Host DNA Synthesis-Suppressing Factor in Culture Fluid of Tissue Cultures Infected with Measles Virus

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Host DNA synthesis is suppressed by the culture fluid of cell cultures infected with measles virus. This activity in the culture fluid is initiated somewhat later than the growth of infectious virus. Ninety percent of host DNA synthesis in HeLa cells is inhibited by culture fluid of 3-day-old cell cultures of Vero or HeLa cells infected with measles virus. This suppressing activity is not a property of the virion, but is due to nonvirion-associated component which shows none of the activities of measles virus such as hemagglutination, hemolysis, or cell fusion nor does it have the antigenicity of measles virus as tested by complement-fixation or hemagglutination-inhibiting antibody blocking tests. Neutralization of the activity of this component is not attained with the pooled sera of convalescent measles patients. This component has molecular weights of about 45,000, 20,000, and 3,000 and appears to be a heat-stable protein. The production of host DNA suppressing factor (DSF) is blocked by cycloheximide. Neither UV-inactivated nor antiserum-neutralized measles virus produce DSF. Furthermore, such activity of nonvirion-associated component is not detected in the culture fluid of cultures infected with other RNA viruses such as poliovirus, vesicular stomatitis virus, or Sindbis virus.

"Cut off" phenomenon of host DNA synthesis was reported as due to various viruses (1, 8, 9). Measles virus also suppresses host DNA synthesis (2, 10). In our previous paper (2), it was reported that measles virus suppressed host DNA synthesis but not RNA and protein syntheses. This phenomenon was dependent upon the multiplicity of infection but not upon the cellular growth phase. Furthermore, this activity of measles virus was resistant to ultraviolet-irradiation and heating for 2 h at 56 C, but was lost by the treatment of virus with proteolytic enzymes. It was assumed that the envelope protein played an important role in the suppression of host DNA synthesis. We afterward found that the purified virions of measles virus did not have the capacity to suppress host DNA synthesis. In the present paper, the characteristics of the factor showing the suppression of host DNA synthesis is investigated.

MATERIALS AND METHODS

Cell cultures. HeLa and Vero cells were cultivated in Eagle minimum essential medium (MEM) supplemented with 0.5% lactalbumin hydrolysate (ML medium) and 5% fetal bovine serum in plastic petri dishes (Falcon Plastics Co., Calif.).

Virus stocks. Measles virus (Toyoshima strain) was prepared by inoculating the virus into Vero cell

cultures. After the infected cultures were frozen and thawed three times and clarified by low-speed centrifugation, virus preparations were concentrated about 20-fold by dialysis against 15% polyethylene glycol G-6,000 (Nakarai Chemicals Co.) and pelleting virions on a cushion of 50% CsCl at 25,000 rpm for 2 h. Virus titers were approximately 10^6 PFU/ml and 128 hemagglutinating units (HAU)/ml.

Sucrose density-gradient centrifugation. A 2-ml amount of the concentrated measles virus suspension was layered on the top of 27 ml of continuous gradients of sucrose from 50 to 10%. The tubes were centrifuged in a swinging-bucket rotor (RPS 25; Hitachi) at 24,000 rpm for 16 h at 2 C. After centrifugation, about 20 fractions of equal volume were collected and dialyzed against phosphate-buffered saline (PBS) and again against ML medium.

Gel filtration through Sephadex G-200 columns. The gel filtration of 2 ml of the concentrated measles virus was carried out on Sephadex G-200 columns (2.6 by 36 cm) using 0.01 M PBS (pH 7.4). The effluents were collected in 5-ml fractions, measured for absorbance at 280 nm and assayed for biological activities after dialysis against ML medium. The molecular weight of the factor which showed the suppression of host DNA synthesis was approximated by using bovine serum albumin (molecular weight 67,000; Armour) and cytochrome c (molecular weight 12,900; Sigma) as markers.

