Human Chromosome 21 Dosage: Effect on the Expression of the Interferon Induced Antiviral State

Abstract. *Human primary skin fibroblasts trisomic for chromosome 13, 18, or 21 and diploid human skin fibroblasts were induced for an antiviral response with human interferon. The cells that were trisomic for chromosome 21 were three to seven times more sensitive to protection by human interferon than the normal diploid or trisomic 18 or 13 fibroblasts. The differential response in trisomic 21 cells is consistent with the known assignment of the human antiviral gene to chromosome 21.*

Several human disorders result from abnormalities of chromosome number and, of course, trisomy 21 (Down's syndrome) is the most common. While it is assumed that the extra chromosome is the direct cause of the developmental and metabolic defects observed in trisomy 21, the mechanism by which they are produced is unknown. Of several mechanisms that may be postulated, the simplest is that the extra chromosome results in a linearly proportional increase of those products controlled by genes on chromosome 21. This increase then produces an imbalance between the products determined by chromosome 21 and the gene products determined by other chromosomes which, in turn, results in the developmental and metabolic abnormalities. Similar proposals would also apply to partial trisomies and deletions.

Unfortunately, while well established for microorganisms, a direct correlation between gene dosage and the amount or activity of gene products is relatively poorly substantiated in mammalian systems. Attempts to use changes in the activities of various enzymes in cells of aneuploid individuals to establish the linkage of the genes for these enzymes have failed and have led to the realization that aneuploidy can affect the physiology of cells so that enzyme activities are altered, regardless of gene dosage or the particular chromosome involved. In contrast, it has been possible to demonstrate X linkage of the genes that code for hypoxanthine guanine phosphoribosyltransferase (HGPRT) (E.C. 2.4.2.8) and glucose-6-phosphate dehydrogenase (G6PD) (E.C. 1.1.1.49) in the mouse by showing a direct correlation between the activities of these enzymes in oocytes in which inactivation by the X chromosome does not occur and the number of X chromosomes present (*1*). Furthermore, Mayeda *et al.* (*2*) have shown a decrease in the relative amount of lactate dehydrogenase-1 (LDH-B) (E.C. 1.1.1.27) in starch-gel electropherograms of hemolyzates from a patient showing a deletion in the short arm of chromosome 12. Analysis of cell hybrids has established that the LDH-B locus is on chromosome 12 (*3*). However, the LDH data do not indicate whether the decrease was in LDH-B subunit production or whether it was proportional to the loss of one of the two LDH-B loci. With the use of electrophoretic variants of glucose phosphate isomerase (E.C. 5.3.1.9) and isocitrate dehydrogenase (E.C. 1.1.1.42), a better correlation between the relative activity on starch gels and the number of chromosomes bearing the specific locus was independently observed by Ruddle (*4*) and Farber (*5*) in heteroploid mouse cell lines. In these latter instances, the genetic linkage of the enzymes under study had been determined by other methods, and the results served to confirm these linkages. We report here on the use of differential gene dosage in normal and trisomic cells to confirm the assignment of the gene for antiviral protein (AVP) to chromosome 21 and to assess the effect of 21 trisomy on the expression of this gene.

Tan *et al.* (*6*), utilizing mouse-human cell hybrids, have provided evidence for the assignment to chromosome 21 of the genes for the human dimeric form of indophenol oxidase (superoxide dismutase; IPO-A) and the AVP induced by human interferon. Independently Nabholz and Merigan (*7*) also suggested the synteny of IPO-A and AVP; the latter is the presumed protein factor that initiates specific inhibition of virus replication. A natural sequel to the assignment of AVP to chromosome 21 was to compare the antiviral responses of trisomic 21 (T-21) and diploid (D-21) cells in an attempt to detect an effect related to chromosome (gene) dosage.

Fibroblast cultures were established from skin biopsies of patients with Down's syndrome and from their normal siblings (*8*). To minimize nonchromosomal differences, biopsies were taken from identical sites, and siblings of the same sex and as close in age as possible were selected. The cell cultures were also matched as nearly as possible with regard to their total number of cell generations in culture. Matched pairs of D-21 and T-21 cultures were coded and then processed; a double-blind procedure was used. All cell cultures were free of mycoplasma and bacterial contamination (*9*).

