distinct clinical syndromes, the polyps all possess varying degrees of potential for malignant degeneration. The study of various polyps will allow a comparison of heritable versus nonheritable tumor lesions at a similar site and also offers an opportunity to observe the phenotypic changes in tissue during malignant transformation. The finding of multiclonal origin of polyps in patients with Gardner syndrome does not permit us to predict whether carcinomas subsequently found in the patients will be of monoclonal or polyclonal origin. A recent report on neurofibromatosis, however, indicates that the benign lesion (neurofibroma) is polyclonal and the malignant lesion (neurofibrosarcoma) is monoclonal (*26*). Studies of tumors originating from known polyps in black females with Gardner syndrome and who are heterozygous for G6PD should provide experimental data to test the hypothesis of a multistage process of human carcinogenesis and the possibility that the malignant neoplasm in the human probably always develops from a single cell (*27*).

SUSAN H. HSU
Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205
GORDON D. LUK
Department of Medicine and *Oncology Center, Johns Hopkins University School of Medicine*
ANNE J. KRUSH
Department of Medicine, Johns Hopkins University School of Medicine
STANLEY R. HAMILTON
Department of Pathology, Johns Hopkins University School of Medicine
HERBERT H. HOOVER, JR.
Department of Surgery and *Oncology Center, Johns Hopkins University School of Medicine*

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Cell-to-Cell Transfer of Interferon-Induced Antiproliferative Activity

Abstract. *Interferon-treated cells rapidly and efficiently transferred the antiproliferative activity of interferon to untreated cells. This phenomenon was not due to the carry-over of interferon by the interferon-treated cells. Thus, to evoke an antiproliferative state, interferon did not directly contact each cell in a population. The results suggest a novel mechanism by which interferon may indirectly regulate cell growth, and suggests that cells other than those of the immune system may play a role in controlling tumor growth in tissue where cell-to-cell contact occurs.*

Interferons (IFN's) can induce a wide range of activities in cells and the mechanism for these responses is generally attributed to the direct interaction of IFN with membrane receptors on each target cell (*1*). Recently, IFN was shown to have an amplification system in which IFN-treated cells indirectly induce antiviral activity (*2–6*) in other cells. It was hypothesized that a secondary messenger molecule is transferred from the IFN-induced cell to the recipient cells which then express the antiviral activity of IFN (*7*). Here we show that the antiproliferative activity of IFN can also be transferred between cells.

For these experiments we used two cell systems in which the effect of IFN was transferred across the species preference barrier of IFN (to xenogeneic cells) and the recipient cells could be separated from the donor cells prior to the assay of antiproliferative activity. Figure 1A shows that cocultivation of mouse L1210 lymphoblastoid cells with human IFN-treated WISH cells resulted in the transfer of significant antiproliferative activity to the L1210 cells, as measured by reduced incorporation of [^{3}H]thymidine into cellular DNA. Both recombinant IFN-γ (*8, 9*) and partially purified IFN-α were able to induce an effective dose response. Likewise, mouse IFN-treated L cells transferred antiproliferative activity to human lymphoblastoid Daudi cells (Fig. 1B). In all cases, the amount of antiproliferative activity transferred followed asymptotic dose-response curves of similar magnitude. The reduction in growth rate was not due to the direct effect of IFN since donor cells were thoroughly washed before coculturing with recipient cells. Assay of supernatant fluids at the end of the 4-hour coculture period failed to detect carry-over of IFN unless more than 1000 IU had been used to induce the cells, in which case only 1 to 3 U/ml were detected. Also, the phylogenetic barrier of IFN was shown to be effective because direct treatment of L1210 cells with 100 IU of human IFNα or Daudi cells with 100 U of mouse IFN-α and -β resulted in no decrease in [^{3}H]thymidine incorporation (Fig. 1). In our system the incorporation of [^{3}H]thymidine into DNA was shown to be an accurate measure of cell growth, both in lymphoblastoid cells that were cocultured with IFN-treated cells or lymphoblastoid cells cultivated alone in dilutions of IFN (*10*). An assay of specific chromium release (*11*) suggested that nonspecific cellular cytotoxicity was not responsible for the observed reduction in cellular growth rate (*12*).

We also tested the ability of other human IFN's to induce antiproliferative transfer. When human IFN-β (specific activity 10^6 U per milligram of protein) or IFN-γ (specific activity 10^5 U/mg) were used to induce the transfer of antiproliferative activity to L1210 cells, the dose-response curves were similar in shape and magnitude to those in Fig. 1 (data not shown).

