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Intrinsic Interference: a New Type of Viral Interference[1]

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The hemadsorption-negative plaque test has revealed a new type of viral interference, termed intrinsic interference. Several unrelated types of noncytopathic viruses were shown to induce in infected host cells a state of interference unique in being directed solely against superinfection by Newcastle disease virus (NDV). The NDV-refractory state arises only in those individual cells of a population actually infected by the inducing virus, and presumably results from the action of a protein(s) coded for by the viral genome. Thus, intrinsic interference differs fundamentally from that mediated by an extrinsic protein detectable under conditions favoring resistance to a broad spectrum of viruses and characteristic of interference induced by interferon, the latter being coded for by the cell genome. Intrinsic interference is defined as a viral genome-induced cellular state of resistance to challenge by high multiplicities of NDV, coexistent with a state of susceptibility to a broad spectrum of other viruses, similarly tested at high multiplicities. The capacity to induce intrinsic interference was demonstrated with rubella virus, Sindbis virus (arbovirus, group A), West Nile virus (arbovirus, group B), poliovirus (MEF, type 2), the lactic dehydrogenase virus (Riley's agent), and an unidentified nonhemadsorbing, noncytopathic adventitious virus. A state of intrinsic interference was also observed in the V5 line of mouse cells carrying a murine leukemia virus, probably resulting from some heretofore unsuspected contaminating virus. The molecular basis for intrinsic interference is not known, but it appears to involve a step in the NDV growth cycle beyond that of viral attachment, entry, and eclipse.

The hemadsorption-negative plaque test (16) was developed as a means of accurately assaying rubella virus as a nonhemadsorbing, noncytopathic virus. The test has proved to be highly reliable, and is based on the fact that cells infected with rubella virus are completely refractory to superinfection by Newcastle disease virus (NDV), while remaining susceptible to many other viruses. Cells refractory to NDV may be scored as (i) hemadsorption-negative (HAD^-) plaques standing out against a background of NDV-susceptible, hemadsorption-positive (HAD^+) cells; (ii) individual HAD^- cells in a population of widely dispersed single HAD^+ cells; or (iii) as cell survivors (colonies)—the principle characteristic used to score the rubella virus-infected cell being its absolute resistance to NDV. This state of refractoriness is termed *intrinsic interference* (16). This communication extends these studies to show that the capacity to induce intrinsic interference is not restricted solely to rubella virus but may be distributed ubiquitously in nature, and that it involves several kinds of seemingly unrelated noncytopathic viruses and also possibly some virulent types. This paper also presents evidence that intrinsic interference (i) is an intrinsic property of the virus-infected cell which does not extend to uninfected cells in the culture, (ii) is induced under conditions that completely rule out interferon-mediated interference, and (iii) is presumably brought about by the action of a protein(s) coded for by the genome of the inducing virus. Intrinsic interference is defined as a viral genome-induced cellular state of resistance to NDV, coexistent with a state of susceptibility to a broad spectrum of other viruses.

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MATERIALS AND METHODS

Cell cultures and medium. Primary green monkey kidney (GMK) cells were obtained from Microbiological Associates, Inc. (Baltimore, Md.) or from BBL as confluent monolayers. These were trypsinized [0.05% trypsin plus 0.005 M ethylenediaminetetraacetic acid (EDTA)] and used in HAD^- plaque tests (16) as confluent monolayers on 60-mm plastic petri dishes (Falcon Plastic, Div. of B-D Laboratories, Inc., Los Angeles, Calif.) or plated as single cells on 35-mm dishes. Other cell types were used in a similar way. Their specific designations are noted below along with the individuals to whom we are grateful as initial suppliers: baby hamster kidney, BHK21/W1-2 (A. Vaheri); L cells (W. Joklik); fathead minnow line (5), FHM (S. Silverstein, L. Sturman); V5 line of mouse cells carrying Rauscher leukemia virus (R. Bases, B. Wright). GMK, BHK, and L cells were grown in NCI solution (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 6% calf serum [attachment solution (16)] and 3% fetal bovine serum. The concentration of fetal bovine serum was raised to 10% for cultures of FHM cells, and incubation for growth was always at 34 C (5) for this poikilothermic line. V5 cells were grown in F10 medium (6) with 15% fetal bovine serum. Just prior to, and during, challenge with NDV, all cell cultures were rinsed and then incubated at 37 C in attachment solution (16).

Virus stocks and preparation. Rubella virus-F8 (M. R. Balsamo) stocks were prepared as described previously (16) and in BHK cells to give 10^5 to 10×10^5 HAD^- plaque-forming particles (PFP) per milliliter (16). Sindbis virus (J. F. Enders) was grown in primary chick embryo cell (CEC) monolayers incubated in Simpson-Hirst medium (Carver and Marcus, Virology, *in press*), to give titers of about 10^9 PFP or HAD^- PFP per ml. NDV stocks of 10^9 to 5×10^9 PFP/ml were prepared from chick allantoic fluid. The California strain of NDV was used in most experiments, but three other strains behaved similarly: Beaudette, Mass.-HiK, and a vaccine strain. Vaccinia virus (7N) was harvested from HeLa cell monolayers or the chorioallantoic membrane of eggs. Poliovirus-MEF1, type 2 (J. F. Enders), was grown in GMK cell monolayers. Vesicular stomatitis virus (VSV; S. Silverstein) was propagated in CEC monolayers to give stocks of about 10^8 PFP/ml. Influenza B virus (R. Simpson) was propagated in eggs, and encephalomyocarditis virus (EMC; R. Bases), in Krebs ascites cells. Lactic dehydrogenase virus (Riley's agent, LDH virus; R. Bases, V. Riley) was used as supplied in high titer by V. Riley and J. Loveless. A noncytopathic, hemadsorbing simian virus (BTV) was isolated as a contaminant from one lot of primary GMK cells and was assayed by hemadsorption foci (8) by use of bovine or turkey erythrocytes. Virulent poliovirus, type 1, was supplied by D. Summers.