Determination of biological activities: HA activity. The microtechnique was employed for the titration of hemagglutinin (HA). Twofold serial dilu-

tions were prepared in 0.025 ml of PBS with standard loops, and 0.025 ml of 1% suspension of green monkey (*Cercopithecus aethiops*) erythrocytes was added with the aid of a dropper to each cup. The HA titer was determined after 2 h of incubation at 37 C.

CF antigen activity. A microtechnique was used for the titration of complement-fixing (CF) antigen. Two units of a human measles convalescent serum and two units of commercial guinea pig complement were employed. All reagents were used in a volume of 0.025 ml. The plates were placed at 4 C for 16 h. Then, 0.05 ml of 1% sensitized sheep red cells was added and the plates were placed at 37 C for 1 h. The dilution factor of antigen in the last drop mixture exhibiting at least partial inhibition of hemolysis was taken as the titer.

HL activity. One milliliter of sample, dialyzed against PBS to remove sucrose, was mixed with 0.25 ml of a 5% suspension of green monkey erythrocytes in PBS, and the mixture was incubated at 37 C for 3 h. Hemolytic (HL) activity was expressed as the optical density of the liberated hemoglobin at 540 nm.

Hemagglutination-inhibiting antibody blocking (HI blocking) test. HA antigens were titrated by the antibody blocking test for HA by the method of Webster and Darlington (7).

Assay of infectivity. The plaque assay was performed in Vero monolayer cultures with 0.1 ml of virus dilutions. After virus adsorption by shaking for 30 min, the monolayers were overlaid with 5 ml of ML medium containing 1% Difco agar, 0.15% bicarbonate, 5% bovine serum, and 0.002% neutral red. Plaques were counted on the 10th day after inoculation, and the infectivity was expressed in PFU per milliliter.

DNA synthesis of host cells. A total of 10^6 cells of HeLa were seeded in a 35-mm Falcon dish and cultivated for several days in a CO₂ incubator. Before the cultures became confluent, they were inoculated with 1 ml of culture fluid to be tested, incubated at 37 C for 16 h, and then labeled with [6-³H]thymidine (2 μ Ci/ml, 15 Ci/mmol) at 37 C for 30 min. The labeled cultures were washed three times with cold PBS, and after the addition of 1 ml of distilled water were frozen at -20 C. Thawed culture was solubilized with 2 ml of 1% sodium dodecyl sulfate (SDS), 0.1 M NaCl, 0.01 M EDTA, and 0.01 M Tris-hydrochloride buffer (pH 7.4), and 0.5 ml of 50% trichloroacetic acid was added. The acid-insoluble material was collected on a Whatman glass fiber filter (GF/C, no. 1) and washed three times with 5% trichloroacetic acid. The filter was dried and placed in 10 ml of toluene containing 2,5-diphenyloxazole (6 g/liter) and 1,4-bis-2-(5-phenyloxazolyl)benzene (200 mg/liter). Retained radioactivity was measured in a Horiba LS-551 liquid scintillation spectrometer. In all experiments, data of counts per minute express mean values of duplicate tests. The activity of DNA suppressing factor (DSF) was calculated as the following: (counts per minute of control minus counts per minute of experiment)/(counts per minute of control) $\times$ 100 (%). The reference to counts per minute applies to [³H]thymidine.

UV-irradiation of measles virus. Virus material was placed in a 90-mm petri dish (0.16 ml/cm²) and irradiated by a germinal lamp at a distance of 30 cm

with agitation for 2 min. The infectivity of irradiated measles virus dropped to less than 10 PFU/ml.

Measles antiserum. Sera of five convalescent measles patients which were pooled and inactivated at 56 C for 30 min showed an HA-inhibiting titer (HIU) of 1:256 and neutralizing titer of 1:128.

Reagent. Cycloheximide (Nakarai Chemicals Co.) was employed in these experiments.

