FAILURE TO CLEAVE MEASLES VIRUS FUSION PROTEIN IN LYMPHOID CELLS
A Possible Mechanism for Viral Persistence in Lymphocytes*

By ROBERT S. FUJINAMI AND MICHAEL B. A. OLDSTONE

From the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California 92037

In several model systems (1) used to examine persistent viral infections, cells are clearly infected, yet they may produce only little amounts of infectious virus. One explanation for this phenomenon was furnished by Scheid and Choppin (2) and Homma and Ohuchi (3), whose experiments provided evidence that the infected host tissue or cell lacked an appropriate function for virus maturation, resulting in the failure to produce infectious virions. From their studies using Sendai virus, investigators concluded (4–7) that proteolytic cleavage of one particular virus glycoprotein, the fusion protein, was required for infectivity, biologic function and pathogenicity.

Measles virus, a paramyxovirus that infects humans, is structurally similar to Sendai virus in that measles virus expresses two surface glycoproteins, hemagglutinin (HA) and fusion (F) protein, and shares other common structural features (8). For measles virus infection, these two polypeptides are important in related disease. Typically, in acute infection of humans, the HA and F proteins of measles virus appear on the surfaces of infected cells where they act as immunogens, as recognition sites for antibody, complement, and/or immune lymphocytes (reviewed in 9 and 10). However, unlike acute infection in persistent measles virus infection, subacute sclerosing panencephalitis, both HA and F are usually absent from the cell's surface (11, 12). In such persistent infection, measles virus genome is found in cells of the central nervous system and/or lymphoid tissues. Virus may be recovered from these cells after extensive cocultivation with feeder cells capable of replicating measles virus (13–17). This suggests that although brain or lymphoid cells become infected with measles virus, they lack some function or are defective in such a way that a productive virus infection does not usually occur (6).

To better understand the pathobiology of measles virus infection, we have studied measles virus-lymphoid cell interactions. Lymphoid cells were selected because these cells can harbor viruses during natural acute and persistent infection (17–19). Further aberrant immune responses occur during measles virus infection (20, reviewed 21). We evaluate how the surface glycoproteins HA and F of measles virus function in human lymphoid cells during virus infection. To answer this question, we infected...
several lymphoid cell lines with measles virus and compared their susceptibilities to infection and expression of cell surface viral glycoproteins. We found that some lymphoblasts do not cleave the F protein and produce only small amounts of infectious virus. The defect in F cleavage can be reconstituted by fusing these infected lymphoblasts with uninfected cells possessing the appropriate cleaving enzyme(s) or by treatment with a proteolytic enzyme like trypsin.

Materials and Methods

**Virus.** The Edmonston strain of measles virus was plaque purified twice on Vero cells and passed at a low multiplicity of infection (MOI)—0.05 plaque-forming units (PFU)/cell—in HeLa cells, after which a stock virus pool was made in Vero cells. The passage history of this virus has been reported (11, 22).

**Cell Lines.** The human lymphoblastoid cell lines Daudi, Victor, Seraphine, Raji, Wi-L2, RPMI 8866, and Ramos Tubar 40379, originally obtained from George Klein, Karolinska Institute Stockholm, Sweden, are well-established in culture and were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS).

Nonlymphoid cells HeLa, Vero, and BSC-1 were used, respectively, to compare measles virus replication with that in lymphoid cells, to grow the virus stocks, and to enumerate virus titers. These cell lines were cultured as previously described (22). Expression of measles virus antigen-infected cells was documented by immunofluorescence. The procedure and reagents used are defined (11, 22, 23). Electron microscopy was also performed on such cells (11).

**Radioiodination.** The procedure of Phillips and Morrison was used with some modifications (24). At least $2 \times 10^7$ lymphoblastoid or HeLa cells were washed twice with phosphate-buffered saline (PBS). After centrifugation at 250 g for 10 min, pellets were removed, resuspended in 250 µl of PBS, and transferred to 1.5-ml Eppendorf tubes. 1 U (10 µl) of lactoperoxidase (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) was added to the cells followed by 1 mCi of $^{125}$I (Amersham Corp., Arlington Heights, Ill.) The reaction was initiated by adding 10 µl of 0.03% H$_2$O$_2$. After 2 min, 10 more µl of 0.03% H$_2$O$_2$ was added. The reaction was allowed to proceed for 2 more min and was stopped by dilution to 50 ml with PBS. Cells were washed once with minimum essential medium (MEM), and a 5-µl fraction was counted to ensure that labeling was satisfactory.

