cell strain, GM 137, also obtained from the Mammalian Genetic Mutant Cell Repository at Camden. The cells were grown in Eagle's minimal essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS).

Interferon preparations. Human fibroblast interferon (HuIFN-β) was prepared in diploid human cells by a superinduction procedure involving poly(I)·poly(C), cycloheximide, and actinomycin D (20); it was further purified by chromatography on controlled pore glass and concentrated by dialysis against polyethylene glycol. Its specific activity was $10^{6.0}$ units/mg protein. Human immune interferon (HuIFN-γ) was induced by staphylococcal enterotoxin A (21) in human spleen lymphocytes (22); it was further purified by chromatography on controlled pore glass and concentrated by dialysis against polyethylene glycol. Its specific activity was $10^{3.6}$ units/mg protein. Murine fibroblast interferon (MuIFN-β) was induced by Newcastle disease virus (NDV) in mouse L-929 fibroblasts [essentially as described by Knight (23)], and partially purified by fractional precipitation with $(NH_4)_2SO_4$. Its specific activity was $10^{6.0}$ units/mg protein. Murine immune interferon (MuIFN-γ) was derived from mouse spleen cells induced by *Staphylococcus aureus* lysate (24). It was partially purified by fractional precipitation with $(NH_4)_2SO_4$ and had a specific activity of $10^{3.0}$ units/mg protein.

The interferon titers of HuIFN-β and MuIFN-β are expressed in terms of the NIH (National Institutes of Health) reference standards G023-902-527 and G002-904-511, respectively. The interferon titers of HuIFN-γ and MuIFN-γ are expressed in arbitrary laboratory units.

Antiviral activity. Confluent human T-21, D-21, or M-21 cell cultures in 55-mm Falcon plastic petri dishes were incubated with serial dilutions of interferon in EMEM + 3% FBS (2 ml/petri dish). After 18 hr incubation, the cell monolayers were washed three times with EMEM and inoculated with 10^4 PFU (plaque-forming units)/ml of vesicular stomatitis virus (VZV, Indiana strain, propagated in mouse L-929 cells). After a 1-hr adsorption period, residual virus was removed, the cells were washed with EMEM, replenished with EMEM + 3% FBS (3 ml/petri dish), and further incubated for 24 hr. The cells were then frozen–thawed and their virus content was determined by plaque formation in mouse L-929 cells.

Anticellular activity. Human T-21, D-21, and M-21 cells were suspended in EMEM containing 10% FBS and serial dilutions of interferon, and seeded in 7-mm Linbro microtray wells at a density of 30,000 cells per well. To each well 0.5 μCi [*methyl*-^{3}H]-thymidine (specific radioactivity 43Ci/mmol; The Radiochemical Centre, Amersham, England) was added and the cells were allowed to proliferate for 18 hr. The cells were then fixed with 5% trichloroacetic acid (TCA) at 0°, washed twice with 5% TCA (0°) and once with ethanol, and after drying, the wells were cut out, immersed in Lipoluma tissue solubilizer (Lumac Systems AG, Basel, Switzerland), and analyzed for radioactivity in a liquid scintillation spectrometer.

Results. *Antiviral activity.* Both HuIFN-β and HuIFN-γ caused a dose-dependent inhibition of VSV yield in human T-21, D-21, and M-21 cells (Fig. 1). Irrespective of the cell strain studied, the dose–response curve of HuIFN-β was markedly steeper than the dose response of HuIFN-γ. This implies that at a given dose, HuIFN-β effected a much greater reduction in virus yield than did HuIFN-γ. For example, at a dose of 10^3 units/ml HuIFN-γ reduced virus yield by 10^3- to 10^4-fold (only 30-fold in M-21 cells), whereas at the same dosage level HuIFN-β reduced virus yield by more than 10^7-fold. Some differences were noted in the cells' sensitivities to the antiviral action of HuIFN-β and -γ. The