Hemadsorption-producing particle (HDP) assay. The HAD^- plaque technique has been described in detail (16). The procedure used to detect HDP activity of a virus preparation on single cells has been described less thoroughly (15). One ramification of this latter technique is its use to detect individual cells that manifest intrinsic interference. This was accomplished as follows. Cells, usually in monolayer culture, were exposed to the virus inducing intrinsic interference and were then incubated under various test conditions as reported in the text. To determine the fraction of cells refractory to NDV, i.e., showing intrinsic interference, the infected cell monolayer first was challenged with NDV at a multiplicity $\simeq$ 10 PFP. (In a typical experiment, 10^8 NDV-PFP in a volume of 0.3 ml was added to a monolayer containing 5×10^6 cells and adsorbed for 30 to 60 min at 37 C.) Nonadsorbed NDV was then washed off, and any surface-situated, nonengulfed virus was neutralized with NDV antiserum to eliminate any background of spurious red blood cell binding in the single-cell hemadsorption test (16). Immediately after treatment with antiserum, monolayers were trypsinized to obtain monodisperse cells. These cells were plated at low densities (usually 10^5 cells per 35-mm petri dish), incubated for about 15 hr to allow NDV hemagglutinin incorporation into the plasma membrane (15), and differentiated as to HAD^+ or HAD^- cells by the single-cell hemadsorption test as illustrated in Fig. 1. The steps taken to detect intrinsic interference in individual cells are listed below and will be referred to collectively as the intrinsic-interference test: (i) infection of cells with virus inducing intrinsic interference; (ii) incubation of virus-cell complexes to develop state of intrinsic interference; (iii) challenge of infected cells with NDV at a multiplicity $\simeq$ 10 PFP; (iv) trypsinization of challenged cells, followed by plating as single cells; (v) performance of single-cell hemadsorption test and determination microscopically of the fraction of cells manifesting intrinsic interference by counting HAD^- (NDV-refractory cells) and HAD^+ (NDV-susceptible) cells.

Ultraviolet-light inactivation of Sindbis and rubella virus. Ultraviolet-light (UV) survival curves for rubella and Sindbis viruses were carried out under conditions during which T2 phage and NDV could be used as biological actinometers (16). Rubella virus survivors were assayed as HAD^--PFP and Sindbis virions as PFP on young CEC monolayers grown in Simpson-Hirst medium. All survival curves showed single-hit inactivation kinetics. The 37% survival dose, D_0, defined as the dose of UV required to reduce the number of infectious particles to e^{-1} (0.37 survivors = one lethal hit), was the same for both viruses, 110 ergs mm^{-2}. For comparison, D_0 values for NDV and T2 phage irradiated in the same medium (attachment solution) were 42.5 and 25 ergs mm^{-2}, respectively. UV-inactivated virus, usually at 5×10^{-5} survival level, was used immediately after irradiation.

Infective-center assay. Control cells, and cells infected with viruses inducing intrinsic interference, were tested for their capacity to act as infective centers for NDV. NDV was adsorbed to cell monolayers (multiplicity $\simeq$ 10 PFP), the free virus was washed away, the surface-situated virions were neutralized with NDV antiserum, and the cells were dispersed with trypsin, all within a 90-min period at 37 C. Monodisperse cells were counted and plated as infective centers as described previously (17). Triplicate plates

were run for each assay, and dilutions of virus-cell complexes were adjusted to obtain 50 to 150 plaques per plate. The results were expressed as the percentage of maximal NDV plaque count relative to control cultures.

RESULTS

Sindbis virus can be obtained readily in high titer compared with the other noncytopathic viruses used here; it is easy to assay, and its reactions are typical of viruses that induce intrinsic interference. Consequently, Sindbis virus has been used in most of the experiments reported here. All viruses listed as possessing the capacity to induce intrinsic interference (Table 2) have been tested in experiments similar to those described below for Sindbis virus, and have been shown to react in the same manner unless specifically noted. For obvious technical reasons, this statement does not apply to some of the viruses for experiments involving high multiplicities.