**Cell-Cell Fusion.** Uninfected, unlabeled Victor, Raji, or Daudi cells washed twice with serum-free MEM were mixed with surface-labeled infected Daudi cells at a ratio of one infected Daudi cell to two uninfected cells. The cell mixtures were centrifuged at 250 g for 10 min, and the supernatant fluids were discarded. The pellets were gently resuspended in 1 ml of 50% polyethylene glycol 1,000 (J. T. Baker Chemical Co., Phillipsburg, N. J.) and incubated at room temperature for 1 min. 1 ml of serum-free MEM was added, followed after 1 min by another 2 ml of serum-free MEM. 2 min later, 4 ml of MEM supplemented with 20% FBS was added to stop the reaction. Counting with a hemacytometer indicated that ~60–70% of the uninfected, unlabeled Victor, Daudi, or Raji cells were fused with the infected, labeled Daudi cells.

**Enzyme Treatment.** The $2 \times 10^7$ to $4 \times 10^7$ infected, labeled Daudi or HeLa cells used as controls were incubated with various dilutions of TPCK-trypsin or chymotrypsin (Worthington Biochemical Corp., Freehold, N. J.), ranging from 0.01 to 300 µg/ml. Briefly, infected cells were first radiiodinated as described above and then washed once with serum-free MEM and resuspended in 4 ml serum-free MEM containing the selected enzyme. The cell-enzyme mixture was incubated for 15 min at 37°C and then combined with 4 ml of MEM containing 20% FBS. The cells were washed twice with MEM-20% FBS and placed on ice. Finally, the enzyme-treated, labeled viral polypeptides were obtained by immunoprecipitation and analyzed by polyacrylamide gels (see below).

To see whether enzyme treatment of virus could increase infectivity, supernatant fluids harvested from cultures of HeLa, Victor, or Daudi cells 24 h after infection were treated with enzyme in the following manner. Measles virus containing supernates were diluted 1:2 with serum-free MEM containing the appropriate enzyme (trypsin, chymotrypsin, or pronase) (Calbiochem-Behring Corp.) to final enzyme concentrations ranging from 0.1 to 500 µg/ml.
Table I

| Cell line | PFU/ml |
|-----------|--------|
| HeLa      | $1.5 \times 10^6$ |
| Victor    | $2.5 \times 10^5$ |
| Raji      | $2.5 \times 10^5$ |
| Ramos     | $2.0 \times 10^4$ |
| Daudi     | $1.2 \times 10^4$ |

*Cells were infected at an MOI of 1, and 24 h later supernatant fluids were harvested. The amount of measles virus present in fluids was assayed by plaquing on BSC-1 monolayers and enumerated as PFU/ml supernatant fluid.

The virus-enzyme mixture was then incubated for 10 min at 37°C, after which serial tenfold dilutions were made in PBS containing 5% FBS. Infectivity was measured immediately by plaque assay on BSC-1 cells and enumerated as PFU.

**Immunoprecipitation and Electrophoresis.** Enzyme treated, surface-labeled infected lymphoid or HeLa cell pellets were resuspended in 100 μl of human serum containing antibodies to measles virus polypeptides including both HA and F proteins, as published (22). The mixture was placed on ice. After 30 min, the cells were washed once with serum-free MEM and solubilized in 0.5 ml of 2% NP-40 containing 1 mM para-methylsulfonylfluoride in Tris sodium chloride EDTA buffer. This mixture was incubated on ice for 15 min with intermittent vortexing. After the nuclei and insoluble cell debris were pelleted by centrifugation at 1,000 g for 15 min, the supernatant fluids were removed and mixed with 125 μl of a 10% *Staphylococcus aureus* (*Staph A*) suspension to precipitate virus antigen-antibody complexes (22, 25, 26). The complexes bound to the *Staph A* were washed extensively, resuspended in sample preparation buffer with or without 2-mercaptoethanol (ME) (Eastman Kodak Co., Rochester, N. Y.), and immersed in boiling H2O for 2 min. The mixture was then centrifuged at 1,000 g for 15 min to pellet the *Staph A*. The supernatant fluid was removed, and a fraction was counted for radioactivity, after which samples were loaded onto 10.5% sodium dodecyl sulfate polyacrylamide gels (22, 27).

