I antigen alone. In the experiments of Faustman *et al.* (*1*), the immunogenic cells were eliminated by the treatment with antiserum to Ia; in our experiments Ia positive cells were left intact (even though the Ia antigens were identical between donor and recipient) with class I disparities alone and thus the class I antigenic disparity could be presented in an immunogenic manner leading to graft rejection. We cannot rule out that treatment with antiserum to Ia (*1*) also reduced the load of class I antigens in the transplanted tissues since passenger cells eliminated by such treatment would also express class I antigens.

It is not clear why the K-region disparity studied by us (B10.AQR to B10.A) and the K + D region disparities evaluated led to rejection in the great majority of grafts whereas the D-region disparity [B10.A(2R) to B10.A] did not. Further experiments will have to be performed to evaluate the relative immunogenicity of K- or D-encoded antigens and the effect of both recipient and donor genotype on transplant success.

Thus we have found that mice differing at the H-2K or H-2K + H-2D histocompatibility loci (encoding class I antigens) alone are capable of rejecting pancreatic islet allografts. It appears that the elimination of allogeneic stimulation from I-region (class II) antigens alone is not sufficient to ensure islet allograft survival. Rather, we believe that the results of our experiments in vivo are consistent with the hypothesis that it is the Ia^+ cells in a graft that are immunogenic whether the Ia antigens are foreign to the recipient or not.

CHARLES E. MORROW*
DAVID E. R. SUTHERLAND
Department of Surgery, University of Minnesota Health Sciences Center, Minneapolis 55455
MICHAEL W. STEFFES
Department of Laboratory Medicine and Pathology, University of Minnesota Health Sciences Center
JOHN S. NAJARIAN
Department of Surgery, University of Minnesota Health Sciences Center
FRITZ H. BACH
Immunobiology Research Center, University of Minnesota Health Sciences Center

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* Address correspondence to Box 314, University of Minnesota Hospital, 420 Delaware Street S.E., Minneapolis 55455.

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Tunicamycin Enhances the Antiviral and Anticellular Activity of Interferon

Abstract. *The inhibitory effects of interferon on virus multiplication and cell growth are significantly enhanced by treatment with tunicamycin. Potentiation of antiviral activity was found only with enveloped viruses and not with nonbudding viruses. Changes in the plasma membrane of treated cells may account for this effect, since enveloped viruses bud from the cell surface as a terminal step.*

Interferons are proteins secreted by animal cells in response to viruses and a number of other stimulating substances. Originally described as antiviral (*1, 2*), interferons have many other biological effects, such as the formation of defective virus particles (*3–5*), inhibition of cell growth (*6*), and regulation of the immune system (*7, 8*).

The cell surface (*9*) is important in several phases of interferon's action. Binding of interferon to specific receptors on the surface results in chemical, morphological, physical, and immunological alterations in the plasma membrane. These include changes in plasma membrane density (*10*) and cell surface charge (*11*), an increase in the number of intramembranous particles (*10*), an altered capacity to bind thyroid-stimulating hormone or cholera toxin (*12*), an increase in the binding of lectin (*13*) and in the toxicity of lymphocytes to target cells (*14*), an increase in the expression of cell surface antigens (*14*), an altered exposure of surface gangliosides (*15*), an increase in the concentration of intracellular adenosine 3′5′-monophosphate (*16, 17*), changes in thymidine uptake (*18*), inhibition of cap formation (*19*), cytoskeletal changes, and a decrease in plasma membrane fluidity (*20*). The relation between these diverse effects on cell membranes and the antiviral and antigrowth activities of interferons remains to be established.