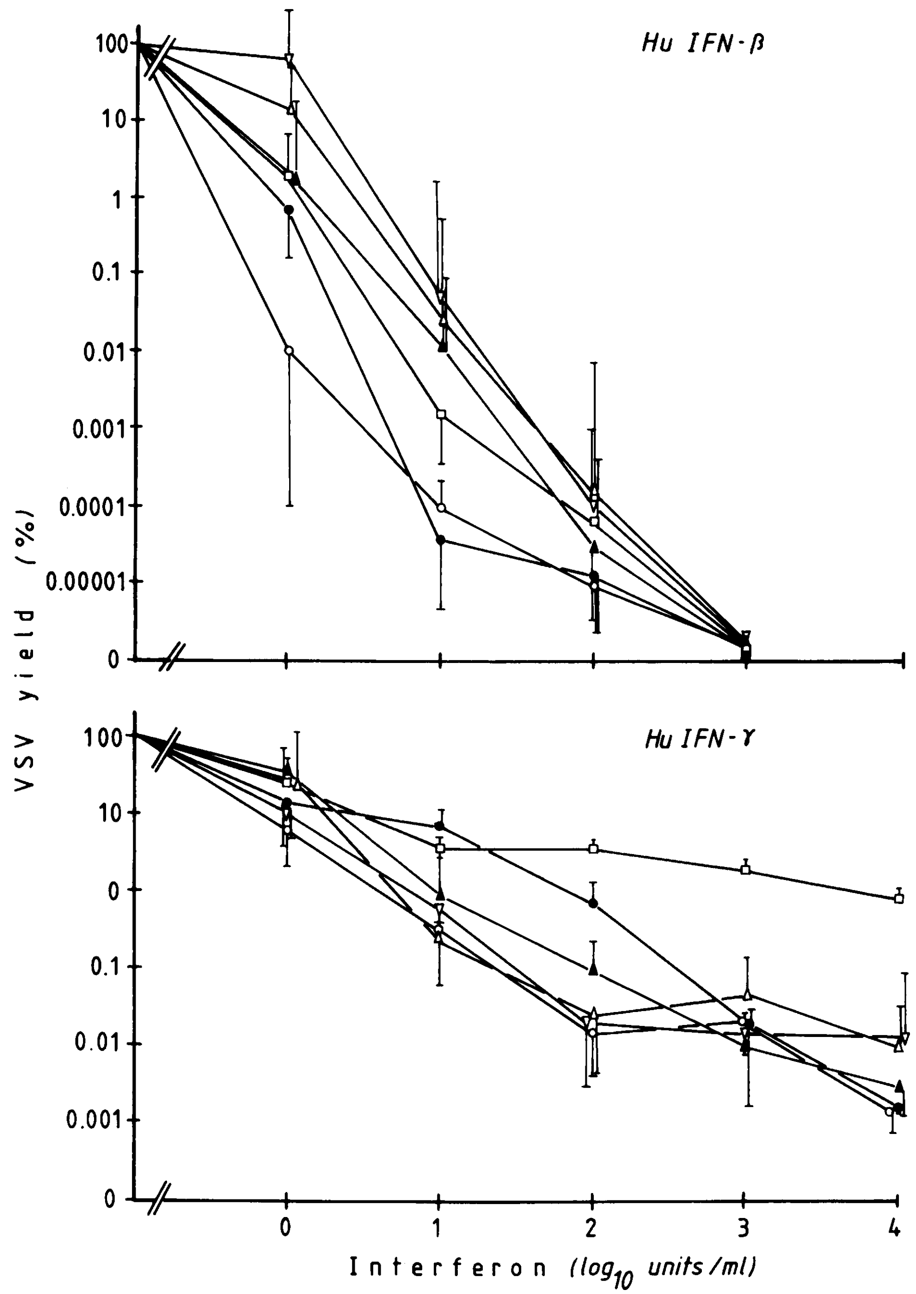

FIG. 1. Antiviral activity of HuIFN-β (upper panel) and HuIFN-γ (lower panel) in T-21 cells [strain LR (●), strain GM 2504A (○)], D-21 cells [strain VGS (▲), E_1SM (△), E_3SM (▽)], and M-21 cells [strain GM 137 (□)]. The data represent the average values (±SD) for three separate experiments.