**Plaque Assay.** Materials to be tested were plaqued on BSC-1 cell monolayers (22).

### Results

**Replication of Measles Virus in Lymphoblastoid Cell Lines.** The seven lymphoblastoid cell lines infected with measles virus at an MOI of 1 included Daudi, Victor, Seraphine, Raji, Wi-L2, RPMI 8866, and Ramos (2 × 10⁷ cells assayed). 24 h after addition of virus, cells from the various lines were compared for their ability to express measles virus antigens. By immunofluorescence, virtually 100% of the cells from the seven different lines expressed large amounts of virus antigens on their surfaces.

The supernatant fluids were collected from these measles virus-infected cells and quantitated for the amount of infectious virus present. Four representative lymphoblastoid cell lines are shown in Table I. Infected Victor cells produced the highest titers of infectious virus and Daudi cells produced the lowest. Ramos cells most often produced amounts of infectious virus intermediate in titers. On a per cell basis, Victor cells produced at least ten times as much infectious virus as Daudi cells. These results were confirmed in repeated experiments. Victor, Seraphine, Raji, Wi-L2, and RPMI 8866 produced equivalent amounts of infectious virus.

**Cell Surface Virus Polypeptides.** To study measles virus antigens on the surfaces of infected lymphoblastoid cells, 2 × 10⁷ cells were surface-labeled with ¹²⁵I. The labeled viral polypeptides were then immunoprecipitated and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under nonreducing or reduc-
ing conditions. The various lymphoblastoid cells were compared to HeLa cells (Fig. 1), whose surface polypeptides have recently been characterized (28). With HeLa cells under nonreducing conditions (ME-), most of the HA migrated as a dimer with a 160,000 mol wt, although occasionally some HA was also seen as an 80,000 mol wt monomer. The F₀ protein migrated as a 64,000 mol wt polypeptide. Under reducing conditions (ME+), the HA migrated as an 80,000 mol wt monomer, and F protein now migrated at a 42,000 mol wt position (F₁). With respect to all the lymphoblastoid lines studied, like HeLa cells, most if not all of the HA appeared in a dimeric form that converted to a monomer upon reduction. Similarly, unreduced F₀ protein migrated as a 64,000 mol wt polypeptide in most lymphoblastoid cell lines, i.e., Victor, Raji, (shown in Fig. 1), Wi-L2, RPMI 8866, and Seraphine (not shown), and upon reduction it converted to a 42,000 mol wt moiety. In contrast to these lymphoblastoid cell lines, in Ramos cells ~50% of the F protein appeared in the uncleaved form, and 50% was in the cleaved form, as shown by ME treatment. In contrast to the other cell lines, the F protein from Daudi cells, under both reducing and nonreducing conditions, migrated only as a 64,000 mol wt polypeptide. Thus, the F protein was not cleaved or was poorly cleaved on Daudi cells. The failure to cleave F₀ to F₁ on Daudi cells paralleled a low production of infectious virus in repeated experiments.

Cleavage of the F Protein. To see whether the defect in Daudi cells could be overcome by fusion with other cell lines having the ability to cleave F protein, the following experiment was conducted. Infected, surface-labeled Daudi cells were fused with uninfected Victor, Raji, or Daudi cells. These viable fused cells were then mixed with anti-measles virus antibody to precipitate the surface viral polypeptides. After solubilization and Staph A treatment, the labeled antigens from these complexes (HA and F originally on the infected Daudi ceils) were identified by electrophoretic analysis and quantitated by removing individual bands and determining their radioactive counts. By this assay we determined that well over one-half of the F protein from labeled infected Daudi cells that fused with uninfected Victor cells migrated at the cleaved position, i.e., F₁, 42,000 mol wt. By comparison with Daudi cells plus Raji cells, this value was slightly less than one-half, but with infected Daudi cells plus

![Graph showing surface-labeled measles virus polypeptides.](image)

**Fig. 1.** Surface-labeled measles virus polypeptides. Viral antigens were labeled with ¹²⁵I, immunoprecipitated, and analyzed electrophoretically. ME samples electrophoresed in the absence of reducing agent (ME); ME+ samples containing 1% ME.
uninfected Daudi cells, just 1/10 of the F0 input was cleaved (Table II). Therefore, Victor and Raji cells that ordinarily have the ability to cleave F protein enabled infected Daudi cells (deficient in this cleavage activity) to which they were fused to now cleave the F protein.