Tunicamycin, a glucosamine-containing antibiotic (*21*) produced by *Streptomyces lysosuperficus*, inhibits lipid biosynthesis by blocking the transfer of *N*-acetylglucosamine-1-phosphate from uridine diphosphate *N*-acetylglucosamine to dolichylmonophosphate (*22*). Many important biochemical functions are inhibited by tunicamycin, such as glycoprotein synthesis in yeast (*23*); the bacterial synthesis of polyisoprenol sugars (*24*), peptidoglycans (*25*), procollagen (*26*), and polymeric cell walls (*27*); cell division in *Tetrahymena pyreformis* (*28*); secretion of immunoglobulins A and E by plasma cells (*29*); and virus multiplication (*30*). Tunicamycin also has profound effects on the morphology and surface properties of prokaryotes and eukaryotes and causes changes in membrane organization (*31*). We have reported (*32, 33*) that treatment of L_B cells with interferon or tunicamycin reduces the production of infectious vesicular stomatitis virus (VSV) particles, decreases the amount of glycoprotein and membrane protein in VSV released from treated cells, and inhibits an early step in the formation of asparagine-linked oligosaccharide chains, that is, the incorporation by membrane preparations from treated cells of *N*-acetylglucosamine into glycolipids with the properties of dolichol derivatives.

The cell surface–altering properties of tunicamycin and interferon, coupled with the biological activities mentioned above, prompted us to investigate the role of these substances in the inhibition of virus multiplication and cell growth. We found that the antiviral and antigrowth effects of interferon are significantly enhanced by tunicamycin. Potentiation of antiviral activity was found only with enveloped viruses. Tunicamycin had no effect on the antiviral activity of interferon against encephalomyocarditis (EMC) virus, which is nonbudding.

The Indiana strain of VSV, Sindbis (SB) virus, and EMC virus (originally

Table 1. Effect of interferon and tunicamycin on division of 3T3 cells. On day 2 of culture the cells were washed and treated in triplicate with interferon (IFN) or tunicamycin (TM) or both for 24 hours, at which point the medium was removed. The cells were then trypsinized and enumerated with a Coulter counter.

Treatment	Total number of cells*	Inhibition (percent of control)
Control	5.1×10^5	—
IFN (100 U/ml)	4.3×10^5	15
TM (0.1 μg/ml)	4.2×10^5	18
IFN (100 U/ml) + TM (0.1 μg/ml)	7.6×10^4	85

*Per 33-mm-diameter petri dish.

obtained from C. Buckler) were plaque-purified and passaged at low multiplicities. Virus titers were expressed as median tissue culture infectious doses by monitoring cytopathic effects or plaques in microtiter plates. Vero cells were used for VSV, L_B cells for SB and EMC viruses, and baby hamster kidney (BHK) cells for herpes simplex virus type 1 (HSV-1). The L_B mouse cell line (obtained from D. Burke) is highly sensitive to mouse interferon. Mouse interferon was prepared and partially purified on an antibody affinity column (*34*). (The specific activity of the preparations was greater than 4×10^7 mouse reference units per milligram of protein). Interferon was assayed by determining its inhibition of VSV-induced cytopathic activity. BHK and Vero cells (originally obtained from the NIH media section) were maintained in Eagle's minimum essential medium (MEM) supplemented with 10 percent fetal bovine serum. Swiss 3T3 mouse cells (obtained from the Imperial Cancer Research Fund, England) were maintained in Dulbecco's MEM with 10 percent fetal calf serum.

Figure 1 shows the effect of tunicamycin on the antiviral activity induced by interferon in L_B cells against three distinct groups of animal viruses. At the concentration used, tunicamycin alone had no significant inhibitory effect against replication of any of the viruses. However, when used in combination with various doses of interferon, the drug potentiated interferon's activity against an enveloped ribovirus (VSV) and against a deoxyribovirus (HSV-1). Similar enhancement of the antiviral activity of interferon was seen against SB virus. Tunicamycin did not enhance interferon's antiviral activity against EMC, a naked ribovirus.

We also studied the effect of tunicamycin on the antimitogenic effects of interferon (Fig. 2). When tunicamycin and interferon were used in combination, the inhibitory effect on DNA synthesis was greater than that obtained when either substance was used singly. In Fig. 2 we have plotted on an arithmetic scale the dose of each drug that alone or in combination produced a 50 percent reduction in the synthesis of DNA stimulated when quiescent 3T3 cells were exposed to fresh serum. The dashed line represents the dose of interferon or tunicamycin necessary to inhibit DNA synthesis 50 percent. Data points below this line represent the values obtained when various combinations of the two agents were used to achieve the same response. If interferon and tunicamycin had acted additively these points would be closer to the straight line. However, the points are closer to the origin, indicating a synergistic interaction between the two drugs. We also observed that tunicamycin enhances the inhibitory effect of interferon on 3T3 cell growth by a factor of 3 to 4 (Table 1).