Scoring intrinsic interference (NDV-refractory, HAD⁻ cells). The fraction of NDV-refractory cells was determined as described in Materials and Methods. Figure 1 shows the appearance of HAD^+ and HAD^- GMK cells that were infected with Sindbis virus at a multiplicity of 5 PFP, incubated for 11 hr, and then challenged with NDV as in the five-step procedure outlined for the intrinsic-interference test. After the 15-hr incubation period allowed for NDV hemag-

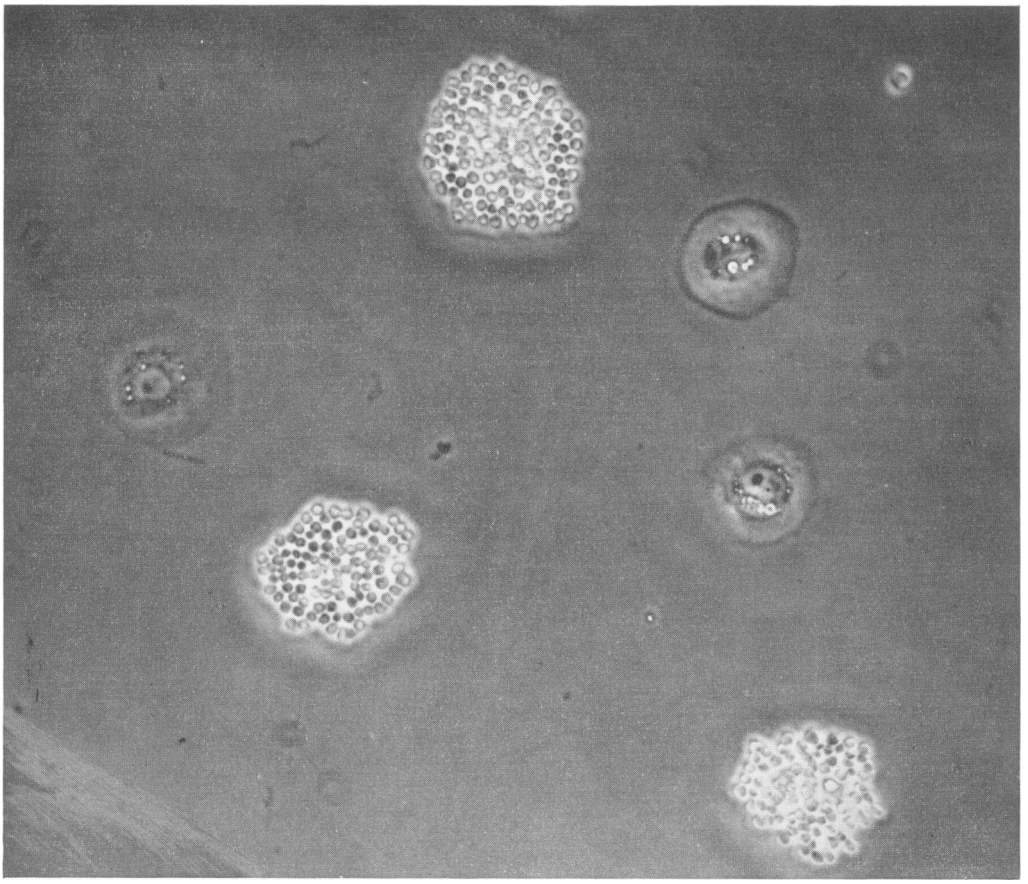

FIG. 1. *Single-cell hemadsorption test to detect individual cells rendered refractory to NDV, i.e., hemadsorption-negative (HAD⁻), by a virus inducing intrinsic interference. Green monkey kidney cell monolayers were infected with Sindbis virus (multiplicity, 5 PFP), challenged 11 hr later with NDV (multiplicity, 10 PFP), trypsinized and plated as single cells, incubated an additional 15 hr, and tested for their hemadsorption reaction. Cells susceptible to NDV, i.e., not manifesting intrinsic interference, are represented by the three HAD⁺ cells covered entirely with bovine erythrocytes. Cells demonstrating intrinsic interference are refractory to NDV and appear as three HAD⁻ cells. Bovine erythrocytes have a diameter of about 6 μ.*

glutinin incorporation into the surface of infected cells, essentially all of the HAD$^+$ cells were saturated with respect to the number of adsorbed red blood cells (Fig. 1) as in control preparations, and the contrast between HAD$^+$ and HAD$^-$ cells was maximal. Thus, hundreds of cells may be scanned rapidly, and the fraction of NDV-refractory (HAD$^-$) cells may be ascertained to a high degree of accuracy. This experimental procedure has been used to define several characteristics of intrinsic interference which are documented below.

Basic characteristics of intrinsic interference: rate of induction—a function of multiplicity. The rate of induction of intrinsic interference and the dependence of rate on multiplicity were determined as follows. GMK cell monolayers were infected with various multiplicities of Sindbis virus, the monolayers were challenged with NDV at different intervals, and the fraction of NDV-refractory cells was determined in the standard manner. The results from such an experiment are illustrated in Fig. 2, where each point represents a count of at least 400 cells. The fraction of HAD$^-$ cells increased linearly from time zero at a rate dependent upon the multiplicity of the inducing virus; the higher the multiplicity, the shorter the time of induction to interference. For example, the time required to induce intrinsic interference in 50% of the cells at a multiplicity of 1, 5, 25, and 100 PFP was 18, 11, 8, and 5 hr, respectively. Although no lag was apparent in the induction process under the conditions of virus attachment used, e.g., 0.3 ml of virus per 60-mm plate for 30 min at 37 C, when Sindbis virus was adsorbed at 4 C and the temperature was then raised to 37 C, all curves extrapolated to a lag of about 0.5 hr. When cells infected at a multiplicity of 1 PFP were incubated for a few hours beyond 24 hr (not shown), the fraction of HAD$^-$ cells increased only slightly above the 62% noted at 24 hr—a value consistent with the fraction of cells calculated from the Poisson distribution to receive ≥ 1 PFP. The 3% point at zero-time represents a base line of control cells physiologically noninfectable at the time of NDV challenge, an observation reported previously (14). The fraction of noninfectable cells varied from ≤ 1 to 10% with different lots and kinds of cells and was determined as a control in each experiment.