In order to quantitatively assess the antiviral state induced by interferon in T-21 and D-21 cells, five matched pairs of fibroblast cultures were exposed for 20 to 24 hours to different concentrations of human interferon, ranging from a 1 : 1,000,000 to a 1 : 1,000 dilution of a stock interferon preparation (*10*). The cells were subse-

Table 1. Units of human interferon required to inhibit viral replication. The values shown represent inhibition of 50 percent.

Cell line	Inhibition by human interferon (units) of	
	Virus-induced CPE	Viral RNA synthesis
	Trisomic	
Set I	0.007	0.008
Set II	0.007	0.011
Set III	0.010	0.009
Set IV		0.009
Set V		0.013
	Diploid	
Set I	0.042	0.044
Set II	0.052	0.047
Set III	0.069	0.072
Set IV		0.032
Set V		0.055

Table 2. Induction of antiviral state in human fibroblasts trisomic for chromosome 13, 18, or 21. Two sets of duplicate confluent human fibroblasts in 35-mm dishes were induced with human interferon and processed as described in legend to Fig. 1c. Results are expressed as units of interferon.

Cell line	Inhibition by interferon of viral RNA synthesis
Trisomic 21	0.008, 0.009
Trisomic 21	0.007, 0.009
Trisomic 18	0.049, 0.049
Trisomic 18	0.052, 0.052
Trisomic 13	0.056, 0.045
Diploid	0.044, 0.042
Diploid	0.056, 0.047

quently challenged with vesicular stomatitis virus (VSV) and examined 36 to 48 hours later for virus-induced cytopathogenic effects (CPE). In addition, in some experiments, the cells challenged with virus were examined 14 to 16 hours later for the total amount of viral RNA synthesis, which was measured by the incorporation of tritiated uridine into the trichloroacetic acid–precipitable fraction in the presence of actinomycin D (*11*). With both assays for the inhibition of VSV replication, the concentration of human interferon required to inhibit viral replication by 50 percent was found to be three to seven times higher for the D-21 than for the T-21 cells (Fig. 1, a and c).

In the second set of experiments, the five matched pairs of T-21 and D-21 fibroblast cultures were tested for their sensitivity to human interferon, and the T-21 cells were more sensitive than the D-21 cells to the action of interferon (Table 1). To demonstrate that this is an effect specific for trisomy of chromosome 21 and not a generalized trisomic effect, we measured the concentration of human interferon required to induce the antiviral state in trisomic 18 and 13 (T-18 and T-13) fibroblasts. The concentration required to induce an antiviral state in T-18 and T-13 fibroblasts was in the same range as that required to protect D-21 fibroblasts (Table 2).

Induction of the antiviral state was repeated with subinducing concentrations of polyinosinate · polycytidylate [poly(I) · poly(C)]. At these concentrations, poly(I) · poly(C) induces only an antiviral state that is thought to be mediated by the induction of a low concentration of intracellular interferon which is not detected in the medium. The T-21 cells were three to seven times more sensitive than D-21 cells to the antiviral action of poly(I) · poly(C) (Fig. 1b). These results are consistent with the observations (Fig. 1, a and c) concerning the sensitivity of T-21 and D-21 cells to human interferon.

To establish that the increased sensitivity to interferon or poly(I) · poly(C) is not in some way due to a nonspecific increase in interferon induction, we compared the interferon production in five pairs of matched D-21 and T-21 fibroblast cultures. No significant difference in interferon production [32 to 64 units of poly(I) · poly(C)–induced human interferon per 2×10^5 T-21 or D-21 fibroblasts] was observed, an indication that differential interferon production is not responsible for differences between T-21 and D-21 fibroblasts.

