JOURNAL OF BACTERIOLOGY, Dec., 1966
Vol. 92, No. 6

Printed in U.S.A.

Persistent Infection of Cells in Culture by Measles Virus

II. Effect of Measles Antibody on Persistently Infected HeLa Sublines and Recovery of a HeLa Clonal Line Persistently Infected with Incomplete Virus

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Received for publication 16 July 1966

ABSTRACT

RUSTIGIAN, ROBERT (Tufts University School of Medicine, Boston, Mass.). Persistent infection of cells in culture by measles virus. II. Effect of measles antibody on persistently infected HeLa clonal line persistently infected with incomplete virus. J. Bacteriol. **92**:1805–1811. 1966.—The effect of viral antibody on persistent infection of HeLa cells by the Edmonston strain of measles virus was investigated by culturing cells from three persistently infected clones in medium supplemented with human immune globulin. The three infected HeLa clones were isolated from a persistently infected parent line. Two sublines which were grown in the presence of measles antibody developed a nonyielder state, wherein there is no detectable virus infectious for normal HeLa cultures. There is, however, continued synthesis of intracellular viral antigen and formation of viral intracytoplasmic inclusion bodies. The development of a nonyielder state was associated with a marked decrease in the degree of hemadsorption in cultures of both sublines. Further studies of the viral properties of nonyielder HeLa cell populations were made with a clone obtained from one of these sublines by plating under antibody. Persistent infection in this line was characterized by synthesis of incomplete virus even when the cells were cultured thereafter in antibody-free medium. This was evidenced by (i) failure to recover infectious virus from the clonal population despite continued formation of intracellular viral antigen and viral intracytoplasmic inclusion bodies in a majority of the cells, (ii) the presence of only a few cells with surface viral antigen(s) including hemagglutinin, and (iii) the relatively weak antibody response to viral envelope antigen(s) after injection of cells into guinea pigs.

Some characteristics of HeLa cell clones persistently infected with measles virus and grown in antibody-free medium have been described in the preceding paper (15). The effect of measles antibody on viral synthesis in cells from each clonal line has been determined. It was reported in a preliminary communication (14) that infectious virus could be recovered from the cells but not the culture fluid of one of these sublines, K11-HG, after 39 serial passages in the presence of measles antibody. Studies on the recovery of infectious virus from this subline have now been made after an additional 39 serial passages in medium containing viral antibody. It was found that the cell line was eventually transformed to a nonyielder state, wherein there is no detectable infectious virus. Clonal lines of K11-HG cells developed in antibody-containing medium were all nonyielders. One of these, K11A-HG-1, was also reported in the earlier communication. It was not known, however, whether the nonyielder state was due to the lack of synthesis of complete virus or to the production of a substance(s) which blocks the detection of complete virus.

This report presents evidence that persistent infection in K11A-HG-1 is characterized by the synthesis of incomplete virus. It also describes the development of a nonyielder state in the parent K11-HG line. The viral properties of K11-HG are described first. Finally, evidence is presented that persistent infection of HeLa cells by measles virus may vary in different cell populations grown in the presence of measles antibody.

MATERIALS AND METHODS

HeLa lines. The recovery, growth, and viral properties of cultures of three infected clonal lines, K8, K11, and K13, in antibody-free medium (CE medium) have been described (15). The effect of measles antibody on persistent infection in these lines was determined by culturing 40,000 trypsinized cells of K8 in their 6th subculture, of K11 in their 9th subculture, and of K13 cells in their 10th and 108th subcultures in tubes with 1 ml of CE medium supplemented with approximately 0.16% human immune globulin (CE-HG medium). Subcultures were then made in CE-HG medium 1 week later, and thereafter at 1 to 8 weeks to obtain cell lines K8-HG, K11-HG, K13-HG1, and K13-HG2. The fluids were changed at least three times weekly. When large numbers of cells were needed for certain experiments, 150-ml, flat-side bottles were prepared with 500,000 to 1,000,000 cells in 8 ml of CE-HG or CE medium, and their fluids were changed daily. To obtain clonal lines, 20 to 40 cells were plated in CE-HG medium, and single colonies were isolated after further growth in the same medium. Five batches of human immune globulin, supplied by the American Red Cross, were used, and each batch in a concentration of 0.16% in CE medium neutralized over 99% of Edmonston virus.