RESULTS

Appearance of activity of suppression of host DNA synthesis in culture fluid after infection with measles virus. Vero cell cultures were infected with measles virus (Toyoshima strain) at the multiplicity of infection (MOI) of 1.0. At the indicated times after the infection, the culture fluids of two infected cultures were harvested and two cultures were frozen at -20 C. The cultures then thawed and centrifuged at 5,000 rpm for 30 min. The infectivity and the activity of suppression of host DNA synthesis in the culture fluids and the supernatant of the thawed cultures were assayed. As shown in Fig. 1, the activity of suppression of

FIG. 1. Appearance of the activity of suppression of host DNA synthesis and the production of progeny virus in culture fluids of Vero cells infected with measles virus. After adsorption of measles virus at the multiplicity (MOI) of 1.0, Vero monolayer sheets were washed with MEM and then maintained with ML medium supplemented with 2% bovine serum. At various intervals after inoculation, culture fluids of two infected and uninfected cultures were harvested and centrifuged and cells were discarded. At the same time, two bottles of the infected and the uninfected cultures were frozen at -20 C, thawed, and centrifuged at 5,000 rpm for 30 min to remove cell debris. Viral infectivity and the activity of suppression of host DNA synthesis in supernatants of culture fluids and frozen cell extracts from infected cultures were assayed by the methods described in the text. Average percentage of suppression of host DNA synthesis by frozen cell extract (●), and by culture fluid (○). Infectious virus in frozen cell extract (▲).

host DNA synthesis in culture fluid appeared somewhat later than the growth of infectious virus 24 h after inoculation of the virus and reached the maximum 3 days later. Ninety percent of host DNA synthesis was inhibited by the 3 days' culture fluid. Since this activity found in the culture fluids of infected cells may be mediated by some substances released from cells destroyed by infection of measles virus, the frozen cell extracts of uninfected cultures were examined. The frozen cell extracts as well as the culture fluids of uninfected cells did not contain the activity to suppress host DNA synthesis compared with ML medium control. Furthermore, this activity found in the frozen cell extracts of the infected cells was of the same order as that found in the infected culture fluids.

In addition to the experiments of Vero cell systems, culture fluids of HeLa and KB cells infected with measles virus under the same conditions as above experiments also contained the activity to suppress host DNA synthesis. On the other hand, culture fluids of Vero cells infected with UV-inactivated or antiserum-neutralized measles virus contained neither infectious virus nor the capacity to suppress host DNA synthesis (Table 1).

Sucrose density-gradient centrifugation of crude measles virus. As shown in Fig. 2, HA, HL, CF antigenic and HI antibody blocking

TABLE 1. Suppression of host DNA synthesis by culture fluids of various cells infected with measles virus^a

Culture fluid from	PFU/ml	Suppression of DNA synthesis (%)
Vero cells infected with MV	2.8×10^5	80.0
HeLa cells infected with MV	5.1×10^4	81.8
KB cells infected with MV	1.0×10^6	84.1
Vero cells infected with UV-MV	$<10^1$	4.0
Vero cells infected with AS-MV	$<10^1$	8.2

^a HeLa, Vero, and KB cells were infected with measles virus (MV) at an MOI of 1.0, and as control uninfected HeLa, Vero, and KB cells were cultivated at 37 C for 3 days. Vero cells infected with UV-inactivated virus (UV-MV) and antiserum-neutralized virus (AS-MV) were similarly cultivated. All culture fluids were harvested and centrifuged at 5,000 rpm for 30 min. The infectivity and the percentage of suppression of host DNA synthesis by these culture fluids were determined by the methods described in the text.