Figure 2, A and B, shows the results of two typical experiments in which antiviral activity was transferred to L1210 or Daudi cells concurrently with antiproliferative activity. In both cell systems, transfer of antiproliferative and antiviral activity correlated well with the IFN dose, and the results of many experiments showed that high antiproliferative

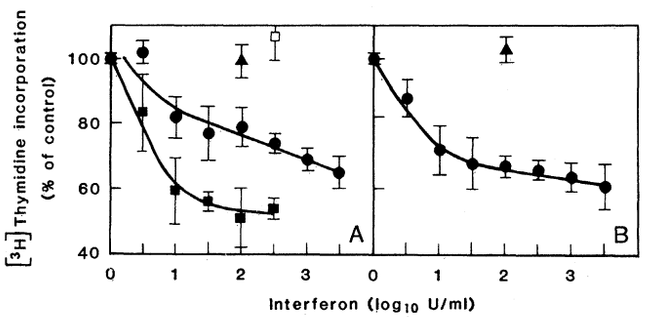
activity was transferred together with high antiviral activity. This suggests that these distinct biological activities may be induced by a similar mechanism.

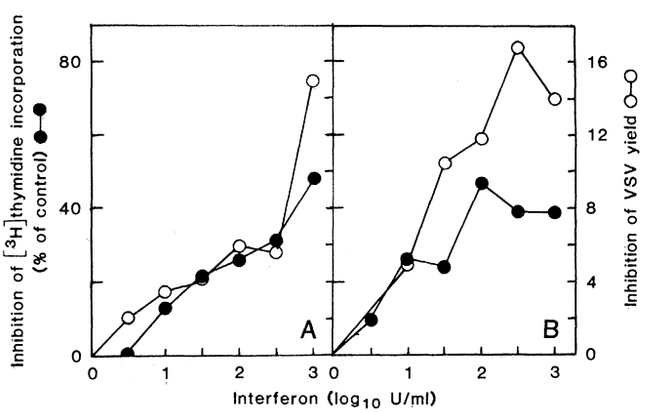
Figure 2C shows that antiproliferative activity was rapidly transferred to Daudi cells, that is, within 30 minutes of the initiation of coculturing with donor cells. By 2 hours the maximum activity had been transferred and the activity was stable through at least 14 hours of coculture conditions. These data agree with previous reports concerning the time in coculture necessary to transfer antiviral activity between cells (*7*).

We have previously shown that the transfer of antiviral activity is not mediated by soluble factors released by cells and is dependent on cell contact (*13*). To investigate whether soluble mediators induce antiproliferative activity, we treated WISH or L cells with IFN and washed them as described in Fig. 1. They were then incubated for 12 hours. Then L1210 or Daudi cells, respectively, were incubated for 6 hours in the supernatants from the WISH or L cells and found to incorporate the same levels of [^{3}H]thymidine as the controls. Interferon was not detectable in the supernatants (data not shown). Thus, the transfer of antiproliferative activity is probably not mediated by the release of soluble factors but does appear to depend on some type of attachment or intimate contact between inducer and receptor cells, since the transfer of antiproliferative activity was prevented by gently rocking cocultures of IFN-treated L cells and Daudi cells.

The data suggest that the transfer of antiproliferative activity is specifically induced by treatment of homologous cells with IFN. Not only did recombinant IFN-γ effectively induce the transfer of antiproliferative activity in a dose-dependent manner (Fig. 1A), whereas control supernatants did not, but the other types of IFN tested thus far also induced antiproliferative transfer. Each of these IFN's was prepared from different cell types, and four were highly purified (specific activity 10^5 to 10^6 U per milligram of protein). Another experiment showed that the transfer of antiproliferative activity from L cells to Daudi cells was abolished by prior treatment of the mouse IFN-γ with antiserum to IFN-γ (*14*) (data not shown).

We have also found that several other types of cells, including human leukocytes, are capable of participating in the transfer of antiproliferative activity. The levels of antiproliferative activity transferred are comparable to the direct effect of IFN on L1210 or Daudi cells both in our experiments (data not shown) and those of others (*15, 16*). The transfer of antiproliferative and antiviral activity appears to occur by the same or a similar mechanism. Although IFN is specifically required to induce the transfer of anti-