Clonal line K11A-HG-1 was established from a single colony of K11-HG cells in their 20th passage in antibody-containing medium (14). At the time of the initial report, this clonal line had been serially subcultured 17 times in the presence of viral antibody. This population will be referred to as K11A-HG-1, passage A. In addition, a sample of cells from the 12th subculture in antibody-containing medium had been subcultured 18 times in antibody-free medium. This population will be referred to as K11A-HG-1, passage B.

Viral properties of the HeLa lines. Most of the methods for determining the viral properties of the cell lines, including the method of assay for infectious virus and expression of the infectious virus yields, have been described (15). The culture fluid and cells for infectivity assays were obtained from 4- to 5-day-old bottle cultures containing approximately 5,000,000 cells. The titrations at some passage levels were made after preliminary subculture of a sample of cells in antibody-free medium to insure removal of antibody. Cell extracts were first prepared by combined homogenization and freezing and thawing (14). Later, only homogenization was used, because it was found that combined homogenization and freezing and thawing resulted in some loss of infectivity. The test cultures inoculated for the recovery of infectious virus from each of the test materials were held for 60 to 95 days. Blind passages were also made. Hemadsorption tests were made with either fixed cell cultures (16) or cell suspensions (15). Hemagglutination tests were performed by the method of Rosen (13) with 10^7 rhesus monkey red cells. Resistance of the infected cell lines to superinfection by standard measles virus (15) and resistance of K11A-HG-1 to infection by vaccinia virus (Clark, M.S. Thesis, Tufts University, Boston, Mass., 1962) were measured by determining the susceptibility of these cells in tube cultures to cytopathic effects (CPE) as compared with uninfected HeLa cultures. The cultures were inoculated with virus after removal of antibody either by washing or after a number of subcultures in antibody-free medium. The tests were made with K11-HG cultures between their 12th and 78th passages and with K11A-HG-1, passage B cultures between their 22nd and 40th passages.

Experiments were also performed in which the viral antigenicity of K11A-HG-1 nonyielder cells was compared with that of K11-HG yielders. K11A-HG-1 nonyielder cells between their 41st and 50th subcultures and K11-HG yielder cells between their 54th and 60th subcultures in antibody-free medium were collected by trypsinization. The respective cell suspensions were mixed with Bayol F and Arlecel A (9) to obtain concentrations of 10^7 to 1.7×10^7 cells per milliliter of mixture. The mixtures were then homogenized by means of a Teflon homogenizer in an ice bath. Each of three guinea pigs, which had been previously bled to obtain preinjection sera, was given four intramuscular injections with 1 ml of each cell-adjuvant mixture at 17- to 21-day intervals. Based on intracellular immunofluorescence, the total number of infected K11-HG-1 cells injected was within 6% of the total infected number of injected K11-HG cells.