FIG. 2. Biological activities of measles virus fractionated by sucrose density-gradient centrifugation. Two milliliters of the concentrated measles virus (1.4×10^6 PFU/ml and 128 HAU/ml) was layered on the top of 27 ml of a continuous sucrose gradients from 50 to 10%. After centrifugation at 24,000 rpm for 16 h at 2 C, 20 fractions of equal volume were collected and dialyzed against PBS. After dialysis these fractions were tested for infectivity (PFU/ml) and activities of HA, HL, CF, and HI blocking of measles virus, and further dialyzed against ML medium for the DNA synthesis-suppressing activity of these fractions. HeLa cell cultures were inoculated with 1 ml of each fraction, incubated at 37 C for 16 h and then labeled with [³H]thymidine (2 μ Ci/ml). Symbols: $\blacktriangle$, percentage of the suppression of host DNA synthesis; $\blacksquare$, infectivity ($\log_2$ PFU/ml); $\bullet$, HL activity (OD_{540}); Δ , HA activity (HAU/ml); ∇ , CF antigen activity (CFU/ml); $\circ$, HI blocking activity (HI blocking titer/ml).

activities of measles virus were shown to be present in fractions 2 to 5 (1.20 to 1.16 g/ml), but the suppression of host DNA synthesis was demonstrated only in the top fraction (<1.08 g/ml). As infectivity remained in the top fraction in which other biological activities of measles virus could not be detected, we carried out the following experiments to separate the infectivity from the top components which contained the activity to suppress host DNA synthesis. Crude measles virus was centrifuged at 40,000 rpm for 2 h to remove the infectious virus. The supernatant contained no viral infectivity and antigenicity but did contain the capacity to suppress host DNA synthesis. On the other hand, the resuspended pellet of virion did show no suppression of host DNA synthesis

(Table 2). Figure 3 shows the time course of the suppression of host DNA synthesis by the supernatant and the resuspended pellet. Host DNA synthesis was rapidly inhibited by the supernatant, whereas by the resuspended pellet the suppression of host DNA synthesis was detected 24 h later and to a lesser degree. This delayed suppression by the resuspended pellet coincided with the appearance of a suppressing activity newly induced by inoculation of the resuspended pellet. But this factor affected the infected cells themselves less effectively than the uninfected cells which were inoculated by the factor. The reason why the factor did affect less effectively the infected cells is not clear at present. Thus, it appears that the activity of suppression of host DNA synthesis in culture fluid was the property of the nonvirion-associated component which we propose to call the host DNA suppressing factor. The DSF activity could not be neutralized with the pooled sera from convalescent measles patients (Table 3). The higher the concentration of antiserum used to incubate with crude measles virus, virus-removed culture fluids, and ML medium, the more host DNA synthesis occurred by them. Antiserum may contain some serum factors which activate host DNA synthesis as reported by Temin et al. (6). However, about 90 and 85% of host DNA synthesis was suppressed by crude measles virus and virus-removed culture fluid, respectively, even after treatment with antiserum. On the other hand, such nonvirion-associated component (DSF) was not detected in the culture fluids of Vero cells infected

FIG. 3. DNA synthesis of HeLa cells inoculated with the supernatant and the resuspended pellet. The supernatant and the resuspended pellet obtained in the ultracentrifugation of measles virus and shown in Table 2 and control medium were inoculated into HeLa cells. At various intervals they were removed and the cultures were labeled with [3 H]thymidine (2 μ Ci/ml) for 30 min to determine the percentage of DNA synthesis in cultures of HeLa cells inoculated with the supernatant (●—●) and the resuspended pellet (○) as compared with DNA synthesis in control HeLa cells. Culture fluids of HeLa cells inoculated with the resuspended pellet were examined if they had the activity to suppress host DNA synthesis, and we determined the percentage of suppression of host DNA synthesis by them according to the method described (●...●).

with other RNA viruses such as poliovirus, vesicular stomatitis virus, or Sindbis virus, but was detected with mumps virus and Newcastle disease virus (Table 4).

Gel filtration of crude measles virus. As shown in Fig. 4, HA, HL, and CF antigen activities of measles virus eluted at the peak of void volume. These fractions of the eluate did not suppress host DNA synthesis. The suppression of host DNA synthesis was seen mainly in eluate fractions 39, 44, and 60 of molecular weight 45,000, 20,000, and 3,000, respectively. Fractions 35 to 45 in Fig. 4 were pooled and concentrated by ultrafiltration, and then refiltered on Sephadex G-200 column. The suppression of host DNA synthesis was shown to be contained in the same fractions as in those shown in Fig. 4 (Fig. 5). Activity of this component was destroyed by proteolytic enzymes Pronase and trypsin, but withstood heating for 2 h at 56 C and treatment with DNase, RNase, phospholipase C and D, and neuraminidase, as was shown previously (2). From these results, the factor which showed the suppression of host DNA synthesis seemed to be a nonvirion-associated and heat-stable protein.

