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The Inhibition of Virus Adsorption by Substances Induced by DEAE-Dextran and Poly I:C (37917)

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Interferons produced in embryonated eggs and in animals by attenuated viruses do not block viral adsorption or penetration (1, 2). Cells in culture are protected by interferon because the viral genome cannot be translated (3). In 1967, it was demonstrated that the double-stranded polymer of inosinic and cytidylic acids (poly I:C) could induce interferon production *in vivo* and *in vitro* (4). To achieve the response *in vitro,* DEAE-dextran was used to facilitate the uptake of poly I:C (5). While studying the activity of interferon in cell cultures, we noted that the product of the induction could inhibit viral adsorption. The study herein reported was designed to assess the adsorption-inhibiting properties of preparations containing interferon-inducing substances and the products of induction.

Materials and Methods. Reagents. The sodium salt of poly I:C (P-L Biochemicals, Milwaukee, WI) was used from a stock solution containing 1 mg/ml of distilled water. DEAE-dextran with an approximate molecular weight of 2×10^6 daltons (Pharmacia Corp., Piscataway, NJ) was used from a stock solution containing 40 mg/ml of distilled water.

L-M cell growth. L-M cells were grown in 300-ml suspensions in KLM-3 medium in 1-liter screw-cap saline bottles (McGaw Laboratories, Milledgeville, GA) on a gyrotory incubator shaker (New Brunswick Scientific Co., New Brunswick, NJ) at 110 rpm and 35°. KLM-3 medium is composed of auto-pow MEM for suspension cultures, supplemented with yeast extract (0.05%), bacto-peptone (0.5%), and lactalbumin hydrolysate (0.25%). Other additions include $NaHCO_3$ (0.17%) and methylcellulose (0.12%) (6). L-M cells were also grown in stationary cultures on 30-ml plastic flasks (Falcon Plastics, Oxnard, CA) in medium 199 supplemented with 0.5% bacto-peptone (199P).

Virus growth. Five million L-M cells growing in the logarithmic phase as cell sheets on plastic flasks were infected with mengovirus. After a 30-min adsorption period at room temperature, the excess fluid containing unadsorbed virus was removed and 5 ml of medium 199P was added. After an 18-hr incubation period at 35°, the supernates from a number of flasks were harvested and pooled. This virus pool was clarified by centrifugation at 800 g, for 10 min. The virus pool retained the same infectivity titer for extended periods when stored at 2°.

Interferon induction. Interferon was induced in L-M cells growing in suspension cultures in KLM-3 medium with DEAE-dextran and poly I:C as previously described (7). When induction was carried out in stationary cultures, the inducers were dissolved in medium 199P. In some stationary cultures, the medium was replaced with fresh medium 199P after a 2-hr induction period.

Plaque assay. Suspension cultures containing between 1 and 1.2×10^6 L-M cells/ml were supplemented with 5% calf serum. Five-milliliter replicates from the cell suspension were dispensed into 60-mm plastic petri dishes (Falcon Plastics, Oxnard, CA).

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Confluent sheets of L-M cells were formed in 30–60 min at 35° in an atmosphere of 5% CO_2 in air. These cell sheets were used immediately for plaque assays or after preincubation with interferon-inducing reagents or interferon preparations, as previously described (7).

Virus adsorption assay. In order to determine if an antiviral preparation affected virus adsorption, the following procedure was used. The plaque assay procedure described above was used, except that after the initial 30-min adsorption period, the excess fluid containing any unadsorbed virus was transferred to a freshly prepared sheet of L-M cells. Adsorption on this plate was carried out for 30 min at 35° in an atmosphere of 5% CO_2 in air. At this point, the excess fluid was removed and an agar overlay was placed over the cell sheet. In the experiments reported here, the first plate is called the primary plate and the second plate is referred to as the transfer plate. To determine the rate of virus adsorption, a similar procedure was used, but samples were taken at various times to plaque assay the unadsorbed virus.