Several studies (*35–37*) have shown

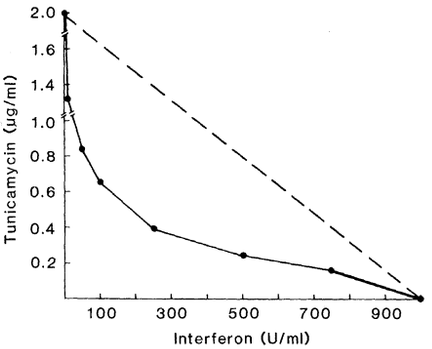

Fig. 2. Effect of interferon and tunicamycin on DNA synthesis. Quiescent 3T3 cells were serum-stimulated and treated with various concentrations of the two drugs. All cells were then labeled with [^{3}H]thymidine for 24 hours, at which point the amount of radioactivity incorporated into acid-precipitable DNA was determined (*41*).

that interferon treatment is associated with the induction of two double-stranded RNA–dependent enzymes, a 2′5′-oligoadenylate synthetase and protein kinase. The 2′5′-oligoadenylate system is one possible biochemical pathway whereby interferon could effect its diverse actions. In fact, the system has been implicated in the interferon-mediated inhibition of certain viruses and of cellular growth. Therefore we examined the possible role of the 2′5′-oligoadenylate system in the enhancement by tunicamycin of the antiviral and antigrowth activities of interferon. Preliminary data suggest that the antibiotic does not enhance the levels of interferon-induced 2′5′-oligoadenylate synthetase and endoribonuclease. This is not surprising in view of the lack of a synergistic effect of tunicamycin against EMC virus, and may indicate that the 2′5′-oligoadenylate system is not involved in the enhancement by tunicamycin of interferon's antiviral and anticellular activity.

We propose that the changes reported to occur in the plasma membranes of cells treated with interferon and tunicamycin may account for the potentiating effect of the antibiotic against VSV, SB virus, and HSV-1. These viruses bud from the cell surface as a terminal step. Perhaps the most interesting effect of interferon on cell membranes is its inhibition of assembly and release of murine leukemia virus (MLV) particles. Billiau *et al.* (*38*) reported that while interferon inhibited production of MLV in various cell lines, none of the intracellular events (such as synthesis of RNA, reverse transcriptase, and protein) were significantly affected. In some cases, interferon-treated cells showed an increased number of membrane-associated particles, suggest-

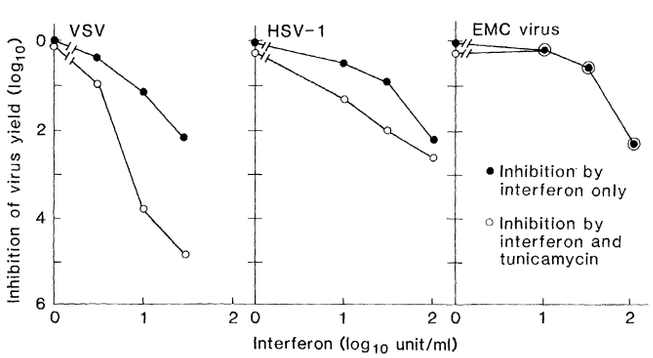

Fig. 1. Inhibition of virus yield by tunicamycin and interferon in L_B cells. The cells were set up in microtiter plates and treated with 3 to 100 U of interferon per milliliter for 14 hours; all monolayers were then washed three times to remove residual interferon and infected with VSV, HSV-1, and EMC virus at a multiplicity of 5 to 10 median tissue culture infectious doses per cell. Tunicamycin (0.2 μg/ml) (obtained from Calbiochem and the National Cancer Institute) was added after 1 hour. All monolayers were further incubated for 24 hours. Culture fluids were then harvested and assayed for virus yield. VSV was titered in Vero cells, EMC virus was titered in L_B cells, and HSV-1 was titered in BHK cells. Inhibition of virus titer was calculated in comparison to controls.