TABLE 2. Biological activities of supernatant obtained by ultracentrifugation of measles virus^a

Test material	Counts/ min $\times$ 10 ³ / culture ^b	PFU/ ml	HAU/ ml ^c	CFU/ ml ^d
Crude measles virus	6	1.4×10^6	64	16
Supernatant from crude measles virus	9	$<10^1$	<2	<2
Resuspended pellet	45	5.9×10^4	32	8
ML medium control	47			

^a After 5 ml of crude measles virus was centrifuged at 40,000 rpm in a swinging-bucket rotor (RPS40: Hitachi) for 2 h at 2 C, the upper two-thirds of supernatant was removed from tube for titration. Pellet was resuspended with ML medium and centrifuged at 40,000 rpm for 2 h. The resultant pellet was resuspended in ML medium. Infectivity and the capacity of suppression of host DNA synthesis of these samples were determined.

^b Counts per minute of [3 H]thymidine incorporated in trichloroacetic acid-insoluble fraction.

^c HAU, Hemagglutinin units.

^d CFU, Complement-fixing antigen units.

TABLE 3. *Effect of crude measles virus and virion-free culture fluid treated with anti-measles serum on HeLa cell DNA synthesis^a*

Substance added to sera	Counts/min $\times 10^2$ /culture				
	Dilution of anti-measles serum				Serum-free control
	1:4	1:16	1:64	1:256	
Crude measles virus	16 (10.5)	8 (6.7)	6 (9.4)	5 (8.8)	5 (10.0)
Virion-free culture fluid	26 (16.7)	17 (14.3)	8 (12.5)	7 (11.9)	7 (14.0)
ML medium control	153 (100)	119 (100)	64 (100)	59 (100)	50 (100)

^a After crude measles virus, virion-free culture fluid, or ML medium was mixed with an equal volume of diluted, pooled human convalescent sera containing 256 HIU/ml or serum-free medium, they were incubated for 2 h at 37 C. Then the mixtures were inoculated into HeLa cell cultures. The cultures were incubated for 16 h at 37 C and then labeled with [³H]thymidine (2 μ Ci/ml) for 30 min. Radioactivity of trichloroacetic acid-insoluble fraction of labeled HeLa cell cultures was counted as described in the text. The numbers in parentheses indicate the percentage of host DNA synthesis as compared with ML medium control.

TABLE 4. *Production of DSF-like substance in culture fluids of Vero cells infected with various viruses^a*

Virus	PFU/ml	Percent in controls ^b
Poliovirus	2.8×10^6	107
VSV	9.0×10^6	114
SDV	2.1×10^6	148
NDV	2.2×10^5	38
Mumps virus	8.8×10^5	42
Measles virus (Schwarz strain)	5.5×10^3	60
Measles virus (Toyoshima strain)	1.5×10^6	16

^a After Vero cells were adsorbed with poliovirus type 1 (Mahoney strain), vesicular stomatitis virus (VSV) (Indiana strain), Sindbis virus (SDV), mumps virus (Ec-2a strain), Newcastle disease virus (NDV) (Miyadera strain), attenuated measles virus (Schwarz strain), and wild measles virus (Toyoshima strain), these cell cultures were maintained with ML medium supplemented with 2% bovine serum. When the cytopathic effect of each virus appeared at the maximum, culture fluid was harvested and centrifuged at 5,000 rpm for 30 min. The infectivity of each supernatant was assayed. After two centrifugations at 40,000 rpm for 2 h to remove virus, the upper two-thirds of each supernatant was removed and it was assayed for remaining virus. The supernatants containing no infectious virus were inoculated into HeLa cells, and the suppression of host DNA synthesis was determined.