two T-21 cell strains proved more sensitive to the antiviral effect of HuIFN-β than the D-21 or M-21 cell strains, as could be most clearly seen at a dose of 10 interferon units/ml (Fig. 1, upper panel). However, the T-21 cell strains appeared equally sensitive as the D-21 cell strains to the antiviral effect of HuIFN-γ and both the D-21 and T-21 cell strains were more sensitive to the antiviral activity of HuIFN-γ than was the M-21 cell strain (Fig. 1, lower panel).

Like HuIFN-β, MuIFN-β brought about

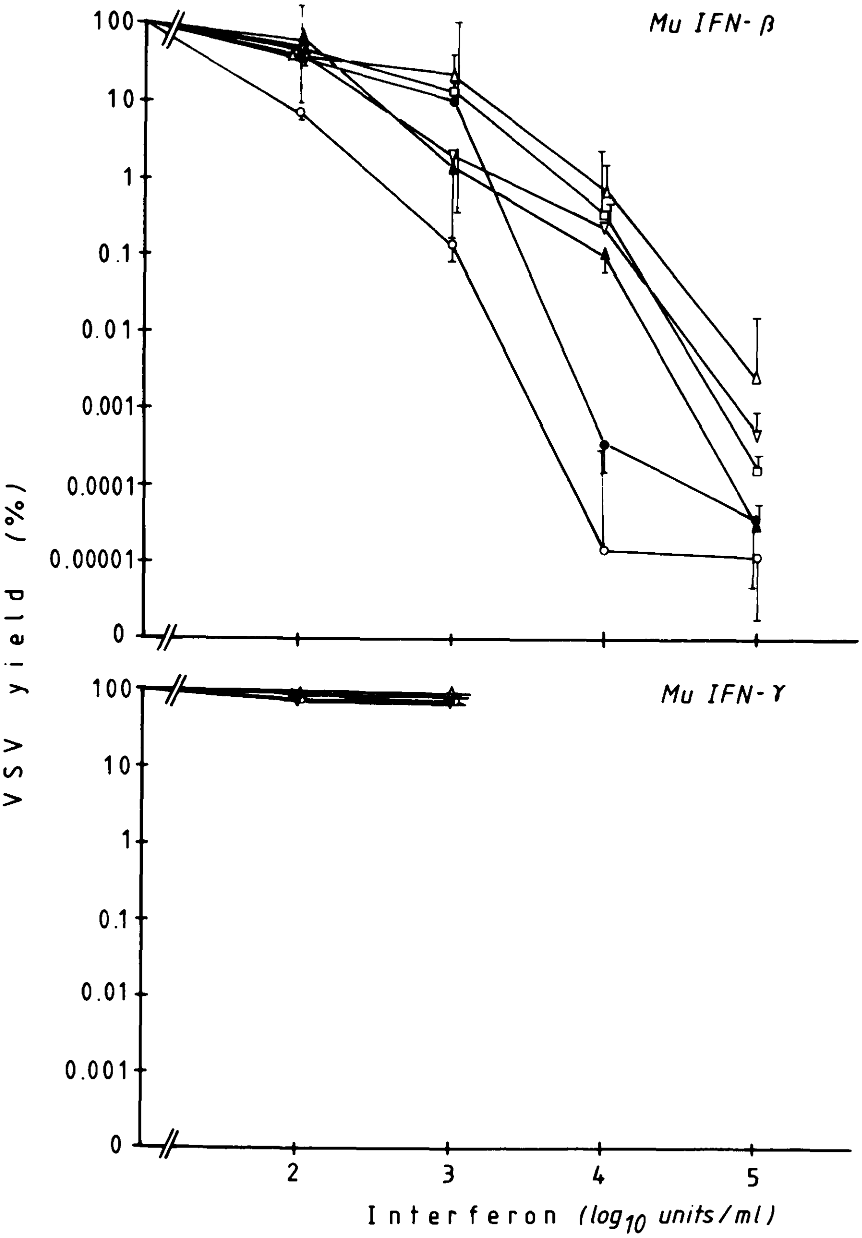

FIG. 2. Antiviral activity of MuIFN-β (upper panel) and MuIFN-γ (lower panel) in T-21 cells [strain LR (●), strain GM 2504A (○)], D-21 cells [strain VGS (▲), E_1SM (△), E_3SM (▽)], and M-21 cells [strain GM 137 (□)]. The data represent the average values (±SD) for two separate experiments.