We interpret our observation of increased response to interferon and interferon inducers by T-21 cells to mean that the extra chromosome 21 in these cells was responsible for the enhancement of protection by interferon. This interpretation is consistent with the assignment of the gene for the expression of the antiviral state to chromosome 21. However, if the enhanced antiviral response in T-21 cells were strictly a consequence of gene dosage, only a 1.5-fold increase would be expected, in contrast to the observed three- to sevenfold enhancement observed. One possible explanation is that a regulator element controlling the expression of the antiviral gene (or genes) operates less efficiently in T-21 cells than in normal diploid cells because of an imbalance between the number of AVP genes and "regulator" genes (*12, 13*). For this model to apply it is necessary to postulate the existence of a "regulator" gene, located on a separate chromosome, the product of which regulates the antiviral state by a negative feedback mechanism (*13*). Another possible explanation is that protection by endogenously induced AVP is not linear. Thus, while the amount of AVP gene product might be increased to one and a half times that of normal T-21 cells, the ultimate cellular response in terms of resistance to viral infection could be considerably greater. This question can only be settled by direct measurement of the

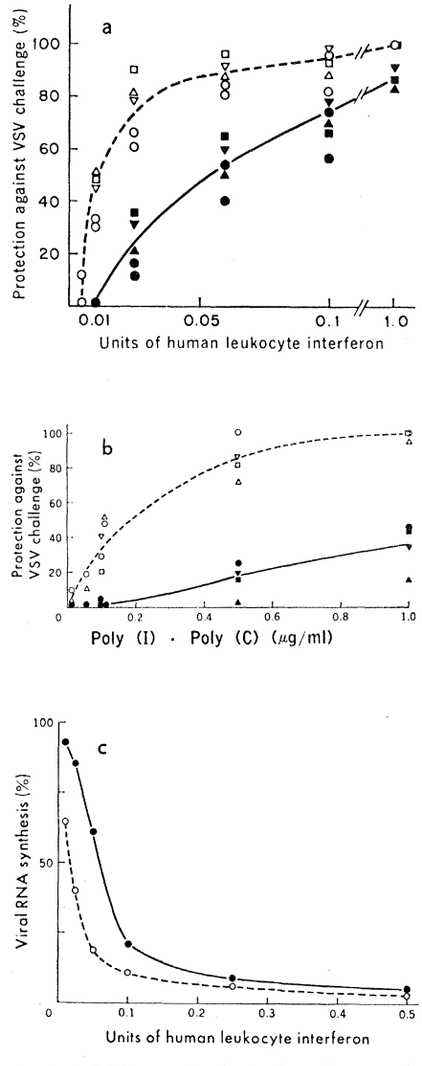

Fig. 1. (a) The induction of the antiviral state in normal diploid fibroblasts and fibroblasts from Down's syndrome patients by (i) human leukocyte interferon. (b) Induction by poly(I)·poly(C). Wells (micro) of a test (semimicro) dish (Linbro) were seeded with 2×10^4 cells per well and treated with the indicated concentrations of interferon or poly(I)·poly(C) 24 hours later. Each set of triplicate wells was exposed to 0.2 ml of human interferon or poly(I)·poly(C) for 20 hours. The culture medium was decanted to remove the interferon or poly(I)·poly(C) and the cultures were challenged with 0.2 ml of VSV suspension containing 1×10^7 plaque-forming units (PFU) of virus per milliliter. The plates were examined 36 to 48 hours later for virus-induced cytopathogenic effect (CPE). For each T-21 and D-21 pair tested, a triplicate set was used for a virus control and another set for a cell control. The percentage protection against a viral challenge is measured by the inhibition of virus-induced CPE after human interferon treatment in comparison with the CPE observed in the untreated viral control. (c) The induction of the antiviral state in normal and Down's syndrome fibroblasts by human interferon. Petri dishes (35 mm) were seeded with 1×10^5 cells per dish. For each test one set of duplicate dishes was exposed to 0.5 ml of human interferon at the indicated concentrations for 20 hours. The cultures were washed to remove the interferon; we then added 2 ml of culture medium containing 2.5 μg of actinomycin D, 2 μc of [^{3}H]uridine, and 1×10^7 PFU of VSV for 14 to 16 hours. The cultures were then washed to remove the excess [^{3}H]uridine and exposed to 2 percent cold trichloroacetic acid to precipitate the newly synthesized virus RNA. The percentage of protection against a viral challenge was measured by the inhibition of total viral RNA synthesis in interferon cultures compared to the amount of viral RNA synthesis in the viral controls. Open symbols, T-21; closed symbols, D-21. Cultures from each donor pair are indicated by common open and closed symbols.

chromosome 21–linked AVP gene product.