We next determined whether enzyme treatment of infected Daudi cells allowed cleavage of the F protein and enhanced production of infectious virus. Surface proteins expressed on viable infected Daudi cells were treated with trypsin (0.01 to 500 μg/ml, incubated at 37°C for 15 min, and the cleavage of F0 to F1 was studied. As shown in Fig. 2, minimal cleavage of the F protein occurred at 0.01 μg/ml, but all of the F0 protein was degraded to F1 after treatment with 1 μg/ml of trypsin. When a concentration of 10 μg/ml of trypsin was used, the HA was degraded. Chymotrypsin

### Table II

**Cleavage of F Protein after Cell-Cell Fusion**

| Fusion of Infected-125I | Uninfected | Cleavage % |
|-------------------------|------------|------------|
| DAUDI                   | DAUDI      | 10         |
| DAUDI                   | RAJI       | 40         |
| DAUDI                   | VICTOR     | 60         |

*Viable, infected Daudi cells were radiolabeled with 125I using the lactoperoxidase method and then fused with uninfected Daudi, Raji, or Victor cells. Fusions were incubated at 37°C for 2 h and thereafter conversion of F0 to F1 was analyzed using immunoprecipitation and 10.5% sodium dodecyl sulfate gels.*

![Fig. 2. Autoradiogram of 125I surface-labeled measles virus polypeptides immunoprecipitated and analyzed electrophoretically. Lane C depicts infected Daudi cells incubated without trypsin. The next lane represent cells incubated with 0.01 μg trypsin/ml, and other lanes represent tenfold increases in trypsin concentration.]
had no effect on cleavage of F₀ to F₁ because untreated and chymotrypsin-treated, surface labeled, infected Daudi cells reacted similarly over the same range of enzyme concentrations used above. However, with 10 μg/ml or greater of chymotrypsin, a reduction of both the HA and F₀ was observed.

Production of Noninfectious Virus. Electron microscopy studies revealed that 24 h after infection with measles virus, Daudi, Victor, and Raji cells all contained nucleocapsid in their cytoplasms, and virus particles were budding from all of these cells' plasma membranes. The amount of virus budding from Daudi cells appeared no different than the amount budding from Victor to Raji cells, yet the infectious virus made by Daudi cells was 1/10 that of Victor or Raji (Table I). This suggested that the defective production of infectious virus from Daudi cells might also be related to the inability to cleave F₀ to F₁. To test this possibility, we treated measles virus harvested from Daudi cells with trypsin. As shown in Table III, trypsin increased the infectivity of Daudi-grown virus by almost 2 logs compared with that of the untreated virus from these cells. This increased titer caused by trypsin treatment approximated that of HeLa- or Victor-grown virus. In contrast to its effect on Daudi cells, at none of these concentrations did trypsin increase the titer of measles virus grown in Victor or HeLa cells, although concentrations of >100 μg/ml decreased the infectivity of virus from any of the three cell lines. Subjecting Daudi- or HeLa-grown measles virus to chymotrypsin (100 μg/ml or less) neither increased nor decreased the ordinarily observed titers of virus. Daudi-grown measles virus was shown to increase in infectivity by ~50% when incubated with pronase (50 μg/ml), whereas HeLa-grown virus actually decreased by 50%. These experiments indicate that trypsin can cleave the F protein on cells or virions, rendering the F protein functional and, thus, fully capable of fusing with and infecting other cells. In contrast, chymotrypsin lacked this ability.

Lack of Temperature-sensitive Mutants. It was previously reported (29) that lymphoblastoid cell lines persistently infected with measles virus generated temperature-sensitive mutants. To check for this possibility in our system, supernatant fluids from infected HeLa, Victor, and Daudi cells were plaqued on BSC-1 monolayers at 33°C or 39°C. No difference in titers was found between the cultures incubated at 33°C and those incubated at 39°C.