Sera obtained 6, 7, and 17 days after the fourth injection were inactivated at 56 C for 30 min and titrated for neutralizing antibody, hemagglutinin-inhibiting antibody, and antibody for fluorescent staining. The antibody titers were measured against standard Edmonston virus. The neutralizing antibody titer was defined as the highest dilution which reduced the number of plaque-forming units in primary human amnion cultures by 40 to 60%. The hemagglutinin-inhibiting antibody titer was expressed as the highest dilution which completely or almost completely prevented hemagglutination by 2 hemagglutinating units of virus. For this test, 1 ml of each serum at a 1:4 dilution was first adsorbed with kaolin (12), followed by adsorption with rhesus monkey red cells. The adsorption with the red cells was performed with 10^8 packed cells for 1 hr in a 37 C water bath. The fluorescent-antibody titers were measured by the indirect test with various dilutions of the test sera and undiluted fluorescein-labeled rabbit anti-guinea pig globulin (from Antibodies, Inc., Davis, Calif.). A 1-ml amount of each serum, at a dilution of 1:16, was first adsorbed three times with 3×10^7 to 6×10^7 uninfected HeLa cells which had been frozen at −25 C for several weeks. The adsorptions were performed for 30 min in a 37 C water bath. One cover slip culture with primary infection by standard Edmonston virus was used to test each serum dilution. The fluorescent-antibody titer was defined as the highest dilution which resulted in fluorescent staining of the infected cells after further treatment with the conjugate. No fluorescent-antibody staining was observed either in uninfected cultures treated with each of the adsorbed immune sera or in infected cultures treated with any of the preinjection sera at a 1:16 dilution.

RESULTS

Persistent infection in K11-HG. Persistent infection has been maintained, to date, in K11-HG after 111 subcultures (more than 300 cell generations) in medium containing measles antibody. The viral properties at different passage levels of K11-HG are shown in Table 1. For comparison, the viral properties at certain passage levels of the parent K11 line, grown in antibody-free medium, are included. The amount of infectious virus recovered from the culture fluid and trypsinized cells of K11-HG passed serially 34 times in antibody-containing medium and subsequently 7 times in antibody-free medium was essentially the same as the yields from the parent K11 line. The amount recovered from extracts of K11-HG was somewhat lower than the amount from extracts of K11. The degree of hemadsorption in fixed cell cultures of K11-HG after these passages was the same as in fixed cell cultures of K11. After 53 subcultures of K11-HG, little or no infectious virus was recovered from any of the test materials. Criteria for the lack of infectious virus in K11-HG after these passages included the absence of viral immunofluorescence, hemadsorption, and CPE in the normal HeLa cultures inoculated with the test materials. Moreover, the test HeLa cultures, 95 days after inoculation of materials from K11-HG which had been serially passaged 78 times in antibody-containing medium and subsequently 5 times in antibody-free medium, were still susceptible to infection by about 100 $TCID_{50}$ of standard Edmonston virus.

Hemadsorption tests also demonstrated a change in the virus-host cell relationships of K11-HG, as shown by a decrease in the degree of hemadsorption, from +++ aften 34 serial passages to + after 53 serial passages. However, the pattern of intracellular immunofluorescence in K11-HG was still similar to that in cells of the parent K11-HG line (15), except that after 43 passages the number of cells which showed coarse granular staining was reduced. The number of cells with intranuclear inclusions was less than 0.2%.

Resistance of K11-HG cultures to CPE by standard Edmonston virus. There was complete resistance of K11-HG cultures to CPE of about 10,000 $TCID_{50}$ of virus, and even with 100,000 $TCID_{50}$ only weak CPE developed within 4 to 11 days and then disappeared within 1 to 2 weeks after its appearance.

Persistent infection in clonal line K11A-HG-1, passage B cultures; attempts to recover infectious virus. It was reported previously (14) that no infectious virus could be recovered from K11A-HG-1 in early subculture in antibody-containing medium (passage A cultures) or in antibody-free medium (passage B cultures), even though up to 60 to 80% of the cells contained cytoplasmic inclusion bodies and showed intracellular viral immunofluorescence. Further studies have been made for infectious virus in K11A-HG-1 with passage B cultures. The nonyielder virogenic state has been maintained, to date, in this population after a total of 92 subcultures. A total

TABLE 1. *Viral properties of K11-HG after subculture in antibody-containing medium (CE-HG medium)*