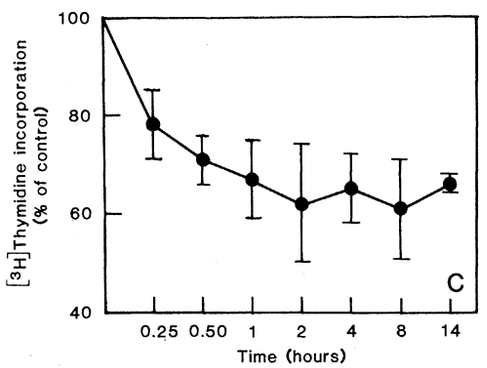


Fig. 1 (left). Transfer of IFN-induced antiproliferative activity to lymphoblastoid cells. (A) Human amnion WISH cells (6×10^4 per well) were plated into Costar 96-well microculture plates in 0.1 ml (per well) of Eagle's minimum essential medium (EMEM) supplemented with 10 percent fetal calf serum (FCS), penicillin (100 U/ml), and streptomycin (100 μg/ml). After overnight incubation, the cells were treated for 4 hours with dilutions of human IFN-α (specific activity 10^6 U per milligram of protein) (●), recombinant human IFN-γ (specific activity 1.7×10^4 U per milligram of protein) (■), or control supernatants for the recombinant IFN (□), then were washed three times with EMEM with 2 percent FCS. L1210 cells in conditioned growth medium (RPMI 1640 with 5 percent FCS and antibiotics) were added to each well (2×10^5 per well) and the cells were cocultured for 4 hours at 37°C in an atmosphere of 5 percent CO_2 (●, ■, □) or cultured alone with human IFN-α (▲). The plastic nonadherent L1210 cells were loosened from WISH cell monolayers by gentle agitation of the supernatant with a Titertek multichannel micropipette and the suspended cells were transferred to a new microculture plate. The L1210 cells were exposed for 1 hour to 2μCi of [^{3}H]thymidine (Amersham) before DNA was precipitated with acid and collected on 25-μm glass-fiber filters (Gelman) for scintillation counting. Human IFN was titrated on WISH cells (*20*) and mouse IFN was titrated on L cells with a slightly modified plaque reduction assay (*21*). (B) The incorporation of [^{3}H]thymidine by human Burkitt lymphoma Daudi cells (American Type Culture Collection) after coculture with L cells treated with virus-induced (*22*) mouse IFN-α and -β. Procedures were as described above, except Daudi cell growth medium was RPMI 1640 with 12 percent FCS, and control Daudi cells were cultured with mouse IFN-α and -β (▲). The data represent the mean and standard error of the mean for five experiments (two experiments with recombinant IFN), each performed in triplicate. Fig. 2 (right). Concurrent transfer of antiproliferative and antiviral activity and kinetics of transfer. (A) Human IFN-α treated WISH cell monolayers were cocultured with L1210 cells for 4 hours and then antiproliferative activity was assessed in the L1210 cells as described in Fig. 1. After the L1210 cells were removed from coculture, antiviral activity was measured by challenging the L1210 cells with vesicular stomatitis virus (*VSV*) at a multiplicity of infection of 0.05. Virus yields were determined 18 hours later by a microplaque assay (*21*). (B) Mouse L cells treated with IFN-α and -β were cocultured with Daudi cells for 4 hours and then antiproliferative activity was assessed in the Daudi cells as described in Fig. 1. Antiviral activity was measured by challenging the Daudi cells with VSV as described above. (C) Kinetics of the transfer of antiproliferative activity from L cells to Daudi cells. L cells (1.5×10^6 per well) in 24-well Falcon dishes were treated overnight with 300 U of mouse IFN-α and -β, then washed three times and overlaid with a 1-ml suspension of Daudi cells (1.5×10^6 per milliliter) at time point 0. At the indicated times points, the Daudi cells were removed from the L cells and exposed to [^{3}H]thymidine as described in Fig. 1B. The data represent the mean and standard error of the mean of three experiments compared to control cocultured cells.

proliferative or antiviral activity, IFN is apparently not the direct effector molecule responsible for inhibiting proliferation in the recipient cells. The evidence is as follows: (i) antiproliferative activity can be effectively transferred between xenogeneic cells that do not respond to heterologous IFN, (ii) transfer will occur if IFN is removed before the cells are cocultured, and (iii) supernatants from IFN-induced cells do not induce antiproliferative activity in recipient cells. Thus the transfer appears to be mediated by a contact-dependent mechanism and the results support the hypothesis that antiviral activity is transferred by means of a secondary messenger, possibly through low-resistance membrane channels such as gap junctions (*7*).

Our results suggest a novel mechanism by which IFN may indirectly regulate cell growth. These cellular interactions could be an important host defense mechanism against neoplasias in tissues, especially where IFN diffusion is poor. Gresser *et al.* reported that a strain of L1210 cells cloned for IFN resistance was still responsive to IFN antitumor therapy in mice (*17*) and attributed these results to the effect of IFN on the host's immune response. The present findings, together with data indicating the probable transfer of antiviral activity in vivo (*18*), suggest that the antitumor effects on IFN-resistant cells may have been due, in part, to the transfer of antiproliferative activity from responding normal mouse cells. Similarly, the human IFN–induced inhibition of human tumor cells in nude mice, which was attributed to a direct effect of IFN only on the human tumor cells, could also have been due to the transfer of activity from mouse effector leukocytes (*19*). Such a defense mechanism suggests that cells other than those of the immune system may play a role in controlling tumor growth where cell-to-cell contact occurs. This mechanism also provides a new system in which basic cell-to-cell communication may be investigated.