Discussion

We demonstrated that several human lymphoblastoid cell lines vary in their ability to produce infectious measles virus and that the amount of infectious measles virus

| Table III |
|-----------|

| Enzyme             | Source of virus |
|--------------------|-----------------|
|                    | Daudi | HeLa |
| Trypsin            | 3.5 × 10⁴ | 9.2 × 10⁴ |
| Chymotrypsin       | 4.0 × 10⁴ | 1.1 × 10⁴ |
| Pronase            | 3.6 × 10⁴ | 4.0 × 10⁴ |
| None               | 7.3 × 10⁴ | 1.4 × 10⁴ |

* Supernatant fluids from infected Daudi or HeLa cells were treated with 50 μg trypsin, chymotrypsin, or pronase/ml for 10 min at 37°C. Serial dilutions were made and these were assayed for infectivity on BSC-1 monolayers.
produced directly correlates with their ability to cleave the F protein. Victor, Raji, Seraphine, Wi-L2, and RPMI 8866 lymphoid cells produce the most infectious virus and readily cleave F₀ to F₁. The quantities of infectious virus made and F protein cleaved were roughly equivalent in these various cell lines. Ramos lymphoid cells made significantly less virus than Victor, Raji, Seraphine, Wi-L2, and RPMI 8866 cells and cleaved quantitatively one-half as much or less F₀ to F₁. Furthermore, Daudi cells produced the lowest titers of virus of any lymphoid cell lines studied and correspondingly cleaved the least F protein. Thus, Daudi lymphoid cells are markedly defective and Ramos lymphoid cells partially defective in their capacity to process the F protein, and this results in a lower production of infectious virus. Cleavage of the F protein of other paramyxovirus leads to the acquisition of infectivity; the result is cell fusion and hemolysis due to conformational changes in the protein (30). It has recently been suggested (31, 32) that both Daudi and Ramos cells represent a less differentiated lymphoid cell population than Raji, Wi-L2, or RPMI 8866. Daudi and Ramos cells that express IgM on their surfaces can be made to express IgG or IgD when given T cell help or infected with Epstein Barr virus, respectively (31, 32).

We next determined whether the inability of Daudi lymphoid cells to cleave F₀ to F₁ was due either to cells lacking appropriate enzyme(s) to cleave F protein or because Daudi cells contained the enzyme but also had an inhibitor that blocked its activity. By fusing surface-labeled, infected Daudi cells with uninfected Victor cells, we found that Victor cells could provide the needed enzyme(s) or functional capacity to cleave the F protein. Therefore, Daudi cells do not contain an inhibitor that interferes with processing of the F protein but are deficient in the enzyme(s) to cleave F protein. These results also suggest that the cleavage event of F protein can occur in close proximity to the infected cell's plasma membrane because the lactoperoxidase labeling technique used is restricted to tyrosine residues exposed at the cell's surface and does not label proteins on the inner surface of a plasma membrane (22, 28). In these experiments, fusion of membranes from two cells was required to cleave the F protein on the Daudi cell surface. Mixing the two cells types together without fusing was not sufficient to cleave the F protein. The results complement those of Silver et al. (33), who demonstrated that adsorption of noninfectious Sendai virus to the surfaces of cells with the ability to cleave F protein did not activate the virus and that the loss of proteolytic activity in monkey kidney cells with serial passage correlated with the inability to make infectious Sendai virus.

The deficiency of Daudi cells for an enzyme to cleave measles virus F protein did not interfere with the assembly or release of budding virus particles. However, the particles produced were largely not infectious. Electron microscopic study of Daudi, Victor, and Raji lymphoid cells 24 h after measles virus infection indicated that all these cells synthesized abundant quantities of nucleocapsids within the cytoplasm and had large amounts of budding virus on their plasma membrane. After being treated with the appropriate enzyme needed to cleave F₀ to F₁, supernatant fluids harvested from Daudi lymphoblastoid cells contained amounts of infectious virus equivalent to those shed by HeLa or Victor cells. These results, in concert with the lactoperoxidase labeling experiments, support the notion that cleavage of F protein can occur as a late event in the course of infection, occurring after F protein is synthesized and transported to the plasma membrane. Our data does not exclude the possibility that cleavage of the F protein may occur within the confines of vesicles transporting F protein to the
cell surface in a suitable cell (34). The small amount of infectious measles virus found in association with infected Daudi cells suggests that Daudi cells do not have an absolute lack of the necessary cleavage enzyme for F protein. Alternatively, such cleavage of F protein may result from enzymes present in FBS (6). These two possible explanations are being studied in current experiments.