ing an inhibition of virus release from the membrane. In other systems, virus particles of low infectivity, such as MLV particles from interferon-treated TB cells (*3*) and VSV particles from interferon-treated L_B cells (*39*), were released. The low infectivity was related to the reduced amount of glycoprotein present in the released viruses and may have been attributable in part to a direct effect of interferon on the cell membrane. Therefore, it is possible that interferon and tunicamycin either markedly inhibit the release of membrane-associated viruses or cause an enhanced release of noninfectious virus particles lacking in glycoproteins. The failure of tunicamycin to enhance interferon's antiviral activity against EMC and mengo viruses (*40*), which have no membrane component, is consistent with both possibilities.

RADHA K. MAHESHWARI
Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814, and *Department of Microbiology, School of Medicine and Dentistry, Georgetown University, Washington, D.C. 20007*

T. SREEVALSAN
Department of Microbiology, School of Medicine and Dentistry, Georgetown University

ROBERT H. SILVERMAN
Department of Pathology, Uniformed Services University of the Health Sciences

JOHN HAY
Department of Microbiology, Uniformed Services University of the Health Sciences

ROBERT M. FRIEDMAN
Department of Pathology, Uniformed Services University of the Health Sciences

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Methoxyindoles and Photoreceptor Metabolism: Activation of Rod Shedding

Abstract. *Using an in vitro eye-cup preparation, we have evaluated a potential relationship between methoxyindole metabolism and photoreceptor disk shedding. Melatonin, 6-chloromelatonin, and 5-methoxytryptophol all activate rod disk shedding in culture. The effect is compound specific since serotonin and* N*-acetylserotonin are without effect, and it is similar to shedding in vivo because it is evoked by light and is quantitatively comparable to a normal intact animal response. The results suggest the involvement of 5-methoxyindoles in the control of rhythmic photoreceptor metabolism.*

The methoxyindoles, melatonin and 5-methoxytryptophol, are found in pineal tissue, where their synthesis and release occur in a circadian pattern with peak activity at night (*1*). The enzyme system for melatonin synthesis also exists in the retina (*2*) where the activity of serotonin *N*-acetyltransferase (NAT) (E.C. 2.3.1.5), a key enzyme controlling pineal melatonin rhythmicity (*1*), varies in a circadian pattern (*3*). The idea that rhythmic aspects of photoreceptor-pigment epithelial metabolism, such as disk shedding, may be controlled by melatonin or a related compound (*4*) is supported by observations that retinal NAT activity (*3*) and photoreceptor disk shedding (*5, 6*) are influenced similarly by alterations in the daily light-dark cycle.

We tested the hypothesis of a relationship between methoxyindoles and photoreceptor metabolism by evaluating the effects of such compounds on disk shedding in eye cups from *Xenopus laevis* (*7*). We based our approach on our previous observations that (i) light-evoked rod shedding occurs in culture provided that the medium-bicarbonate concentration is sufficiently high ($\geq$ 30 m*M*), (ii) that a low-bicarbonate medium (20 to 27.5 m*M*) does not support light-evoked shedding, and (iii) that colchicine activates light-evoked shedding in low-bicarbonate medium (*8*). Melatonin is similar to colchicine structurally and at high concentration has been reported to mimic some of its effects (*9, 10*). We therefore evaluated the effects of methoxyindoles by a procedure analogous to that used previously for colchicine (*8*).

Eye cups were prepared by surgical removal of cornea, iris, and lens in dim red light from eyes of postmetamorphic *X. laevis* that had been maintained in cyclic light (12 hours of light and 12 of darkness) at 25°C for at least 3 weeks before use. Disk shedding was evaluated as described previously (*7*) by counting the dense bodies (phagosomes) within pigment epithelium that were derived from the shed and internalized fragments of photoreceptor outer segments. Melatonin activated light-evoked disk shedding at a concentration of 0.5 m*M* (Fig. 1). The response occurred only in light and did not differ significantly from that