Passages in CE-HG medium	Passages in CE medium[a]	Per cent of cells with viral inclusions	Per cent of cells with intracellular viral antigen	Hemadsorption	Infectious virus ($TCID_{50}$ per 10^4 cells)		
					Culture fluid	Trypsinized cells	Cell extract[c]
20	0	56	ND[b]	ND	<0.01	2,000	ND
30	0	82	Many	ND	<0.01	2,000	ND
34	7	65	94	+++	1	10,000	5
43	0	78	85	ND	<0.01	47	ND
53	0	74	77	ND	<0.01	<0.5	ND
53	5	85	94	+	<0.01	<0.5	≤0.2
78	5	94	93	+	<0.01	<0.5	<0.01
111	0	ND	94	+	ND	ND	ND
0[d]	51	56	95	+++	4	16,000	150
0[d]	81	67	99	+++	≤1.5	5,000	32

[a] K11-HG line. Number of side passages of sample of cells in CE medium prior to tests.

[b] ND = not done.

[c] Combined homogenization and freezing and thawing was used for preparation of all cell extracts with the exception of extracts from K11-HG cells after 78 subcultures in CE-HG medium and 5 subcultures in CE medium. Extracts from K11-HG cells after these passages were prepared by homogenization only.

[d] K11 parent line.

volume of 50 ml of culture fluid, 5,000,000 trypsinized cells, and 20,000,000 cell equivalents have been tested. The test materials were obtained at a number of different passage levels. Each was tested in normal HeLa and in primary human amnion cultures; trypsinized cells were also tested in the stable BS-C-1 monkey kidney line (11). The normal HeLa cultures inoculated with each of the test materials from certain passages of the K11-HG-1 population were examined for infection by immunofluorescence, staining for inclusion bodies, and hemadsorption, in addition to the appearance of CPE. The inoculated BS-C-1 cultures were examined for infection by hemadsorption and the appearance of CPE. The inoculated HeLa cultures, in some experiments, were then challenged after 57 to 90 days with standard Edmonston virus. They were still susceptible to infection by 25 to 100 $TCID_{50}$ doses of virus.

Three lines of evidence were obtained which show that the failure to detect infectious virus in the K11A-HG-1 population was not due to the production of interferon. First, Clark performed tests for the presence of an interferon-type inhibitor in the culture fluid using measles virus as challenge virus in normal HeLa and in primary human amnion, and found negative results when the culture fluid was added to the test cultures 18 to 24 hr prior to inoculation of virus. Secondly, Clark also found that K11A-HG-1 cultures were just as susceptible as normal HeLa cultures to infection by vaccinia virus which is sensitive to the action of interferon (2, 8). Finally, in the present study, an attempt was made to recover infectious virus from K11A-HG-1 cells after their exposure to actinomycin D, because it has been reported that this antibiotic prevents synthesis of interferon (1, 5, 6). A Blake-bottle culture containing about 40,000,000 cells in log phase was exposed for 2.25 hr to actinomycin C (5 μg/ml). Preliminary studies indicate that viral nucleic acid synthesis is not inhibited in K11A-HG-1 cultures by actinomycin D in this concentration (Kajioka et al., *unpublished data*). A total of 1,000,000 trypsinized cells from the treated cultures were tested in BS-C-1 monkey kidney cells with negative results.

Viral immunofluorescence, inclusion bodies, and viral hemagglutinin. These properties of K11A-HG-1 passage B cultures are presented in Table 2. They are compared with those of a population from the parent K11-HG line which, after 34 subcultures in antibody-containing medium, yielded infectious virus (*see* Table 1) and from which infectious virus has been recovered after an additional 51 subcultures in antibody-free medium. This side passage of K11-HG will be referred to as a yielder K11-HG population.