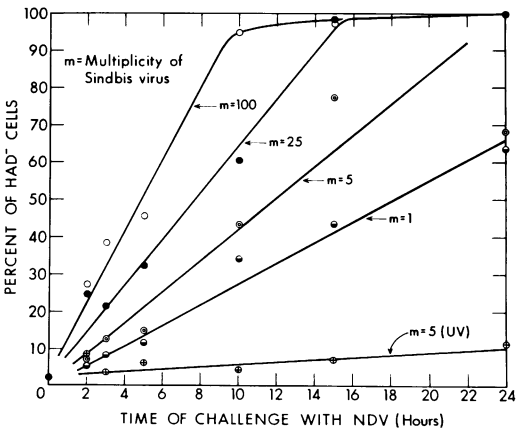

FIG. 2. *Dependence of rate of induction of intrinsic interference on viral multiplicity. Monolayers of GMK cells were infected at various multiplicities (m) of Sindbis virus, and challenged with NDV (m = 10 PFP) at different intervals; the fraction of HAD$^-$ cells was determined 15 hr later by the intrinsic-interference test.*

Half-life of the interference. The NDV-refractory state, once it is reached in 100% of the cells, may persist indefinitely if the cell monolayer is not disrupted and is maintained in a healthy state with adequate changes of medium. For example, rubella virus-infected monolayers of GMK cells remained refractory to NDV for the entire length of a 2-month test period. At the end of this time, the NDV-resistant cells were as susceptible to the other test viruses (Table 4) as were control cells not previously infected with rubella virus. However, the NDV-refractory state has a measurable half-life under certain conditions. When GMK cell monolayers rendered 100% refractory to NDV by prior infection with rubella or Sindbis virus were dispersed with trypsin and plated at lesser cell densities in fresh medium, the HAD$^-$ state was lost. The kinetics of this return to NDV susceptibility may be ascertained by challenging the cells with NDV at daily intervals and determining the number of HAD$^-$ and HAD$^+$ cells. Results of this type are presented in Fig. 3 and 4 for rubella and Sindbis viruses, respectively. The data show that the HAD$^-$ state was maintained in all of the cells for about 1 to 2 days and then was lost at a relatively rapid and constant rate, independent of cell density. In three of the four experiments illustrated, the half-life of the return to an NDV-susceptible state was about 10 to 15 hr. In cells kept for several days beyond the test period (Fig. 3 and 4), there was a reversal of the loss of the HAD$^-$ state and a gradual return to a confluent monolayer of 100% NDV-refractory cells.

Requirement for active virus. Stock preparations of Sindbis virus were inactivated with UV to a survival level of 5×10^{-5} and were compared with active virus in their capacity to induce an NDV-refractory state. The results of our first experiment are presented as the bottom curve in Fig. 2, and those of two other experiments, in Fig. 5. Clearly, the capacity to induce intrinsic interference was lost after inactivation of plaque-forming capacity

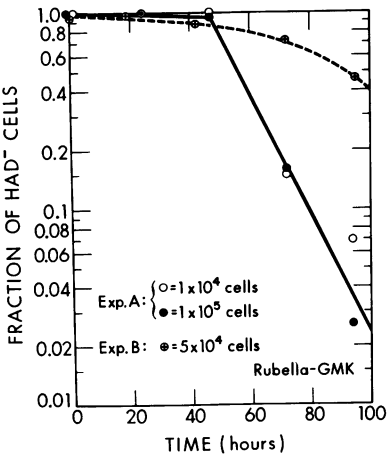

FIG. 3. *Loss of intrinsic interference after subculture of rubella virus-infected cells. Monolayers of GMK cells infected with rubella virus and maintained in a 100% NDV-refractory state for 1 week were subcultured, plated at various cell densities in 60-mm dishes, challenged with NDV at approximately daily intervals, and scored 15 hr later by the hemadsorption test to determine the fraction of HAD⁻ cells.*

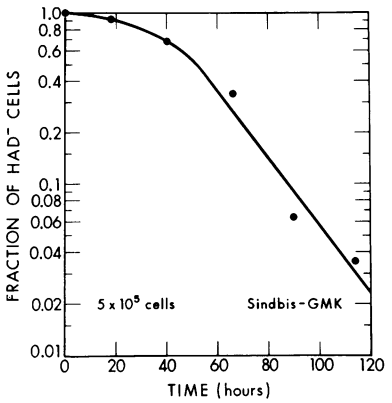

FIG. 4. *Loss of intrinsic interference after subculture of Sindbis virus-infected cells. Monolayers of GMK cells were infected with Sindbis virus to induce 100% NDV-refractory cells, and were then treated as described in Fig. 3.*

with UV. These same preparations of UV-inactivated Sindbis virus were still capable of inducing high levels of interferon production in GMK cell monolayers.