a dose-dependent inhibition of VSV yield in human T-21, D-21, and M-21 cells (Fig. 2). The slopes of the dose–response curves of MuIFN-β were similar to those observed for HuIFN-β but shifted to the right over a distance of approximately 3 log interferon units/ml (compare Fig. 2, upper panel with Fig. 1, upper panel). The two T-21 cell strains proved more sensitive to the antiviral effect of MuIFN-β than the D-21 or M-21 cell strains, as most clearly demonstrated at a dose of 10^4 interferon units/ml

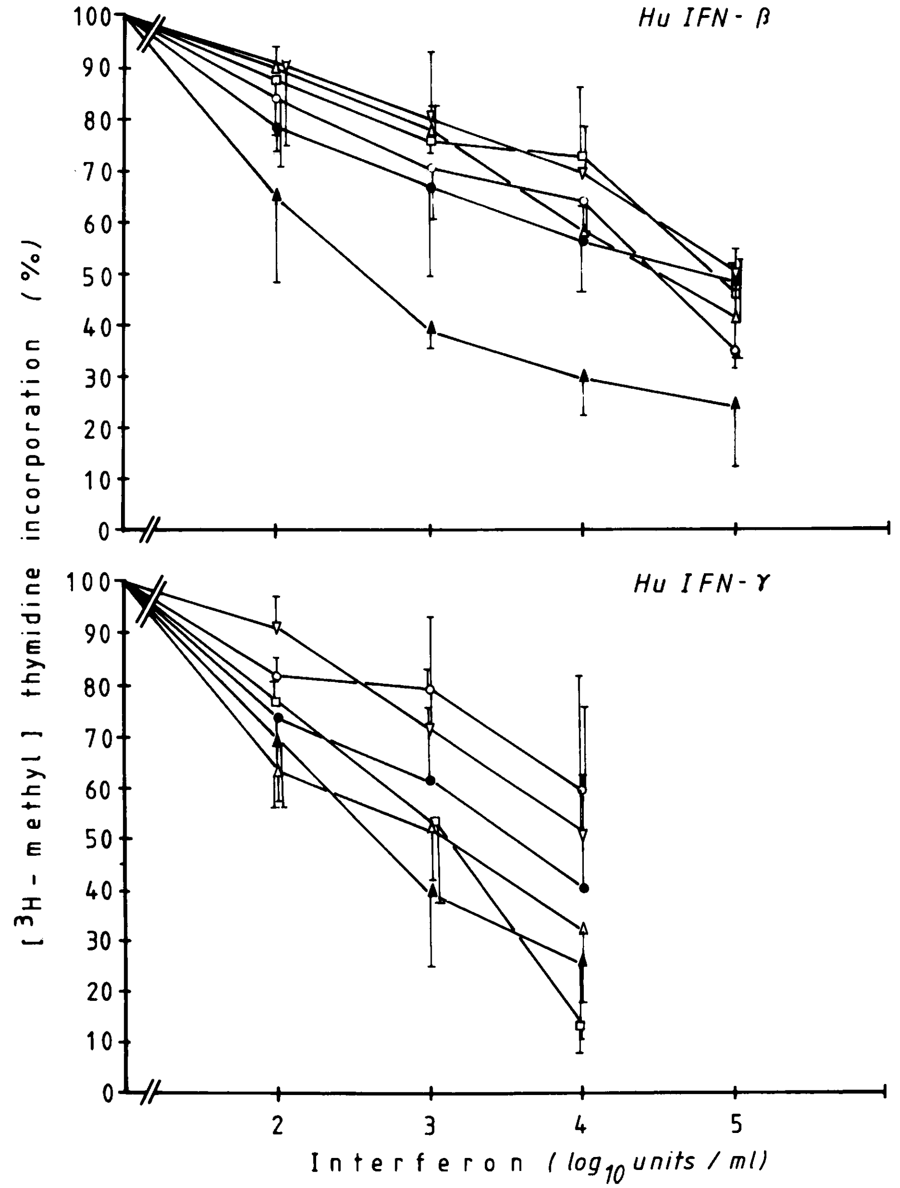

FIG. 3. Anticellular activity of HuIFN-β (upper panel) and HuIFN-γ (lower panel) in T-21 cells [strain LR (●), strain GM 2504A (○)], D-21 cells [strain VGS (▲), E_1SM (△), E_3SM (▽)], and M-21 cells [strain GM 137 (□)]. The data represent the average values (±SD) for three separate experiments.

(Fig. 2, upper panel). For MuIFN-γ no heterologous antiviral activity could be witnessed (Fig. 2, lower panel). Due to the limited amounts of MuIFN-γ that were available, it could not be assayed at a concentration higher than 10^3 units/ml.

Anticellular activity. Both HuIFN-β and HuIFN-γ caused a dose-dependent inhibition of cell proliferation, as monitored by [*methyl*-^{3}H]thymidine incorporation into DNA (Fig. 3). The dose–response curves of HuIFN-γ were generally steeper than the dose–response curves of HuIFN-β, which means that 50% inhibition level was reached at a lower concentration of HuIFN-γ than of HuIFN-β. For example, the inhibitory dose-50 (ID_{50}) of HuIFN-γ for D-21 (E_1SM) and M-21 cells was 10^3 units/ml, as compared to $10^{4.5}-10^{4.8}$ units/ml for HuIFN-β. The two T-21 cell strains were not more sensitive to the cell-growth inhibitory effects of HuIFN-β than the other cell strains. In fact, the most sensitive was VGS, a D-21 strain (Fig. 3, upper panel). VGS cells as well as M-21 cells were also more sensitive to the antiproliferative effects of HuIFN-γ than either of the T-21 cell strains (GM 2504A or LR) (Fig. 3, lower panel).