In light of the above finding, it should be noted that Down's syndrome patients show an increased incidence of leukemia of all types (*14*). Furthermore, in one early study by Shein and Enders primary human cells transformed by SV40 virus showed a high frequency of chromosome rearrangement and monosomy involving the G group chromosomes (*15*). It is possibly significant that the gene that regulates the expression of the antiviral state can be assigned to chromosome 21, and that SV40-induced cell transformation and the incidence of leukemia also involve the same chromosome group.

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16. Supported by PHS grants GM 19952, GM 09966, and GM 18076 and by grant R73-116 from the National Foundation–March of Dimes. We thank Dr. J. Valenta of Smith Kline & French Laboratories for the crude interferon.
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19 March 1974; revised 14 May 1974

Morphine and Ethanol: Selective Depletion of Regional Brain Calcium

Abstract. *Administration of morphine or ethanol to rats produces a decrease in regional brain calcium in vivo. This effect is selectively antagonized by the stereospecific narcotic antagonist naloxone. Reserpine and the dopamine-acetaldehyde conjugate salsolinol also produce a depletion of regional brain calcium, but only the salsolinol depletion is antagonized by naloxone. Experiments with naloxone provide evidence for two calcium-sensitive pools in the central nervous system.*

Calcium ions play an important role in the release of neurotransmitters and in neuronal excitability (*1, 2*). Moreover, calcium plays a key role in mechanisms of excitation–muscle contraction coupling and excitation–neurotransmitter secretion coupling (*3*). Calcium has been suggested as the primary transducer of many hormone receptor interactions (*4*) and as a coupling agent in certain central nervous systems (CNS) (*5*). However, despite the vast amount of research on calcium and neuronal systems in vitro, very little is known about the effects of various drugs, particularly psychoactive drugs, on the in vivo calcium concentrations in the CNS. It has been demonstrated, however, that calcium antagonizes morphine analgesia (*6*) and the development of tolerance to morphine (*7*), and the administration of morphine to mice produces a slight but significant decrease in whole brain calcium (*8*). In addition, morphine stimulates phospholipid metabolism and inhibits transport of calcium facilitated by phospholipids and gangliosides (*9*). Similarly, the effects of ethanol on brain calcium in vivo have received little attention, although it has been reported that both morphine and ethanol antagonize the release of acetylcholine from whole brain and cortex slices (*10*). To further evaluate the role of calcium in psychoactive drug action, we have studied the in vivo effects of morphine and ethanol on regional brain calcium.

Brain calcium concentrations were determined by atomic absorption spectroscopy with a Perkin-Elmer model 303 spectrophotometer. Male rats (Sprague-Dawley, 150 to 200 g) were decapitated after appropriate drug treatment; the brains were immediately removed and rinsed in five washes of ice-cold isotonic saline to remove as much blood as possible. Use of ice-cold saline also facilitated the regional dissection of tissue. After the tissue was blotted dry, regional brain areas were dissected according to the outline of Glowinski and Iverson (*11*). Portions of tissue (25 to 50 mg) were transferred to weighed disposable test tubes, 200 μl of concentrated HNO_3 (analytical grade) was added to each tube, and the contents were evaporated to a dry white ash over a hot plate. The residue was allowed to cool for 15 minutes and resuspended in a solution of 0.5 ml of 0.1*N* HCl plus 4.5 ml of 1 percent lanthanum (as the oxide) in 0.01*N* HCl.

Four regional areas were examined: corpus striatum, cortex, hippocampus, and hypothalamus. As seen from Table 1, administration of morphine sulfate (25 mg per kilogram of body weight) produced a significant decrease in regional brain calcum. This decrease was dependent on dose over the range 5 to 50 mg/kg, linear over the analgesic range 5 to 25 mg/kg, and saturable at doses greater than 50 mg/kg. To determine whether the effect would be classified as a specific narcotic action, we attempted to block the agonist action with a specific antagonist. Naloxone hydrochloride has essentially no agonist effects except at high doses (50 mg/kg) and is highly stereospecific in antagonist activity (*12*). Naloxone inhibited the morphine-induced calcium depletion and showed no significant calcium-depleting effects when administered alone. The specificity of the calcium-depleting effect of morphine was further tested by giving reserpine, which has been reported to lower brain calcium levels (*13*). Although reserpine (5 mg/kg) caused an average regional depletion of 38 percent, the reserpine-