We analyzed the specificity of enzymes that could cleave the F protein of measles virus using both biochemical and biological assays. Infected and 125I surface-labeled Daudi cells were treated with varying concentrations of trypsin. Amounts of \( \geq 0.1 \mu g \) cleaved the F protein, as shown by migration on polyacrylamide gel. Chymotrypsin did not cleave the F protein into a functional moiety over a wide concentration range (0.01–500 μg/ml), although at high concentrations both HA and F were lost from the cell surface. In addition, measles virus harvested from the supernates of infected Daudi lymphoid cells was also treated with trypsin, chymotrypsin, or pronase. Treatment with trypsin markedly enhanced virus infectivity (Table III). In contrast, chymotrypsin did not enhance infectivity titers at a low concentration (0.1 μg/ml) but decreased infectivity when used in a higher concentration (500 μg/ml). Pronase treatment increased the infectivity of measles virus harvested from Daudi lymphoid cells slightly but decreased infectivity of measles virus grown in HeLa cells. Norrby and Gollmar (35) reported that trypsin treatment of measles virus produced particles deficient in hemagglutinin but having normal levels of F protein. Others (36) have reported that paramyxovirus spread, giant cell, and syncytia formation are blocked with antibodies to F protein. These previous results together with our results support the conclusion that cleavage of the F protein is necessary for biological activity of measles virus.

What are the biological implications of our findings? Measles virus acutely infects a variety of cells in vivo during natural infection but persists in lymphoid and neuronal cells (13–19). Furthermore, in vitro studies show that measles virus can replicate in human macrophages, B lymphocytes, T lymphocytes and their subsets, and persist in some of these cells and in cultured lymphoid lines (37–40). An implication of our findings is that measles virus infection of specific type(s) of lymphoid cells or subpopulations may lead to persistence in the host in cells that lack or are deficient in the enzyme that cleaves F protein. Because many lymphocytes are long-lived cells and circulate throughout the body, such an infected lymphocyte with nonfunctional F protein on its surface would contain measles virus genome but persist many years after initial infection. Furthermore, the infected lymphocyte might traffic to distant sites and perhaps ultimately to an area of inflammation where proteolytic enzymes were locally present. In this area, proteolytic enzymes might cleave the F protein, yielding a functional molecule on the plasma membrane. The infected lymphocytes would then fuse with other susceptible cells in the local area, resulting in a new round of virus replication. Finally, resting peripheral blood lymphocytes infected with measles virus do not express viral antigens even though the viral genome is present within the cell. These cells must be stimulated with mitogens before the virus or antigens are detected (37–40). Perhaps during mitogen or antigen-specific activation of lymphocytes, enzymes needed for the production of mature virions are turned on. Once available, such enzymes could process viral F₀ and F₁, leading to a lytic infection with resultant release of infectious virus and subsequent death of the lymphocyte or partial expression of viral components leading to a persistent infection.
Such a mechanism might play a role in the lack of appropriate immune response to tuberculin seen during measles virus infection (20), presumably because the antigenic-specific responder cell is deleted.

Summary

The host-directed cleavage of measles virus fusion protein on infected lymphoid cells was studied to understand the mechanism of viral persistence in lymphoid cells in vivo. Several lymphoblastoid cell lines were infected with measles virus, and the viral glycoproteins expressed on the cell’s surface were radiolabeled and analyzed for cleavage of fusion (F₀) to F₁ by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Daudi and Ramos lymphoblastoid cells were deficient in their ability to cleave measles virus fusion protein and correspondingly produced low titers of infectious measles virus, Daudi cells being more defective than Ramos cells. In contrast, other lymphoblastoid cells studied, Victor, Raji, Wi-L2, RPMI 8866, and Seraphine, cleaved the fusion polypeptide and made significantly more infectious virus. Despite their defect in cleaving F protein, Daudi cells were able to assemble and release (noninfectious) measles virus particles into the fluid phase. The deficit in Daudi cells was corrected by fusing infected Daudi cells with cleavage-competent cells such as Victor or Raji. Furthermore, the cleavage event performed by competent cells could be mimicked at the plasma membrane by treating infected Daudi cells with trypsin, implicating the role of a plasma membrane enzyme in cleaving F₀ to F₁ during measles virus infection. Hence, lymphoid cells deficient in the plasma membrane enzyme required to cleave F protein are permissive for measles virus, maintain viral gene products, produce mostly noninfectious virus, and fail to place the biologic activity F₁ protein on their surfaces.