The inhibitory effects of MuIFN-β on the growth of human T-21, D-21, and M-21 were relatively weak as compared to the anticellular activity of homologous interferon (HuIFN-β or -γ) (Fig. 4). Again, VGS cells proved more sensitive to the antiproliferative effects of MuIFN-β than any other cell strain. Although the cell-growth inhibitory effects of MuIFN-γ could not be fully assessed because of lack of high-titered material, the heterologous anticellular activity of MuIFN-γ tended to be greater than that of MuIFN-β. For example, MuIFN-β did not substantially decrease the growth of D-21 (E_1SM) and M-21 cells when used at a concentration of 10^3 units/ml (Fig. 4, upper panel), whereas at the same dosage level MuIFN-γ reduced the growth of both cell strains by more than 25% (Fig. 4, lower panel).

Discussion. The salient features of the studies presented here could be summarized as follows. (i) T-21 cells were more sensitive to the antiviral activity of HuIFN-β than D-21 or M-21 cells, but the reduction in virus yield effected by interferon was not proportional to the number of chromosome 21. Thus, no gene dosage effect was apparent, and, in this sense, our results diverge from those reported by Tan *et al.* (2, 3).

(ii) The increased sensitivity of T-21 cells to the antiviral action of interferon could, as has been noted in some previous studies (11), be extended to heterologous interferons, since MuIFN-β, like HuIFN-β, effected a greater virus yield reduction in T-21 than in D-21 or M-21 cells. Again, no gene dosage effect was apparent.