RICHARD E. LLOYD
J. EDWIN BLALOCK
G. JOHN STANTON
Department of Microbiology, University of Texas Medical Branch, Galveston 77550

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10. Intracellular thymidine from L1210 cells was measured by high-performance liquid chromatography (HPLC) on an ion-pair reversed-phase column (Serva Feinbiochemica). We used the protocol described for Fig. 1A, except the cell numbers were scaled up 15 times to be performed in 35-mm petri dishes (LUX). The WISH cells treated with 100 U of IFN-α transferred antiproliferative activity effectively and reduced [^{3}H]thymidine incorporation into the DNA of the L1210 cells by 37 percent. However, HPLC analysis revealed no significant changes in the amount of intracellular thymidine from L1210 cells whether they had been cocultured with IFN-treated WISH cells, directly treated with human IFNα, or left untreated. The direct effects of IFN on cellular [^{3}H]thymidine incorporation and cell growth also were compared. L1210 cells or Daudi cells in the log phase of growth were treated directly with dilutions of homologous mouse or human IFN for 48 hours and then cell counts and [^{3}H]thymidine incorporation were determined. When number of cells and the incorporated radioactivity were plotted logarithmically against the units of IFN, parallel curves were obtained which suggested an accurate correlation between [^{3}H]thymidine incorporation and cell growth.,
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12. The specific chromium release assays showed that IFN-treated (1000 IU) WISH or L cells are not cytotoxic toward L1210 cells (3.7 percent specific ^{51}CR release) or Daudi cells (3.3 percent specific ^{51}CR release) even after 22 hours of cocultivation. Furthermore, routine microscopic examination of L1210 or Daudi cells after coculture with IFN-treated cells did not reveal significant cytotoxicity as determined by trypan blue dye uptake.
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Human Fetal Muscle and Cultured Myotubes Derived from It Contain a Fetal-Specific Myosin Light Chain

Abstract. *Human fetal muscles at ages 110, 125, and 132 days contain a fetal-specific myosin light chain. This light chain is absent in adult human muscle, copurifies with myosin, and is identified as a slow light chain because it reacts with purified antibody to chicken slow muscle light chains and does not react strongly with antibody to fast myosin light chains. This light chain is synthesized in cultures of fetal muscle along with normal myosin light chains. The presence of a fetal light chain in culture provides a marker for studies of human muscle disease in which it is important to know when or if the muscle makes a transition from embryonic or fetal expression to true adult phenotype.*

One-dimensional sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis of myosin from human muscle has shown conflicting results concerning the number and distribution of light chains (*1–3*) probably because most human muscles contain mixtures of slow and fast fibers (*4*). Two-dimensional polyacrylamide gel electrophoresis of adult human myosin (*2, 5*) has shown the presence of a slow light chain (SLC_1) with an isoelectric point (PI) and molecular weight closer to that of fast light chain (FLC_1) than in other mammalian species (*6*), although the exact relationship varies possibly because of slightly different analytical conditions. Fetal-specific light chains have been demonstrated in rat skeletal and cardiac muscle (*7, 8*), bovine cardiac muscle (*9*), and in human ventricle (*10*). Volpe *et al.* (*2*) were unable to demonstrate the presence of slow light chains in fetal or neonatal human skeletal muscle and attributed this to the predominance of fast type 2 fibers in these muscles. We now show that a fetal-specific slow light chain (fetal LC_1) is present in human fetal muscle and that it is synthesized in cultures of fetal muscle cells.

Table 1. Synthesis of myosin light chains in cultures of human fetal cells

Light chain fraction	Percent of total light chains*	
	Experiment 1	Experiment 2
Fetal LC_1	27.7	30.9
Slow LC_1	0.9	0.1
Fast LC_1	10.7	13.7
Slow LC_2	5.4	1.0
Fast LC_2	41.1	37.4
Fast LC_3	14.9	16.9

*After electrophoresis and fluorography of [^{35}S]-methionine-labeled myosin from 4- to 5-day-old cultures, stained light chains were cut from the dried gel and extracted with NCS and radioactivity was determined in Omnifluor-toluene scintillation fluid. Corrections for background were made.

Since most human adult muscles contain a mixture of slow and fast fibers (*4*)