^b Percent of counts per minute per culture inoculated with infected culture fluid as compared with counts per minute per culture inoculated with uninfected culture fluid.

Effect of cycloheximide on the production of DSF. Cycloheximide (50 and 5 μ g/ml) was added to the culture fluids of infected and uninfected cultures at 0, 12, 24, and 48 h after inoculation of measles virus (MOI 1.0). The culture fluids were harvested 3 days after virus

inoculation and centrifuged. The supernatants were dialyzed against PBS for 3 days and further against ML medium to remove cycloheximide. Table 5 shows that the production of DSF was inhibited when 5 or 50 μ g of cycloheximide per ml was added within 12 or 24 h after virus inoculation, respectively. Also, the yield of infectious virus was inhibited.

In another experiment, cycloheximide (50 μ g/ml) was added at zero time to block protein synthesis in infected cultures, and at various times the inhibitor was washed out and fresh medium was added. Virus yield and the production of DSF 48 h after virus inoculation were determined and the percentage of virus yield and the activity of DSF were plotted against the time of reversal of cycloheximide inhibition (Fig. 6). There was no decline in virus yield or the production of DSF until 8 h after infection, but thereafter both productions decreased by cycloheximide inhibition. About 20% of virus yield was inhibited 4 h after infection, but the inhibition is not significant. Therefore, this indicates that the productions of viral protein and DSF were produced 8 h after virus inoculation.

DISCUSSION

The DNA synthesis of HeLa cells was suppressed by culture fluids of cells infected with measles virus. The appearance of the activity of suppression of host DNA synthesis in the culture fluid followed the growth of infectious virus. This activity could not be detected in culture fluids of either uninfected cultures or cultures infected with UV-inactivated or antiserum-neutralized measles virus. This suggests that this activity of culture fluid is induced by infection with measles virus. That the measles virus preparations always have the

FIG. 4. Gel filtration (Sephadex G-200) of crude measles virus. Two milliliters of the concentrated measles virus (128 HAU/ml) were layered on the top of a column (2.6 by 36 cm). The effluent was collected in 5-ml fractions, and the absorbance of each fraction was measured at OD_{280} . After these fractions were assayed for biological activities, they were dialyzed against ML medium and inoculated into HeLa cells as described in the text. Symbols: ●, percentage of the suppression of host DNA synthesis; ▲, HL activity; Δ, HA activity; ▽, CF antigen activity; ○, OD_{280} . Arrows show the peaks of marker proteins of bovine serum albumin (BSA) and cytochrome c (Cyt. C). Void volume was estimated by blue dextran.

FIG. 5. Rechromatography of the DSF-containing peak obtained by gel filtration of Sephadex G-200. Fractions 35 to 45 in Fig. 5 were pooled and concentrated with ultrafiltration (Diaflo membrane UM 10), and gel filtration of the concentrated sample was carried out (Sephadex G-200). Symbols: ●, percentage of the suppression of host DNA synthesis; ○, OD_{280} .

capacity to suppress host DNA synthesis was already shown by us in a previous publication (2).

Furthermore, it was shown that the suppression of host DNA synthesis by measles virus was not due to viral particles or envelope components but was due to nonvirion-associated

TABLE 5. Effect of cycloheximide on the production of DSF^a

Hours of treatment with cycloheximide	Counts/min and PFU after treatment with cycloheximide ^b		
	No treatment	5 μ g/ml	50 μ g/ml
0-72	22 (5.3×10^4)	79 ($<10^4$)	76 ($<10^4$)
12-72	20 (1.6×10^5)	67 (1.4×10^4)	70 ($<10^4$)
24-72	18 (9.6×10^4)	46 (5.7×10^4)	66 (2.9×10^3)
48-72	16 (1.2×10^5)	21 (1.4×10^5)	45 (7.2×10^4)
0-72 ^c	81	75	77

