INHIBITION OF MEASLES VIRUS REPLICATION AND RNA SYNTHESIS BY ACTINOMYCIN D

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Summary. Measles virus replication and RNA synthesis in Vero cells are inhibited by actinomycin at concentrations which inhibit cellular RNA synthesis. Drug present from the 2nd to the 24th hr post infection inhibited infectivity but not hemagglutinating activity or cell fusion. Infectivity was much less sensitive to drug added during the second 24-hr period, and 52S RNA was labeled and incorporated into virions during this later time interval.

There are several reports in the literature of enhancement of measles virus growth by low doses (0.01 to 0.1 μg/ml) of actinomycin (1-3). Mirchamsy and Rapp used cell lines and measles strains differing in their ability to produce or induce interferon to study its postulated role in enhancement (3). They concluded that increase in measles titer was primarily a function of suppression of interferon production by inhibition of DNA-dependent host RNA synthesis. The extent to which host RNA or protein synthesis was depressed in the above studies was not reported, however.

Suppression of measles growth by actinomycin at concentrations of 1 μg/ml or more also has been reported, but the effects noted were not considered significant because of toxicity of the drug in the systems studied (3, 4).

Vero cells have been shown to be relatively resistant to toxic effects of actinomycin (5) and to be deficient in interferon production (6). We found that yields of infectious measles virus (Edmonston attenuated) in these cells were suppressed 99 to 99.9% by doses of actinomycin (1 μg/ml or greater) which inhibited host RNA synthesis. Doses which failed to suppress cellular RNA did not inhibit measles virus. Pretreatment of cells with actinomycin had little or no depressive effect indicating that cellular DNA expression is not a requirement for measles virus replication. This is in keeping with earlier

Table 1. Effect of actinomycin D concentration on production of measles hemagglutinin (HA), plaque-forming units (pfu) and cell fusion in Vero cells.

Hours after infection	Assay	Actinomycin concentration (μg/ml) 0[1]	0.1[1]	1.0[1]	1.0[2]
8	HA units	< 10	< 10	< 10	< 10
	Fusion	-	-	-	-
16	HA units	< 10	< 10	< 10	< 10
	Fusion	+	+	+	+
	pfu	$< 10^{2.3}$	$< 10^{2.3}$	$< 10^{2.3}$	$< 10^{2.3}$
24	HA units	20	20	20	20
	Fusion	++	++	+++	++
	pfu	$10^{3.8}$	$10^{3.9}$	10^{2}	$10^{3.8}$
48	HA units	2560	2560	160	3840
	Fusion	++++	++++	++++	++++
	pfu	$10^{5.8}$	$10^{6.2}$	$10^{3.3}$	$10^{5.3}$
8 (Uninfected controls)	cpm[3]	1560	1510	338	

[1]Vero monolayers in plastic flasks were inoculated with a multiplicity of 1.5, washed 3 times with warm medium after a 2-hr absorption period, and warm medium, with or without actinomycin, was added. At times indicated cells from 2 bottles were pooled for each concentration tested, washed, resuspended in 2 ml medium and disrupted by 3 cycles of freezing and thawing.

[2]Actinomycin was added to culture medium 24 hr post infection.

[3]1 μCi/ml ^{3}H-5-uridine added with actinomycin and labeling continued for 6 hr. Determination of uridine incorporation as described previously (10).

studies of measles virus growth in cells irradiated with ultraviolet light (7) or treated with mitomycin (8).

Table 1 shows that drug added after the virus absorption period caused a 100-fold reduction in yields of infectious virus, but no reduction in production of measles hemagglutinin or in induction of cell fusion "from within" (9). On the other hand, drug added 24 hr after infection caused only a 3-fold decrease in infectious virus. Thus, the inhibition of infectivity noted during the first 24 hr after infection cannot be explained by a general