Results. Table I shows that 150 mengovirus plaques developed on control plates where confluent sheets of L-M cells were incubated in medium 199P prior to assay. When suspension culture medium KLM-3 was diluted 1:10 in 199P, the number of plaques on the primary assay plate was reduced to 122. On plates where KLM-3 medium containing DEAE-dextran was diluted 1:10, 93 plaques were noted which represents a 24% reduction. Plaque development was completely abolished on those plates where KLM-3 medium containing DEAE-dextran and poly I:C was diluted 1:10. When diluted 1:25 and 1:50, a reduction, 24 and 5% respectively, was noted when media containing DEAE-dextran was tested, while media containing DEAE-dextran combined with poly I:C showed a 98% reduction when diluted 1:10 and an 80% reduction at a 1:50 dilution.

When KLM-3 media containing DEAE-dextran and DEAE-dextran combined with poly I:C were first incubated with L-M cells in suspension cultures to induce interferon and then tested in a similar assay, a comparable result was obtained. Spent KLM-3 medium tested at a 1:10 dilution also inhibited plaque formation on the primary plate so that only 123 plaques formed. When spent medium in which DEAE-dextran and DEAE-dextran combined with poly I:C were tested at a 1:10 dilution, the number of plaques was reduced by 60 and 100%, respectively, compared to the spent medium control. When tested at 1:25 and 1:50 dilutions, the spent medium did not inhibit plaque formation. However, when spent media in which DEAE-dextran and DEAE-dextran combined with poly I:C were used, plaque formation was inhibited. Even when diluted 1:50, the spent medium which originally contained DEAE-dextran caused a 35% reduction in the number of plaques, and that which contained DEAE-dextran combined with poly I:C inhibited plaque formation by 45%.

Since interferon should only be produced in media containing poly I:C, these data suggest that an additional substance which inhibits plaque development is present in interferon preparations induced in cell cultures. To determine whether this inhibitory substance might block viral adsorption, a transfer plate assay was used to detect virus not adsorbed to cells on the primary plate. The results of the transfer plate assay indicate that 23 plaques or about 12% of the mengovirus is not adsorbed to L-M cells on the primary plate in 30 min when the assay is carried out in medium 199P. In all other cases, the reduction in the plaque number on the primary plate could be accounted for, in part, by unadsorbed virus. Media containing DEAE-dextran incubated with or without cells inhibited virus adsorption at all dilutions tested. In those supernates containing DEAE-dextran combined with poly I:C, as much as 40% of the plaque reduction could be accounted for by adsorption inhibition. When the number of plaques appearing on the primary and transfer plates were totaled, it was evident that the virus could all be accounted for in the various media except for those originally containing poly I:C. In the case of the poly I:C medium, the virus was undoubt-

TABLE I. The Antiviral Activity and Adsorption-Inhibiting Capacity of Supernates from L-M Cell Cultures Exposed to DEAE-Dextran and DEAE-Dextran Combined with Poly I:C[a]

			Plaque assay in medium 199P		KLM-3 media containing inducers tested directly in a plaque reduction assay			KLM-3 media with inducers incubated with L-M cells for interferon induction before the plaque reduction assay		
	μg/ml DEAE-dextran	μg/ml poly I:C	Plaques on primary plate	Plaques on transfer plate	Plaques on primary plate	% Plaque reduction	Plaques on transfer plate	Plaques on primary plate	% Plaque reduction	Plaques on transfer plate
Control	0	0	150	23	—	—	—	—	—	—
	0	0	—	—	122	—	48	123	—	37
1:10	400	0	—	—	93	24	78	54	56	63
	400	10	—	—	0	100	35	0	100	81
	0	0	—	—	151	—	19	144	—	34
1:25	400	0	—	—	116	24	95	128	11	78
	400	10	—	—	3	98	73	26	82	99
	0	0	—	—	150	—	31	151	—	38
1:50	400	0	—	—	144	5	55	98	35	84
	400	10	—	—	30	80	93	83	45	74