Insensitivity to actinomycin D. A slight modification of the intrinsic-interference test was introduced to determine whether Sindbis virus could induce an NDV-refractory state in cells precluded from synthesizing cellular messenger ribonucleic acid (mRNA). GMK cell monolayers were treated with actinomycin D (30 μg per 3 ml of medium per plate) for 20 min prior to infection with a high multiplicity of Sindbis virus. This treatment had no discernible effect on the hemadsorption-producing capacity of NDV, but reduced ^{3}H-uridine incorporation by >95% (17). At various intervals after infection with Sindbis virus, the cultures were challenged with NDV and the fraction of HAD⁻ cells was determined in the usual manner. The results presented in Fig. 6 show that Sindbis virus can induce intrinsic interference in cells treated with actinomycin D and no longer able to synthesize cellular mRNA. Actually, the rate of induction consistently is greater in actinomycin D-treated cells than in untreated cultures.

Requirement for protein synthesis. The effect of protein synthesis inhibition on the induction by Sindbis virus of an NDV-refractory state in GMK cells was examined as follows. Cells were treated with puromycin (50 μg/ml) for 45 min, followed by infection with a high multiplicity (≃20 PFP) of Sindbis virus, and the virus-cell complexes were incubated in the presence of puromycin (*with puromycin*). The drug was washed out, the infected cells were incubated for various intervals *without puromycin*, followed by challenge with NDV, and the fraction of HAD⁻ cells was determined as in the standard intrinsic-interference test. The results illustrated in Fig. 7 show that the percentage of virus-cell complexes which became refractory to NDV (HAD⁻) after a sojourn with and without puromycin was essentially equivalent to that expected if the

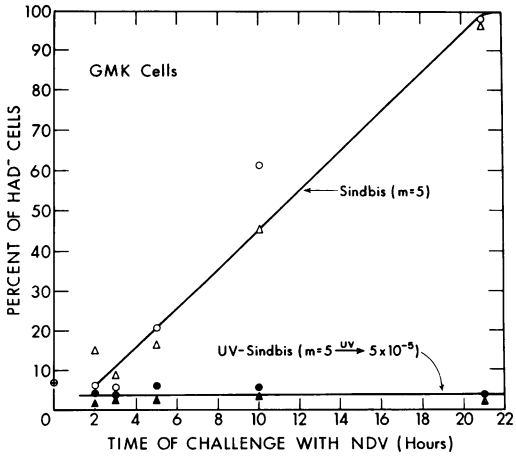

FIG. 5. *Loss of the capacity of Sindbis virus to induce intrinsic interference after UV irradiation. Monolayers of GMK cells were infected with active Sindbis virus (multiplicity, 5 PFP), or the same preparation irradiated with UV to a level of 5 × 10^{-5} PFP survivors. The rate of development of an NDV-refractory state in the cells was determined in the usual manner.*

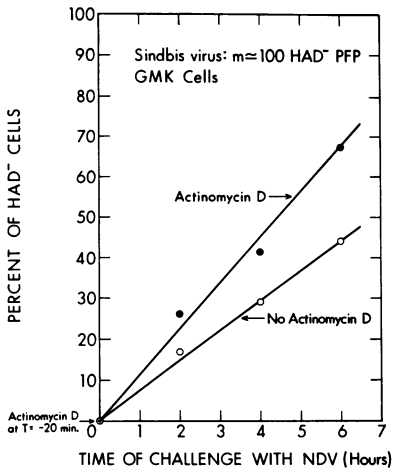

FIG. 6. *Induction of intrinsic interference in the presence of actinomycin D. Monolayers of GMK cells were treated for 20 min with actinomycin D (10 μg/ml), infected with Sindbis virus (multiplicity, 100 PFP), and challenged with NDV after 2, 4, and 6 hr. At 15 hr later, the fraction of HAD⁻ cells was determined as usual.*

inductive process were functioning only during the time of incubation *without puromycin*. The rate of induction of the NDV-refractory state in control preparations of Sindbis virus-cell complexes never exposed to puromycin is shown as the dashed line on the right-hand side of Fig. 7. The interval during which cells were incubated *with* and *without* puromycin is indicated on the left and right, respectively, of the ordinate, with the solid circles representing the percentage of HAD⁻ cells at the end of the 6-hr test period. From the data presented in Fig. 7, we concluded that protein synthesis is required for Sindbis virus to induce an NDV-refractory state in GMK cells.

NDV attachment, infective-center assay, and eclipse. NDV binding to cell monolayers was carried out as described previously (13). The kinetics of NDV attachment to monolayers containing 100% NDV-refractory GMK cells induced by rubella virus is shown in Fig. 8, along with virus adsorption to uninfected control cells. No significant difference in the rates of NDV adsorption was noted. Similar results were obtained with monolayers of FHM cells induced to the 100% HAD⁻ state with Sindbis virus. In this latter instance, it was necessary to prevent plaque formation of residual Sindbis virus by plating NDV in the presence of Sindbis antiserum on aged CEC monolayers (Carver and Marcus, *in press*).