Not only was there continued synthesis of intracellular viral antigen and formation of inclusion bodies in a majority of the K11A-HG-1 cells, but also this population was similar to the yielder K11-HG population with respect to the percentage of cells with these viral properties (Table 2). It was reported previously (14) that there were relatively large amounts of viral complement-fixing (CF) antigen in cell extracts of both cell lines. The K11A-HG-1 contained 12 CF units and K11-HG, 24 CF units, per 10^6 cell equivalents. K11A-HG-1 differed, however, from K11-HG with respect to the percentage of cells with surface viral antigen and hemadsorption, and the presence of viral hemagglutinin in cell extracts. The ratio of cells with surface viral antigen to those with intracellular viral antigen in the K11A-HG-1 population varied from 0.02 to 0.2, as compared with a ratio of 0.8 to 0.9 for the yielder K11-HG at a given passage level. So far as hemadsorption was concerned, not only was the quantity of cells exhibiting hemadsorption different in the two lines, but even those K11A-HG-1 cells which reacted positively adsorbed fewer red cells than the K11-HG yielder cells.

The pattern of viral intracellular immunofluorescence in K11A-HG-1 cells also differed from that of K11-HG yielder cells. Viral intracellular immunofluorescence in the latter popu-

TABLE 2. *Viral properties of K11A-HG-1 nonyielder and K11-HG yielder HeLa cells persistently infected with the Edmonston strain of measles virus*[a]

Cells	No. of serial passages in CE-HG medium	Passages in CE medium	Per cent of cells with				Hemagglutination by cell extracts[b]
			Viral inclusions	Intracellular antigen staining	Surface antigen staining	Hemadsorption	
K11A-HG-1	12	2–96	67–90	61–94	1.6–15	1–10	0
K11-HG	34	2–58	60–75	74–99	77–86	65–95	17–80

[a] Tests were done with 4- to 7-day-old cultures.

[b] Cell extracts were prepared by combined homogenization and freezing and thawing. Results expressed as hemagglutination units per 10^6 cell equivalents.

lation was like that in cells of the parent K11 (15), but, in the K11A-HG-1 population, viral intracytoplasmic immunofluorescence was limited to brightly stained, discrete bodies in almost all of the cells, and intranuclear staining consisted exclusively of weakly stained, small bodies.

Viral antigenicity of nonyielder passage B K11A-HG-1 cells and K11-HG yielder cells. It seemed likely that if the nonyielder state in the K11A-HG-1 line was associated with a defect in the synthesis of viral hemagglutinin and mature infectious virus, there might be little, if any, hemagglutinating-inhibiting and neutralizing antibody in the sera of animals injected with these cells. If, on the other hand, only the detection of viral hemagglutinin and infectious virus was blocked in the K11A-HG-1 line by a cellular substance(s), then the concentration of antibodies to these antigen(s) in the sera from the injected animals should be similar to the concentrations in sera from animas injected with K11-HG yielder cells.

The results of such a study on the comparative viral antigenicity of nonyielder K11A-HG-1 and K11-HG yielder cells provided evidence that the K11A-HG-1 population was associated primarily with the synthesis of incomplete virus. Thus, as shown in Table 3, three guinea pig anti-K11-HG-1 sera had neutralizing-antibody titers 32- to 256-fold less and hemagglutinin-inhibiting antibody titers of 32- to at least 512-fold less than the respective antibody titers in three guinea pig anti-K11-HG sera. There was no significant difference between the fluorescent-antibody titers. The formation of relatively small amounts of neutralizing antibody after injection of the K11A-HG-1 cells may signify the presence of small amounts of infectious virus in this line which could not be detected under the test conditions. It is also possible that this as well as the hemagglutinin-inhibiting antibody may have been induced by a relatively small number of viral envelopes devoid of nucleoprotein.