The authors are indebted to Dr. Evelyn Bounnaud-Toulze and Dr. Peter Lampert for their help with studies involving electron microscopy, to Ms. Alicia Cross for excellent technical assistance, and to Mrs. Susan Edwards for manuscript preparation.

Received for publication 8 June 1981 and in revised form 4 August 1981.

References

1. Stevens, J. G., G. J. Todaro, and C. F. Fox, editors. 1970. Persistant Viruses. Academic Press, Inc., New York.
2. Scheid, A., and P. W. Choppin. 1974. Identification of the biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. Virology. 57:475.
3. Homma, M., and M. Ohuchi. 1973. Trypsin action on the growth of Sendai virus in tissue culture cells. III. Structural differences of Sendai viruses grown in eggs and tissue culture cells. J. Virol. 12:1457.
4. Homma, M. 1971. Trypsin action on the growth of Sendai virus in tissue culture cells. I. Restoration of the infectivity for L-cells by direct action of trypsin on L-cell-borne Sendai virus. J. Virol. 8:619.
5. Homma, M. 1972. Trypsin action on the growth of Sendai virus in tissue culture cells. II. Restoration of the hemolytic activity of L-cell-borne Sendai virus by trypsin. J. Virol. 9:829.
6. Scheid, A. 1976. Activation of parainfluenza viruses through host-dependent cleavage of an envelope glycoprotein. In Animal Virology. A. S. Huang and C. F. Fox, editors. Academic Press, Inc., New York. 4:457.
7. Homma, M. 1975. Host-induced modification of Sendai virus. *In Negative Strand Viruses*. B. W. J. Mahy and R. D. Barry, editors. Academic Press, Inc., London. 685.

8. Choppin, P. W., and R. W. Companis. 1975. Reproduction of paramyxoviruses. *In Comprehensive Virology*. H. Fraenkel-Conrat, and R. R. Wagner, editors. Plenum Press, New York. 95.

9. Sissons, J. G. P., and M. B. A. Oldstone. 1980. Antibody mediated destruction of virus infected cells. *Adv. Immunol.* 29:209.

10. Sissons, J. G. P., and M. B. A. Oldstone. 1980. Killing of virus-infected cells by cytotoxic lymphocytes. *J. Infect. Dis.* 142:114.

11. Lampert, P. W., B. S. Joseph, and M. B. A. Oldstone. 1976. Morphological changes of cells infected with measles or related virus. *In Progress in Neuropathology*. H. M. Zimmerman, editor. Grune & Stratton, Inc., New York. 51.

12. Iwasaki, Y., and H. Koprowski. 1974. Cell to cell transmission of virus in the central nervous system. I. Subacute sclerosing panencephalitis. *Lab. Invest.* 31:187.

13. Morgan, E. M., and F. Rapp. 1977. Measles virus and the associated diseases. *Bacteriol Rev.* 41:636.

14. terMeulen, V., M. Katz, and D. Muller. 1972. Subacute sclerosing panencephalitis: a review. *Curr. Top. Microbiol. Immunol.* 57:1.

15. Horta-Barbosa, L., D. A. Fuccillo, W. T. London, J. T. Jabbour, W. Zeeman, and J. L. Sever. 1969. Isolation of measles virus from brain cell cultures of two patients with subacute sclerosing panencephalitis. *Proc. Soc. Exp. Biol. Med.* 125:272.

16. Payne, F. E., J. V. Baublis, and H. H. Itohashi. 1969. Isolation of measles virus from cell cultures of brain from a patient with subacute sclerosing panencephalitis. *N. Engl. J. Med.* 281:585.

17. Horta-Barbosa, L., R. Hamilton, B. Witteg, D. A. Fuccillo, and J. L. Sever. 1971. Subacute sclerosing panencephalitis: isolation of suppressed measles virus from lymphnode biopsies. *Science (Wash. D. C.).* 1973:840.

18. Robbins, S. J., H. Wrozos, A. L. Kline, R. B. Tenser, and F. Rapp. 1981. Presence of cytopathic paramyxovirus from peripheral blood leukocytes in subacute sclerosing panencephalitis. *J. Infect. Dis.* In press.

19. Wrozos, H., J. Kulecycika, Z. Laskowski, D. Matacz, and W. J. Brzosko. 1979. Detection of measles virus antigen(s) in peripheral lymphocytes from patients with subacute sclerosing panencephalitis. *Arch. Virol.* 60:291.