The results in Table 1 present the plaquing efficiency of NDV as infective centers from GMK monolayers made 100% refractory to NDV (HAD⁻) by infection with rubella virus, compared with that from uninfected control cells (HAD⁺). Although NDV-susceptible and -refractory cells adsorbed NDV equally well, in the latter case there was a 94% loss in plaquing efficiency of the intact infective centers. When infective centers were freeze-thawed three times, the control cells showed the usual >90% loss in plaque count, representing viral eclipse and loss of viral replicative capacity of the cells. The NDV-refractory cells (HAD⁻) showed no further change in plaquing efficiency from the low level observed with the intact virus-cell complexes, indicating that a constant small percentage of noneclipsed and non-neutralized input virus was present in sensitive and refractory cells alike.

Viruses inducing intrinsic interference. The first step used to screen viruses for their capacity to induce intrinsic interference involved a simple test of their ability to form HAD⁻ plaques (16). To date, no virus which has displayed this particular trait has failed to meet the more stringent criteria used as the final determinant of capacity to induce intrinsic interference. These criteria are: (i) the ability of viruses to induce an NDV-refractory state in the presence of actinomycin D, and (ii) the susceptibility of the NDV-refractory cells to a broad spectrum of viruses. Table 2 lists the viruses which have met these criteria, the exception being the agent(s) responsible for the

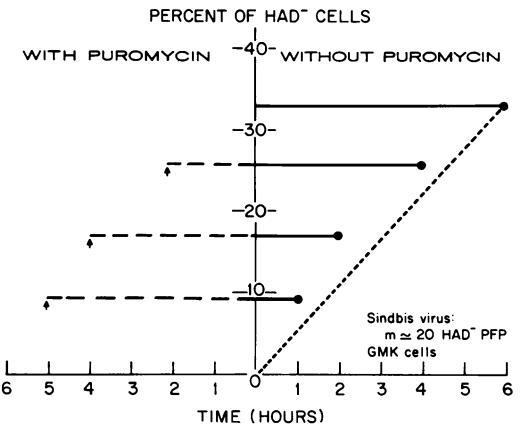

FIG. 7. *Inhibition of intrinsic-interference induction in the presence of puromycin. Monolayers of GMK cells infected with Sindbis virus (multiplicity, 20 PFP) were incubated for 0, 2, 4, and 5 hr in puromycin (50 μg/ml), washed, and incubated for 6, 4, 2, and 1 hr, respectively, in the absence of the drug. At the end of the 6-hr total incubation period, all preparations were challenged with NDV, and the fraction of HAD⁻ cells (solid circles) was determined 15 hr later by the hemadsorption test. The dotted line on the right represents the rate of development of the NDV-refractory state in the complete absence of puromycin.*

NDV-refractory state of the V5 mouse cell line carrying Rauscher murine leukemia virus. Although the HAD$^-$ state in this latter case is maintained in the presence of actinomycin D, it has not been possible thus far to initiate the NDV-refractory condition in another line of cells and, hence, to test inducibility in the presence of the drug. Also included in Table 2 is a virulent strain of type 1 poliovirus that caused a rapid and extensive cytopathic effect (CPE), which precluded testing for an NDV-refractory state. However, in the presence of guanidine, cytopathic effects were delayed long enough so that the cell could be challenged with NDV and tested for hemadsorption. These conditions permit partial expression of the input poliovirus genome but not its replication (2). The following experiment was done in conjunction with D. Summers. HeLa cells from spinner culture were suspended in medium containing actinomycin D (5 μg/ml) and guanidine hydrochloride (50 μg/ml) and infected with poliovirus at an adsorbed multiplicity of 200 PFP. Control cells were treated similarly but were not exposed to poliovirus. Poliovirus-cell complexes were incubated for 1.5 hr at 37 C (a period sufficient to strip most host-cell mRNA from polysomes), followed by chilling to 4 C, and

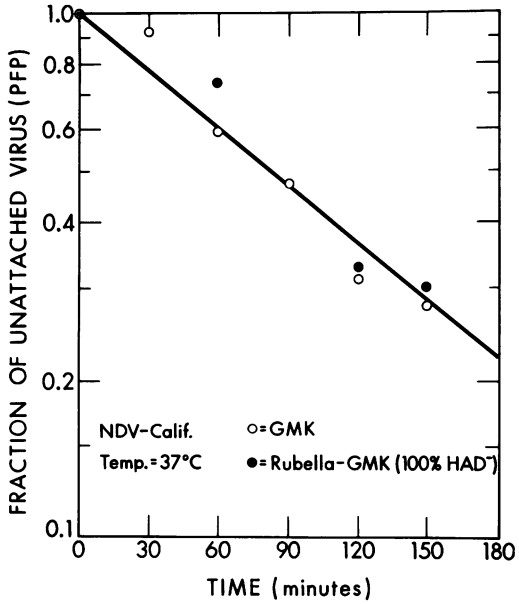

FIG. 8. *Attachment kinetics of Newcastle disease virus on GMK monolayers containing 100% NDV-refractory cells (100% HAD$^-$). GMK cell monolayers infected with rubella virus and incubated to produce 100% NDV-refractory cells were exposed to NDV contained in 0.3 ml of attachment solution. At various intervals, the supernatant fluids were assayed on chick embryo cell monolayers for unattached NDV-PFP.*

TABLE 1. *Infective-center plaquing of rubella virus-infected GMK cells challenged with NDV*

State of cells	Per cent of maximum NDV plaque count on CEC monolayers	
	Infective centers: intact	Infective centers: 3X freeze-thaw
HAD^{+a}	100	8.2
HAD^{-b}	5.9	6.4

[a] Control monolayers of GMK cells. All cells susceptible to NDV, i.e., score as HAD$^+$ when challenged.