Resistance of K11A-HG-1 passage B cultures to superinfection by standard measles virus. There was complete resistance to 100 to 1,000 $TCID_{50}$ and partial resistance to 10,000 to 100,000 $TCID_{50}$ of virus. The latter doses induced weak to moderately strong CPE which appeared within 2 to 4 days and then disappeared about 3 to 4 weeks later. In one experiment by Clark, cultures inoculated with 50,000 $TCID_{50}$ of standard Edmonston virus were examined for infectious virus in the culture fluid at 2, 4, 6, and 11 weeks after inoculation. Infectious virus was recovered in amounts of 300 to 400 $TCID_{50}$ per 0.5 ml at 2, 4, and 6 weeks, but none was recovered at 11 weeks after inoculation.

Persistent infection in K11A-HG-1 passage A cultures. The percentage of cells with intracellular viral immunofluorescence, inclusion bodies, and hemadsorption in passage A was about the same after 61 subcultures in the antibody-containing medium as in passage B cultures of K11A-HG-1 grown in antibody-free medium. However, two subcultures later, passage A showed only 2% of the cells with viral immunofluorescence. Cultures were then prepared in antibody-free medium, and, after three subcultures in this medium, they were examined for viral immunofluorescence, inclusion bodies, and for their susceptibility to CPE caused by standard Edmonston virus. The cultures appeared to be free from virus as evidenced by the presence of viral immunofluorescence and inclusion bodies and their susceptibility to marked CPE by 10 $TCID_{50}$ of standard virus. It will be recalled that, in the parent K11-HG line from which K11A-HG-1 was derived, persistent infection has been maintained, to date, after 111 serial subcultures or more than 300 cell generations in antibody-containing medium.

Variation in persistent infection of HeLa cells by measles virus in different cell populations grown in the presence of measles antibody. Evidence for variation in persistent infection of HeLa cells by measles virus grown in the presence of measles

TABLE 3. *Viral antigenicity of K11A-HG1 nonyielder and K11-HG yielder HeLa cells infected with the Edmonston strain of measles virus*

Cells injected	Guinea pig serum	Day of bleeding after last injection	Titer of		
			Neutralizing antibody	Hemagglutinin-inhibiting antibody	Fluorescent-antibody staining
K11A-HG-1	86202	17th	1:64	1:8	1:256
	86210	6th	1:16	1:16	1:256
	86211	6th	1:64	<1:4	1:512
K11-HG	86201	17th	1:2,048	1:512	1:1,024
	86207	7th	1:2,048	1:1,024	1:512
	86208	7th	1:4,096	1:1,024	1:512

antibody was obtained after cultivation of K8 and two different populations of K13 cells in the antibody-containing medium. The results (Table 4) show, along with the reported occurrence of persistent infection in K11-HG after 111 subcultures in the presence of viral antibody, that: (i) infected cell populations with presumably similar virus-host cell relationships in antibody-free medium differ from each other with respect to the number of subcultures in which persistent infection can occur in the presence of measles antibody; and (ii) in some cases, as evidenced with the K13 populations, persistent infection in a given clonal line grown in the presence of viral antibody may be dependent upon the previous number of subcultures. The virus-free state observed in K8-HG and K13-HG1 after a relatively few passages in antibody-containing medium was confirmed after their cultivation in antibody-free medium. Both lines were just as susceptible as normal HeLa cells to infection by standard measles virus.

The effect of viral antibody on the virus-host cell relationships in K13-HG2 was similar to that in K11-HG with the following exceptions: the cell population by the 10th subculture was transformed to a nonyielder state with weak hemadsorptive capacity (±), and the percentage of cells with intracellular immunofluorescence and viral inclusion bodies was reduced to about 50%. The parent K13 line after a similar number of passages showed intracellular immunofluorescence and inclusion bodies in about 90% of the cells, and infectious virus was recovered from the culture fluid and cells.