(iii) T-21, D-21, and M-21 cells did not markedly differ in their sensitivity to the antiviral activity of HuIFN-γ. This contrasts with the findings of Epstein and Epstein (10) who reported a higher sensitivity, and the findings of De Ley and Billiau (19) who reported a lower sensitivity of T-21 cells to the antiviral activity of HuIFN-γ. A characteristic difference between these three studies was the kind of inducer used to prepare HuIFN-γ; the inducer was phytohemagglutinin (10), concanavalin A (19), and staphylococcal enterotoxin A (this work). Depending on the degree of purity achieved, residual amounts of these inducers may have been present in the interferon preparations used.

(iv) T-21 cells did not prove more sensitive to the anticellular activity of human interferon (HuIFN-β or -γ) or murine interferon (MuIFN-β or -γ) than did D-21 or M-21 cells. So far, only one report has appeared, claiming an increased sensitivity of T-21 cells to the growth-inhibitory properties of human interferon (12). This claim could not be substantiated by the present findings. Relatively important variations were observed in the sensitivity of various D-21 cell strains to the anticellular activities of HuIFN-β or -γ and MuIFN-β or -γ (Figs. 3 and 4), and even greater variations have been noted previously (25) in the antiproliferative effects of interferon on human tumor cells. These variations may obviously mask the impact, if any, of chromosome 21 on the growth-inhibitory effect of interferon.

(v) The antiviral activity induced by HuIFN-γ followed a dose–response curve that differed markedly in slope from the dose–response curve of HuIFN-β (Fig. 1), indicating that, as has been suggested before (26), type I and type II interferons may establish the antiviral state by different (derepressional ?) mechanisms.

(vi) Unlike MuIFN-β, MuIFN-γ did not exhibit any heterologous antiviral activity when assayed at 10^3 units/ml (Fig. 2). This is in keeping with the general trend that type II interferons, whether human or murine, are more species specific in their antiviral action than type I interferons (27–29).