[b] GMK cell monolayers infected with rubella virus and incubated for 3 days. All cells rendered refractory to NDV, i.e., score as HAD$^-$ when challenged.

NDV was attached for an adsorbed multiplicity of 10 PFP. The cells were washed, plated in attachment solution containing guanidine, and incubated at 37 C. After 2 hr, the cells were firmly attached and could be treated with NDV antiserum to neutralize nonengulfed NDV. Periodically, samples were tested for HAD$^+$ and HAD$^-$ cells. The results presented in Table 3 show that 99% of the control cells treated with actinomycin D and guanidine, but not infected with poliovirus, became HAD$^+$ when challenged with NDV, attesting to their susceptibility. In contrast, infection with poliovirus rendered all of the cells refractory to NDV, and these were scored as HAD$^-$.

Viruses insensitive to intrinsic interference. Table 4 lists the challenge viruses that can infect cells made refractory to NDV by the interference-inducing viruses shown in Table 2. All challenge viruses were used at high multiplicities, i.e., $\simeq$10 infectious particles. Cytopathic effects on NDV-refractory cells resulting from the action of these viruses were indistinguishable in their time of onset and severity from control cells challenged at the same time. In the case of the noncytopathic, hemadsorbing simian virus, infectivity was scored by the hemadsorption-positive state it conferred on cells. Rubella virus-infected GMK cells that were completely HAD$^-$ upon challenge with NDV thus became 100% HAD$^+$ when exposed to the simian virus. This produced a cell that was simultaneously expressing the characteristics of the two infecting viruses—NDV-refractoriness and hemadsorption positivity. The four strains of NDV thus far tested (Table 4) are all excluded by intrinsic interference.

DISCUSSION

Data presented here extend our earlier study of rubella virus-induced interference to NDV (16) and demonstrate that several unrelated viruses

TABLE 2. *Viruses that induce intrinsic interference*

Virus	Designation	Host cell
Rubella	F-8 strain and fresh isolates	GMK, L cell, human embryo kidney
Sindbis	Group A arbovirus	GMK, FHM[a], HeLa
West Nile	Group B arbovirus	GMK
Poliovirus	MEF 1, type 2	GMK
Poliovirus[b]	Type I	HeLa
Lactic dehydrogenase virus	LDH virus, Riley's agent	Primary mouse embryo cells
JLS-V5 mouse cell agent[c]	JLS-V5 line carrying Rauscher murine leukemia virus	JLS-V5 mouse cell line
Mouse cell agent[c]		Present in 3 of 5 different lots of primary mouse embryo cell cultures

[a] This line of cells is infected with a *Mycoplasma* sp., but shows 100% HAD$^+$ cells when challenged with NDV.

[b] Cytopathic, see text for special test conditions.

[c] Cells and supernatant fluids were tested by J. Loveless and V. Riley and found to be free from LDH virus. Possible identical nature of all agents in mouse cell cultures has not been ruled out.

TABLE 3. *NDV-refractory state induced by cytopathic poliovirus in HeLa cells*

Cell treatment	Time of NDV challenge (hr after poliovirus attachment)	No. of cells		Fraction of HAD$^-$ cells
		HAD$^+$	HAD$^-$	
Actinomycin D + guanidine	4[a]	426	75	0.18
	6	510	5	0.01
	17	505	3	0.01
Actinomycin D + guanidine + poliovirus[b]	4	0	450	1.0
	6	0	525	1.0
	17	0	750	1.0

[a] At 2.5 hr, i.e., 0.5 hr after treatment with NDV-antiserum, there were no HAD$^+$ cells.

[b] Multiplicity, 200 PFP.

TABLE 4. *Viruses and their cytopathic effect on cells manifesting intrinsic interference*

Virus	Cytopathicity on NDV-refractory cells[a]
Echovirus 11	+[b]
Encephalomyocarditis virus	+
Influenza B	+
Poliovirus	+
Vaccinia	+
Vesicular stomatitis virus	+
Simian virus, noncytopathic, hemadsorbing	+[c]
Newcastle disease virus	
California	−[d]
Beaudette	−
Mass.-HiK	−
Blacksburg, vaccine	−

[a] All cell types listed in Materials and Methods were susceptible to NDV, and most cell types were susceptible to the majority of the test viruses. Notable exceptions are referred to in the text.

[b] Denotes complete cell destruction within 24 hr, indistinguishable from control cultures similarly infected at a high multiplicity.

[c] For this noncytopathic, hemadsorbing virus, + denotes infection scored by hemadsorption.