20. Von Pirquet. 1913. *Das bild der masern ouf der ausseren haut.* Springer, Berlin.

21. Huddleston, J. R., P. Lampert, and M. B. A. Oldstone. 1979. Measles virus infection of T lymphocyte population. *In Virus-Lymphocyte Interactions: Implications for Disease*. M. R. Proffitt, editor. Elsevier North-Holland, Inc., New York. 251.

22. Fujinami, R. S., and M. B. A. Oldstone. 1980. Alterations in expression events in antibody-induced antigenic modulation. *J. Immunol.* 125:778.

23. Joseph, B. S. and M. B. A. Oldstone. 1975. Immunologic injury in measles virus infection. II. Suppression of immune injury through antigenic modulation. *J. Exp. Med.* 142:864.

24. Phillips, D. R., and M. Morrison. 1970. The arrangement of proteins in the human erythrocyte membrane. *Biochem. Biophys. Res. Commun.* 40:284.

25. Kessler, S. W. 1975. Rapid isolation of antigens from cells with a Staphylococcal protein A-antibody adsorbent; parameters of the interaction of antibody-antigen complexes with protein A. *J. Immunol.* 115:1617.

26. Kessler, S. W. 1976. Cell membrane antigen isolation with the Staphylococcal protein A-antibody adsorbent. *J. Immunol.* 117:1482.

27. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680.

28. Sissons, J. G. P., N. R. Cooper, and M. B. A. Oldstone. 1979. Alternative complement
pathway mediated lysis of measles virus infected cells—induction by IgG antibody band to individual viral glycoprotein and comparative efficiency of F(ab')2 and Fab' fragments. J. Immunol. 123:2144.

29. Ju, G., S. Udem, B. Rager-Zisman, and B. R. Bloom. 1978. Isolation of a heterogeneous population of temperature-sensitive mutants of measles virus from persistently infected human lymphoblastoid cell lines. J. Exp. Med. 147:1637.

30. Hsu, M., A. Scheid, and P. W. Choppin. Activation of the Sendai virus fusion protein (F) involves a conformational change with exposure of a new hydrophobic region. J. Biol. Chem. In press.

31. Kishimoto, T., T. Hirano, T. Kuritani, T. Yamamura, P. Ralph, and R. A. Good. 1978. Induction of IgG production in human B lymphoblastoid cell lines with human T cells. Nature (Lond.). 271:756.

32. Spira, G., P. Aman, A. Koide, G. Lundin, G. Klein, and K. Hall. Cell surface immunoglobulin and insulin receptor expression in an EBV negative lymphoma cell line and its EBV converted sublines. J. Immunol. In press.

33. Silver, S. M., A. Scheid, and P. W. Choppin. 1978. Loss on serial passage of adhesive monkey kidney cells of proteolytic activity required for Sendai virus activation. Infect. Immun. 20:235.

34. Palade, G. 1975. Intracellular aspects of the process of protein synthesis. Science (Wash. D. C.). 189:347.

35. Norrby, E., and Y. Golimar. 1975. Identification of measles virus-specific hemolysis-inhibiting antibodies from hemagglutinating-inhibiting antibodies. Infect. Immun. 11:231.

36. Merz, D. C., A. Scheid, and P. W. Choppin. 1980. Importance of antibodies to the fusion glycoprotein of paramyxoviruses in the prevention of spread of infection. J. Exp. Med. 151:275.

37. Joseph, B. A., P. W. Lampert, and M. B. A. Oldstone. 1975. Replication and persistence of measles virus in defined subpopulations of human leukocytes. J. Virol. 16:1638.

38. Huddleston, J. R., P. W. Lampert, and M. B. A. Oldstone. 1980. Virus-lymphocyte interactions: infections of T₀ and T₅ subsets by measles virus. Clin. Immunol. Immunopathol. 15:502.

39. Sullivan, J. L., D. W. Barry, S. J. Lucas, and P. Albrecht. 1975. Measles infection of human mononuclear cells. I. Acute infection of peripheral blood lymphocytes and monocyte. J. Exp. Med. 142:773.

40. Lucas, C. J., J. C. Bels-Postma, A. Rezee, and J. M. D. Galama. 1979. Activation of measles virus from silently infected human lymphocytes. J. Exp. Med. 148:940.