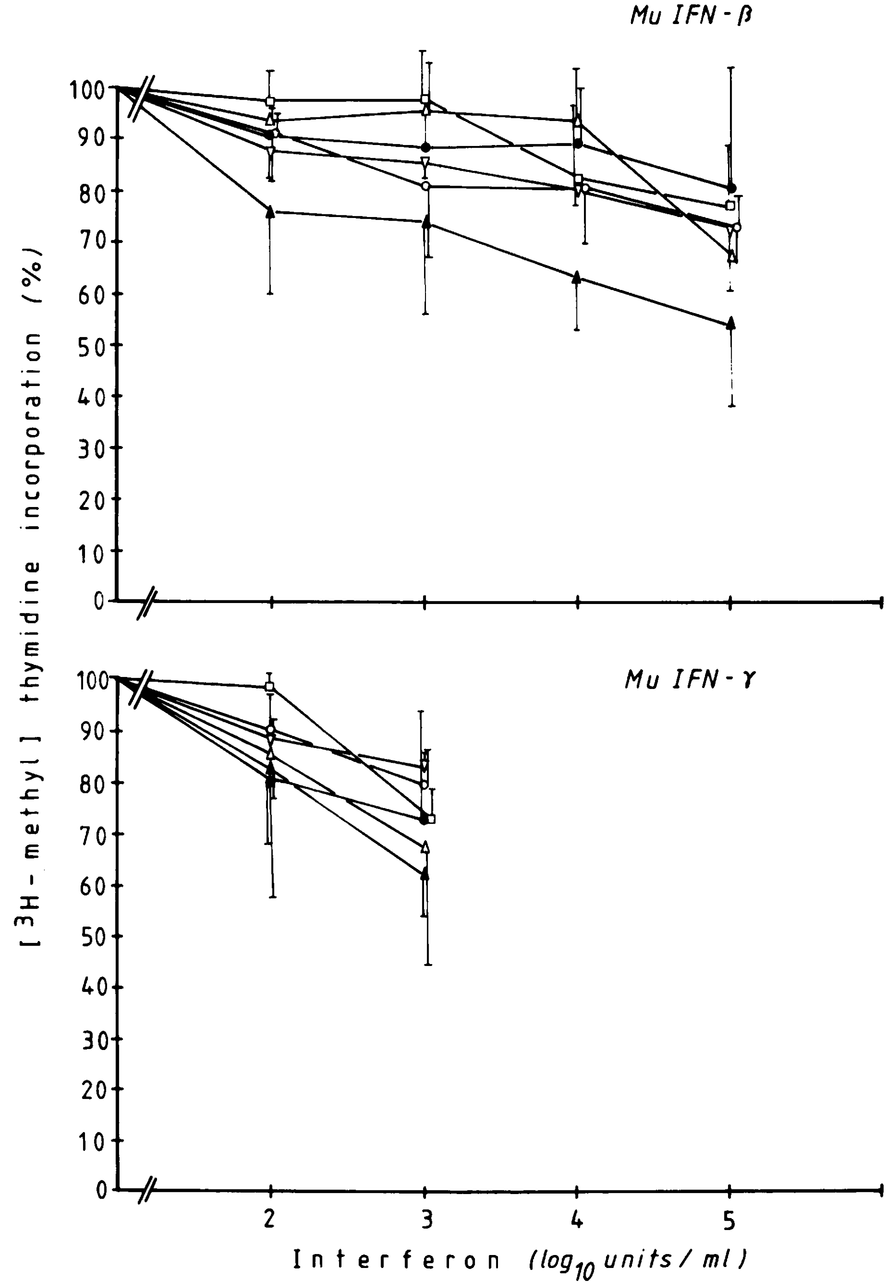

FIG. 4. Anticellular activity of MuIFN-β (upper panel) and MuIFN-γ (lower panel) in T-21 cells [strain LR (●), strain GM 2504A (○)], D-21 cells [strain VGS (▲), E_1SM (△), E_3SM (▽)], and M-21 cells [strain GM 137 (□)]. The data represent the average values (±SD) for three separate experiments.

(vii) Per unit of antiviral activity, HuIFN-γ and MuIFN-γ exerted, at least in some cell strains, a markedly greater cell-growth inhibitory effect than their β counterparts (Figs. 3 and 4). The difference in the ID_{50} of HuIFN-γ and HuIFN-β for D-21 (E_1SM) and M-21 cells was about 30- to 60-fold, a figure which closely corresponds to the differences in anticellular activity that have been reported for HuIFN-γ versus HuIFN-β (30) and MuIFN-γ versus MuIFN-β (31), respectively.

In additional experiments we examined the levels of 2-5A (pppA2′p5′A2′p5′A) synthetase induced as a function of the interferon (HuIFN-β) dose. The 2-5A synthetase activity was measured essentially as described previously (32). No gene dosage effect could be established for the T-21, D-21, and M-21 cells examined (data not shown). In fact, the dose–response curves varied considerably from one D-21 cell strain to another, and even for the same cell strain the dose responses showed important fluctuations depending on the cell passage number and cell density. That the 2-5A synthetase activity is critically dependent on the cell growth conditions has also been demonstrated by other authors (33). Unless these conditions can be standardized rigorously, it would seem difficult to correlate the induction of 2-5A synthetase activity by interferon to the number of chromosome-21 copies present in the cell.

As demonstrated here and previously (5, 18, 19), there are several exceptions to the rule that all interferon activities are better expressed in T-21 cells than in D-21 or M-21 cells. These exceptions indicate that the relationship between chromosome 21 and interferon activity is not a simple one. It may seem premature to postulate that chromosome 21 codes for a receptor common for all interferon effects (16). This premise does not take into account that (i) HuIFN-γ, or at least some HuIFN-γ preparations, are as active, and in some instances (19) even less active, on T-21 than on D-21 cells, and (ii) some effects of interferon, i.e., inhibition of cell growth, are not markedly affected by the number of chromosome 21. If it were to be proven that chromosome 21 codes for an interferon receptor, the latter observations can be accomodated only by assuming that HuIFN-γ is recognized by a different receptor, not coded for by chromosome 21, and/or the multiple effects of interferon can be independently regulated subsequently to its interaction with the putative interferon receptor.

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