PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 167, 83–86 (1981)

# The Development of Balb/c Cells Persistently Infected with Respiratory Syncytial Virus: Presence of Ribonucleoprotein on the Cell Surface[1] (41129)

BRUCE F. FERNIE,[2] EUGENIE C. FORD, AND JOHN L. GERIN

*Division of Molecular Virology and Immunology, Department of Microbiology, Georgetown University Schools of Medicine and Dentistry, Rockville, Maryland 20852*

*Abstract.* Balb/c mouse cells were persistently infected with RS virus. Continued passage of the virus produced in these Balb/c cells resulted in a 10-fold increase in the amount of infectious virus produced. Approximately 80% of the BCH4 cells expressed RNP and "spike" antigen intracellularly and associated with the cell surface.

Respiratory syncytial (RS) virus is a leading cause of lower respiratory disease in young children (1). Although classified as a *pneumovirus* of the paramyxoviridae family (2), RS virus lacks functional markers, such as hemagglutinin or neuraminadase (3), which are associated with other members of this family. It is expected, by analogy with other members of the paramyxoviridae family (4), that a virus-specified, membrane-associated protein is involved in cell fusion, viral maturation, and infectivity.

In view of the importance of such a fusion (F) protein in the cell to cell spread of SV5 (5) and the serious consequences of vaccination with formalin-inactivated RS virus (6) presumably containing nonantigenic F protein (7), the search for the RS "F" protein has intensified. Due to the highly cell-associated (8) nature of RS virus, it was felt that infected cells might provide a better source of RS "F" protein. Attempts were made to establish a reliable source of cell-associated viral antigen. We describe here the development of a persistently infected line of Balb/c cells which produce low levels of virus and express both "spike" and RNP antigens on the cell surface.

**Materials and Methods.** *Cells.* HeLa Ohio and HEp-2 cells were obtained from Flow Laboratories (McClean, Va). A cell line, originally derived from a Balb/c mouse embryo, was obtained from Dr. John Lee (Frederick Cancer Research Center, Frederick, Md.) at passage No. 190. All cell lines were grown in antibiotic-free medium consisting of 45% basal medium (Eagle) with Hanks' salts (Flow Laboratories), 45% minimum essential medium (Eagle) with Earle's salts (Flow Laboratories), 10% heated (56°/45 min) fetal bovine serum (Irving Scientific, Santa Anna, Calif.), and 2 m*M* glutamine (Flow Laboratories). The cell lines are routinely tested for and kept free of mycoplasma contamination.

*Virus.* The Long strain of RS virus was originally obtained from Dr. Robert Chanock (NIH, Bethesda, Md.). RS virus was grown in HeLa cells and plaque-assayed on HEp-2 cells as previously described (9).

*Antisera.* Antiserum to RS "spike" protein was prepared in rabbits using the glycoprotein fragments obtained by treating double isopycnically banded RS virus with bromelain (10). This antiserum had a neutralization titer of 1/6400 as determined by plaque reduction following incubation at 37° for 30 min. Antiserum to the ribonucleoprotein (RNP) complex was prepared in rabbits using cesium chloride-purified, cellular RNP (11). This anti-RNP did not neutralize RS virus. An antiserum against cell-associated viral antigens was prepared in mice using 0.2% glutaraldehyde-fixed RS-infected HeLa cells[3] (mouse anti-RS/HO).

[1] This work was supported by Contract NO1-AI-82572 between Georgetown University and the National Institute of Allergy and Infectious Diseases.

[2] To whom requests for reprints should be sent at: Division of Molecular Virology and Immunology, Georgetown University, 5640 Fishers Lane, Rockville, Md 20852.

[3] P. Cote *et al.*, submitted for publication.



0037-9727/81/050083-04$01.00/0

This antiserum had a neutralization titer of 1/3200. The antisera were adsorbed with rabbit liver powder and uninfected HeLa cells. The mouse antiserum was also adsorbed with Balb/c cells. The rabbit and mouse antisera were heated prior to use for 45 min at 60 and 56°, respectively.

*Immunofluorescence.* Indirect immunofluorescence studies were performed on live or acetone-fixed cells[3] (13). FITC-labeled goat anti-rabbit γ-globulin (Meloy Laboratories, Springfield, Va.) and FITC-labeled goat anti-mouse γ-globulin (Cappel Laboratories, Cochranville, Pa.) were adsorbed with equal volumes of RS-infected HeLa cells and clarified before use.