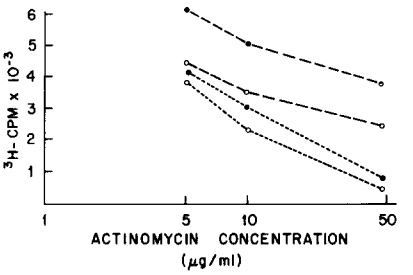

Figure 1. Dose responses of viral and cellular RNA syntheses to actinomycin D. 3 x 10^6 control cells or cells 24 hr post infection were exposed to actinomycin for 1 hr prior to the addition of 50 μCi/ml ^{3}H-5-uridine. After a 90-min labeling period in the presence of drug, cytoplasmic RNA was extracted and assayed for acid-insoluble radioactivity as described previously (10). Closed symbols, Experiment 1; open symbols, Experiment 2. Cellular RNA (----); viral RNA, cpm infected cytoplasm minus cpm control cytoplasm, (——).

toxic effect, since other viral expressions--hemagglutination and fusion--were not inhibited. Also, when drug was present for an equivalent period late in infection, infectivity titers were much less depressed.

Because measles virus growth was inhibited only by drug concentrations which inhibited host RNA synthesis, the effects of actinomycin on cellular and viral RNA syntheses were compared at 24 hr post infection when measles RNA synthesis is maximal (C. Williams, unpublished results). A dose-dependent inhibition of cell-associated viral RNA synthesis was demonstrated. Figure 1 shows that both cellular and viral RNA syntheses were sensitive to the drug, but 24 to 25 hr after infection cellular RNA synthesis was more sensitive.

To test the effect of drug on viral genome RNA, virion RNA was purified as described previously (10). 52S viral RNA isolated from virions labeled with ^{3}H-5-uridine in the presence of 2 μg/ml actinomycin from 24 to 48 hr post infection contained about 50% of the radioactivity associated with 52S RNA from virions labeled in the absence of drug.

If suppression of measles replication is due to an effect on viral RNA synthesis, it is not likely to be due to suppression of genome RNA itself, since actinomycin does not substantially prevent its synthesis late in infection.

Table 2. Effect of actinomycin D added 24 hr post infection on the density distribution of measles hemagglutinating particles released into the medium.[1]

Buoyant density (g/cc)	Hemagglutination titer (HA)		Uridine incorporation (cpm)		Specific activity (cpm/HA)	
	No act.	Act.[2]	No act.	Act.	No act.	Act.
1.18	64	320	180	700	2.8	2.2
1.21	48	96	450	1140	9.0	11.9
1.23	24	60	630	980	26.4	16.3
1.26	4	4	850	200	212.0	50.0

[1]Medium collected at 48 hr was concentrated, banded in sucrose-D_2O gradients and assayed as described previously (10). See Legend, Figure 1, for conditions of infection.

[2]1 μg/ml.

The block cannot be an overall suppression of messenger RNA either, since viral membrane proteins are made in the presence of the drug at both early and late times after infection. It seems more likely that actinomycin acts to inhibit a step prior to the completion of template for 52S genome RNA and might act directly to inhibit the formation of a replicative intermediate. The proposed selective inhibition of measles RNA synthesis is not without precedent. East and Kingsbury found synthesis of cell-associated mumps virus 50S RNA suppressed, but synthesis of small minus strands was unchanged or even enhanced (11). In addition, actinomycin preferentially suppressed synthesis of influenza minus-strand RNA (12).

Another effect was noted when actinomycin was added 24 hr after infection and, in some cases, after cells had been treated with drug prior to infection only. There was an increase in the number of hemagglutinating particles in medium collected at 48 hr (Table 2). Infectivity titers were not increased, however, since most of the hemagglutinating activity was associated with light, non-infectious particles with densities around 1.18 g/cc. These particles have specific activities about one-sixth that of complete virions, densities in the range 1.21 to 1.23 g/cc, also labeled in the presence of actinomycin. This effect on viral budding may be unre-

lated to viral RNA synthesis, but it may bear some relation to enhancement noted with low doses of drug.

In conclusion, our findings and those with mumps virus indicate that resistance to actinomycin D is not a uniform characteristic differentiating all subgroup II myxoviruses from subgroup I viruses (13, 14). In addition, these experiments and those of others showing enhancement of viral replication by low doses of drug demonstrate that actinomycin may produce effects on viral replication which are fundamentally different and may be mediated through different mechanisms.

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