[a] Fifty milliliters of KLM-3 medium was placed into each of six 250-ml Erlenmeyer flasks. DEAE-dextran was added to two of the flasks at a concentration of 400 μg/ml; DEAE-dextran and poly I:C were added to two flasks so that the final concentration ratio would be 400 to 10 μg/ml. The two remaining flasks served as controls. L-M cells were pelleted from suspension cultures and resuspended in the medium from one flask in each of the three sets. The six flasks were incubated at 35° for 24 hr. At this time, the cells in the three flasks were pelleted and the supernates along with the media from the flasks that did not contain cells were assayed for antiviral material. The assay was carried out by treating confluent sheets of L-M cells with the various supernates diluted 1:10, 1:25, and 1:50 in medium 199P, and incubating them for 12 hr at 35° in an atmosphere of 5% CO_2 in air. A plaque reduction assay using approximately 150 PFU per plate was carried out.

edly adsorbed on the primary plate but its development was inhibited by the interferon mechanism. This result is also noted when interferon is induced with DEAE-dextran and poly I:C in stationary cultures.

To prove that the rates of virus adsorption by these preparations were affected in this system, a kinetic analysis of mengovirus adsorption was carried out. Spent media which contained DEAE-dextran and DEAE-dextran combined with poly I:C were used. Media from stationary cell cultures where the inducers were removed after 2 hr were also used. The results (Fig. 1) indicate that the rates of adsorption of virus are inhibited in spent media containing DEAE-dextran and DEAE-dextran combined with poly I:C. More revealing is the fact that spent media that did not contain the inducers also inhibited viral adsorption.

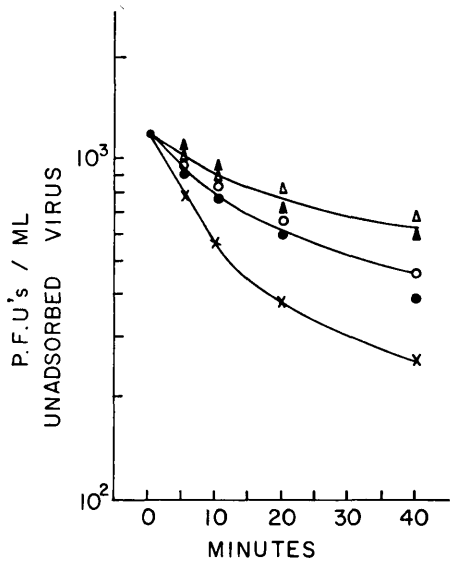

FIG. 1. The rate of adsorption of mengovirus to L-M cells protected with the products formed in the presence of DEAE-dextran and DEAE-dextran combined with poly I:C. Approximately 1200 PFU of mengovirus in 1 ml of phosphate-buffered saline were adsorbed to sheets of L-M cells which had been incubated for the previous 18 hr in the presence of the products of induction diluted 1:10 in medium 199P. Residual unadsorbed virus was assayed on a second sheet of L-M cells at the times indicated.

Control spent medium 199P (×); spent 199P media that contained 400 μg DEAE-dextran for induction (△); spent 199P media that contained 400 μg DEAE-dextran/ml combined with 10 μg poly I:C/ml for induction (▲); spent media from cultures exposed to 400 μg DEAE-dextran/ml for 2 hr and then changed to 199P (○); spent media from cultures exposed to 400 μg DEAE-dextran/ml combined with 10 μg poly I:C/ml for 2 hr and then changed to 199P (●).

Discussion. The amount of interferon produced *in vitro* by poly I:C is increased by the addition of DEAE-dextran. The highest titers are observed when the two polymers are kept in contact with L-M cells for 12 hr (5). The results herein reported show that media containing these inducing components either before or after incubation with L-M cells have the capacity to inhibit the adsorption of mengovirus to L-M cells. DEAE-dextran is suspected to be the inhibitory substance since it probably persists in the medium after incubation with cells. However, since DEAE-dextran is employed to facilitate virus and polymer attachment to cells (8, 9), it is difficult to conceive in these experiments that these molecules are competing with virus for adsorption sites. Thus, it is likely that some other cell protein is released in the presence of DEAE-dextran that exerts this effect.

Summary. The adsorption of mengovirus to L-M cells is inhibited either directly by DEAE-dextran or by substances released into culture media from cells exposed to DEAE-dextran and DEAE-dextran combined with poly I:C. Therefore, preparations containing interferon induced in cell cultures in which DEAE-dextran is used to facilitate the uptake of poly I:C should be appropriately controlled to account for this activity.

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