[d] Denotes the complete absence of a cytopathic effect and cell appearance indistinguishable from uninfected controls.

can induce a similar pattern of interference. Our experiments show that the interference-inducing capacity of these viruses is lost upon irradiation with ultraviolet light, does not take place in the presence of puromycin, but is expressed in the presence of actinomycin D. In concert, these results point out the necessity of protein synthesis, coded for by a functional viral genome, in the induction of the interference. As documented previously (16), the NDV-refractory state is an intrinsic property of the individual cell that has been infected, in contrast to cells manifesting interferon-mediated interference. The latter type of interference is characterized by cellular resistance to a broad spectrum of viruses and may be induced in uninfected cells by the addition of an extrinsic factor, interferon (10, 20). The NDV-refractory state described here is not mediated by the action of interferon, since interference can develop (even at an enhanced rate) in cells blocked by actinomycin D from synthesizing the cell genome-coded, translation-inhibitory protein responsible for the action of interferon (18). Cells refractory to all multiplicities of NDV demonstrate their usual susceptibility to a whole spectrum of other viruses when challenged at high multiplicity. The list of these challenge viruses

has been extended to include vesicular stomatitis virus, encephalomyocarditis virus, and simian virus 5 (Table 4). Whether strains of NDV will continue to constitute the only viruses excluded by this type of interference remains to be established.

Because of these considerations, we term the NDV-refractory state *intrinsic interference* and define it as: a viral genome-induced cellular state of resistance to challenge by Newcastle disease virus, coexistent with a state of susceptibility to a broad spectrum of other viruses, similarly tested at high multiplicity.

The saturated hemadsorption pattern seen on HAD^+ cells at the conclusion of the NDV challenge is significant, and suggests that the NDV-refractory state represents an all-or-none block, since a cell with small amounts of NDV hemagglutinin on its surface, either because of a delay in initiation of hemagglutinin synthesis or a lower level of synthesis, can be detected as a result of its reduced capacity to bind red blood cells (15).

The linear rate of development of intrinsic interference shows a dependence on multiplicity of the inducing virus at the time of infection. Virus added at a later time does not appear to influence the rate of development of interference (Lipschitz, Marcus, and Carver, *in preparation*). We interpret this to mean that only the initial input strands of the viral RNA may function as mRNA for the protein(s) that presumably acts to bring about interference. Consistent with this interpretation is the development of an NDV-refractory state in HeLa cells infected with poliovirus under conditions (presence of guanidine) that prevent replication of viral RNA (2).

When long-standing cultures of NDV-refractory cells are removed from confluent monolayers and set down in fresh medium, intrinsic interference disappears, slowly at first and then with a half-time of about 10 to 15 hr. After the NDV-refractory state is lost in about 99% of the cells, usually over a period of 6 to 7 days, there is a gradual return to the hemadsorption-negative state until all cells are again refractory to NDV. We are studying this process and at present can only speculate that the half-life of the refractory state expressed after subculture is perhaps dependent upon cell division and some dilution effect, or a break in contact-inhibition regulated events. The small fraction of infective centers found in these cultures could represent the source of virus for reinfection once the cells were again susceptible. We have omitted from consideration the possible gradual loss of the inducing virus from the cell population. Clearly, the situation is complex and requires further investigation.

Although no obvious taxonomic relationship is apparent among the different types of viruses that can induce intrinsic interference, they share two characteristics in common: (i) they are noncytopathic [the special test conditions for virulent poliovirus may be thought of as delaying cytopathicity and providing a temporary noncytopathic state (1)], and (ii) they are RNA viruses insensitive to the action of actinomycin D. Only further investigation will reveal the biochemical event(s) common to the capacity of the viruses to induce intrinsic interference. We have not tested noncytopathic myxoviruses as inducers of intrinsic interference because of possible complications attending receptor destruction by viral neuraminidases (3, 14). However, it is apparent that not all noncytopathic viruses manifest intrinsic interference. Thus, cells overtly infected with, or carrying, parainfluenza viruses (7, 12), including simian virus 5 (P. Choppin, *personal communication*) and hog cholera virus (11), appear to be as sensitive, or more so, to NDV infection as the viruses tested. Cells transformed by Rous sarcoma virus (H. Temin, *personal communication*) or SV40 (*unpublished data*) do not appear to be refractory to NDV.

Although NDV attaches to cells manifesting intrinsic interference and enters an eclipse phase in an apparently normal manner, the ultimate fate of the NDV genome is not known, and the mechanism controlling its expression is not understood. We are continuing this aspect of our study. Our approach to this probelm is modeled after experiments which have shown that viral RNA is not translated when complexed with ribosomes from interferon-treated cells (18). This inhibition is brought about by the presence of a trypsin-sensitive, translation-inhibitory protein on the ribosomes of the interferon-treated cell. Experiments are underway to look for a similar, but more specific, translation-inhibitory protein, in this case presumably coded for by the genome of the inducing virus, that acts to block translation of only NDV RNA.

The NDV-refractory state induced by intrinsic-interference viruses represents a unique example of heterotypic interference. However, in the biochemical sense, it is not without precedent that taxonomically unrelated viruses initiate similar biochemical events, e.g., the induction of interferon production, or the turn-off of host-cell macromolecular synthesis. It would not be profitable to compare other types of viral interference with intrinsic interference until more is known about its molecular basis. However, mention of one example is appropriate since it raises the intriguing question of whether viruses inducing intrinsic interference can interfere with

each other. In this connection, P. T. Allen and R. Z. Lockart (Bacteriol. Proc., p. 98, 1965) have reported on interference of a cytopathic strain of Western equine encephalomyelitis virus by a slightly cytopathic strain, in the presence of actinomycin D. Other examples of noninterferon-mediated interference have been reported (4, 9, 19).

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