^a Vero cell cultures which adsorbed measles virus (MOI 1.0) were maintained with ML medium supplemented with 2% bovine serum and cycloheximide added to the medium at various intervals from 0 to 48 h after virus adsorption, and the cultures were maintained for 3 days, after which the culture fluids were harvested and centrifuged at 5,000 rpm for 30 min. The supernatants were dialyzed against 0.01 M PBS (pH 7.4) for 3 days at 4°C and again against ML medium to remove cycloheximide. The infectivity and the activity of DSF in the supernatants were then assayed.

^b Counts per minute $\times 10^3$ /culture. Number in parentheses indicates PFU/ml.

^c Uninfected Vero cell cultures.

moiety with no biological activities of measles virus. The component which was responsible for the suppression of host DNA synthesis was a nondialyzable heat-stable protein. This activity

FIG. 6. Production of DSF and virus yield as a function of the time of reversal of cycloheximide inhibition. After Vero cells were adsorbed with measles virus (MOI 1.0), ML medium containing cycloheximide (50 μ g/ml) was added to cultures. At the indicated times, medium was discarded and the culture was washed four times with MEM to remove cycloheximide, and fresh ML medium was added. At 48 h after virus inoculation, all culture fluids were harvested and centrifuged. The activity of DSF (●) and the infectivity (○) at the time of removal of cycloheximide were assayed and plotted as percentages of that obtained in controls taken as 100 percent.

was demonstrated in fractions of molecular weights of 45,000, 20,000, and 3,000. When the eluate fractions containing the large-molecular-weight components were pooled, concentrated, and refiltered through Sephadex G-200 column, not only large- but also small-molecular-weight components were obtained. Thus, the component of small molecular weight might be monomers of the large-molecular-weight polymer.

The evidence that DSF produced by measles virus was not derived from cells damaged by viral cytopathogenicity was born out by the observation that cells damaged by the cytopathic effect of poliovirus, vesicular stomatitis virus, or Sindbis virus did not produce DSF-like substance. However, mumps virus and Newcastle disease virus, as well as measles virus, produce this substance. Thus, this phenomenon may be common in the paramyxovirus group.

The activity of DSF in culture fluid could not be neutralized with pooled convalescent sera from measles patients. Also, in another experiment, DSF activity was not neutralized with rabbit anti-measles serum to crude measles virus (unpublished data). Therefore, DSF does not seem to induce antibody formation. At present we are attempting to produce antiserum to purified DSF in rabbits.

In any case, DSF appeared to be newly synthesized by infection with measles virus. When infected cells were treated with cycloheximide within 24 h after inoculation of virus, neither DSF nor progeny virus were produced. Furthermore, the experiments of reversal of cycloheximide inhibition show that the synthesis of DSF and the formation of infectious virus are not affected by the inhibition of protein synthesis in the early stage of virus growth (within 8 h after infection). These results do not agree with those of Portner and Bussel (3). They reported that the early protein synthesis within 4 h after infection is necessary to form the infectious virus. The apparent discrepancy may be due to the different speed of maturation of virus strain employed and/or difference in cell line. From our results, it appears that DSF is a late product of infection with measles virus and is produced almost simultaneously with the formation of infectious virus. On the other hand, actinomycin D did not affect the production of DSF and progeny virus (unpublished data). Although actinomycin D inhibits the production of interferon as reported by Smith and Wagner (5) and others, Schluederberg et al. (4) reported that replication of measles virus is blocked by actinomycin D. Then, at the present time, it is not yet possible to be certain whether cellular or viral genome is involved in the production of DSF.

Recently, we observed that DSF, when injected intravenously into mice, decreased the number of circulating leukocytes in the blood of these animals. On the other hand, purified measles virus did not produce this effect (unpublished data). It is conceivable that DSF, if it is produced by the infection with measles virus, may play an important role in immunosuppression in the early stage of human measles as noted by Shishido (personal communication).

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