TABLE 4. *Persistent infection of measles virus (Edmonston strain) in HeLa clonal lines K8 and K13 after growth in the presence of measles antibody*

HeLa line in CE-HG medium	Source and passage level (in parentheses) in CE medium	No. of subcultures in CE-HG medium	Persistent infection[a]
K8-HG	K8 (6)	7	+
		12	−
K13-HG1	K13 (10)	9	+
		13	−
K13-HG2	K13 (108)	28	+

[a] Persistent infection was based on one or more of the following viral properties: intracellular antigen, inclusion bodies, and infectivity. Tests for infectious virus were made by inoculating into each of three to six normal HeLa cultures 1 ml of culture fluid and 10,000 to 40,000 trypsinized cells. The cells prior to trypsinization were washed with CE medium to remove residual antibody and suspended after trypsinization in CE medium.

DISCUSSION

These studies show that, if HeLa cells with persistent infection by the Edmonston strain of measles virus (15) are grown in the presence of viral antibody, cells may be obtained which synthesize incomplete virus rather than infectious virus even when cultured thereafter in antibody-free medium. The synthesis of incomplete virus in a clonal population, K11A-HG-1, was evidenced by: (i) failure to recover virus, infectious for any of three types of susceptible cell cultures, from the clonal population despite continued formation of intracellular viral antigen and intracytoplasmic inclusion bodies in a majority of the cells; (ii) the presence of only a few cells with surface viral antigen(s) including hemagglutinin; and (iii) the relatively weak antibody response to viral envelope antigen(s) after injection of the cells into guinea pigs.

It is possible that in the K11A-HG-1 population there was synthesis of small quantities of complete virus as a noncytopathic variant. However, the findings were negative when attempts were made to detect such virus by immunofluorescence, staining for inclusion bodies, and hemadsorption in test HeLa cultures inoculated with various test materials from K11-HG-1 cultures, and by challenge of the inoculated HeLa cultures with standard measles virus.

The reason for the development of HeLa cells persistently infected with incomplete virus is not known. There are at least two possible explanations. First, variant virogenic cells which produce incomplete virus were present in the K11 population, and after growth of K11 cells in the presence of measles antibody there was selective growth of the variant virogenic cells. It was reported in the preceding paper that changes occurred in the virus-host cell relationships of the persistently infected populations after serial subculture in antibody-free medium. It is therefore reasonable to assume that the K11 line comprises a heterogenous population of cells, some of which synthesize incomplete virus in addition to cells which synthesize complete virus. There is morphological evidence which indicates that measles virus may maturate at the cell surface (3, 10). It is likely that the K11 cells which contain surface viral antigen are those which synthesize complete virus. The combination of viral antibody with surface viral antigen(s) of these cells could conceivably decrease their growth rate relative to the growth rate of cells with incomplete virus. The latter might then emerge as clones after plating under antibody and as the dominant, if not the sole, population after a number of generations in

antibody-containing medium, as suggested by the eventual change of the parent K11-HG line from a yielder to a nonyielder population.

It is also possible that HeLa cells persistently infected with complete measles virus may be transformed by specific antibody to cells which synthesize incomplete virus by means of an intracellular antigen-antibody reaction, or an antigen-antibody reaction at the cell surface which suppresses viral maturation, or both. It has been reported that living HeLa cells may take up antibody by pinocytosis (7), and that specific antibody exerts an effect upon the intracellular stages of infection by influenza virus in chick chorioallantioc membrane cells (Uhlendorf, Ph.D Thesis, Univ. Michigan, Ann Arbor, 1957) and of toxic effects by *Staphylococcus* filtrates in cultured human amnion cells (4). The continued suppression of measles virus maturation in the persistently infected HeLa cells might be accompanied or followed by either the induction of cellular repressors which block viral maturation or irreversible damage of virus-cell complexes involved in viral maturation.

ACKNOWLEDGMENTS

The technical assistance of Carolyn Miethe, Josette Gaudreau, and Marcia Cohen is gratefully acknowledged.

This investigation was supported by Public Health Service research grant AI 01675 from the National Institute of Allergy and Infectious Diseases, and by U.S. Army contract DA-49-193-MD-2528.

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