*Persistently infected cells.* Confluent monolayers of Balb/c cells in 32-oz bottles were infected with RS virus at an input multiplicity of 1. The cells were incubated at 37° for 2 hr, rinsed, and refed with culture medium containing 2% FBS. Four days after infection, the cells showed no sign of syncytia formation or microscopic viral cytopathology. These cells were removed with 0.1% EDTA in Hanks' salt solution without magnesium or calcium, passaged into plastic T75 flasks (Costar, Bellco Glass Co., Vineland, N.J.), and designated BCH01. After the BCH01 cells had been passaged at biweekly intervals for 3 weeks, culture fluid from these cells was used to infect Balb/c cells. Confluent monolayers of Balb/c cells in T75 flasks were infected with 3 ml of culture fluid from BCH01 cells. These cells, designated BCH2, were passaged as described above. In a like manner, culture fluid from BCH2 cells was used to establish the BCH3 cell line, and fluid from BCH3 cells was used to establish the BCH4 cell line.

**Results.** The BCH01 cells showed no morphologic sign of RS infection. Culture fluid was tested for infectious virus by plaque assay on HEp-2 cells and acetone-fixed cells were examined by indirect immunofluorescence for the presence of viral antigens. Preliminary evidence indicated that the BCH01 cells were infected and were producing low levels (about 100 PFU/ml) of infectious virus that formed normal syncytia on HEp-2 cells.

Since RS virus has been adapted for suckling mouse brain (14), the culture of BCH01 was continued in an attempt to further adapt the RS virus to growth in Balb/c cells. This adaptation was accomplished by sequentially passing the RS virus in Balb/c cells. As the virus was adapted to Balb/c cells, the character of the infection changed. BCH01 monolayers are characterized by patchy areas of infection with elongated binucleated cells. The pattern of fluorescent staining is generally restricted to granules with some generalized staining (Fig. 1). In BCH4 monolayers the infection is more generalized. The pattern of large granule (RNP) staining is similar, but the pattern of smaller granule ("spike") staining is less prominent, with a concommitant increase in generalized staining.

The concentration of infectious virus increased from 100 to 1000 PFU/ml of culture fluid as the virus was passaged from BCH01 to BCH3 cells. No further increase in viral concentration was found with continued passage of the virus and further passage was not continued after the BCH4 cell line was established.

The BCH4 cell line has been passaged over 50 times and continues to produce low levels of infectious virus. The percentage of infected cells has increased with passage until at present, cell monolayers consist predominantly (estimated to be 80%) of infected cells. Acetone-fixed BCH4 cells contain both RNP and "spike" antigen as do RS-infected HeLa cells (Fig. 2). RNP staining is characterized by variably sized, cytoplasmic inclusion bodies, while "spike" staining results in a diffuse pattern of fluorescence with small granules. Cells stained with the mouse anti-RS/HO display both types of staining. Live infected HeLa cells display "spike" staining on the cell surface but not RNP staining (15). Interestingly, however, live BCH4 cells display a large amount of granular RNP on the cell surface (Fig. 3).

**Discussion.** A line of Balb/c cells has been developed that is persistently infected with RS virus. This cell line contains a large percentage of cells that contain RS "spike" and RNP antigen in the cell and on the cell

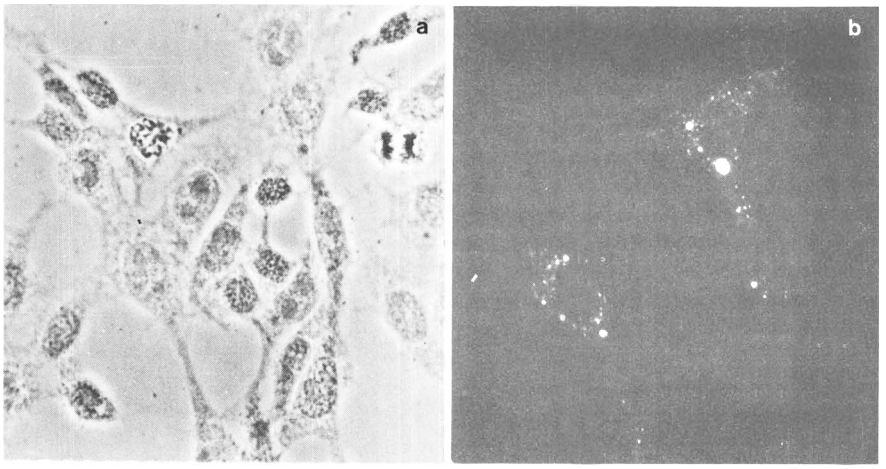


FIG. 1. BCH01 cells at passage level 33 were seeded onto 9 × 22-mm coverslips in 35-mm petri dishes. Two days later, the cells were fixed in acetone (13), incubated with mouse anti-RS/HO, rinsed, and then incubated with FITC-labeled goat anti-mouse γ-globulin. The cells were examined in a Zeiss epifluorescence microscope. (a) Phase contrast; (b) fluorescence. ×400.

membrane. These cells produce low levels of infectious virus that produce normal syncytia when grown in HEp-2 cells. The development of Balb/c cells persistently infected with RS virus should aid the study of RS in several ways. First a convenient and reliable source of RS antigen is now available for the development of assay

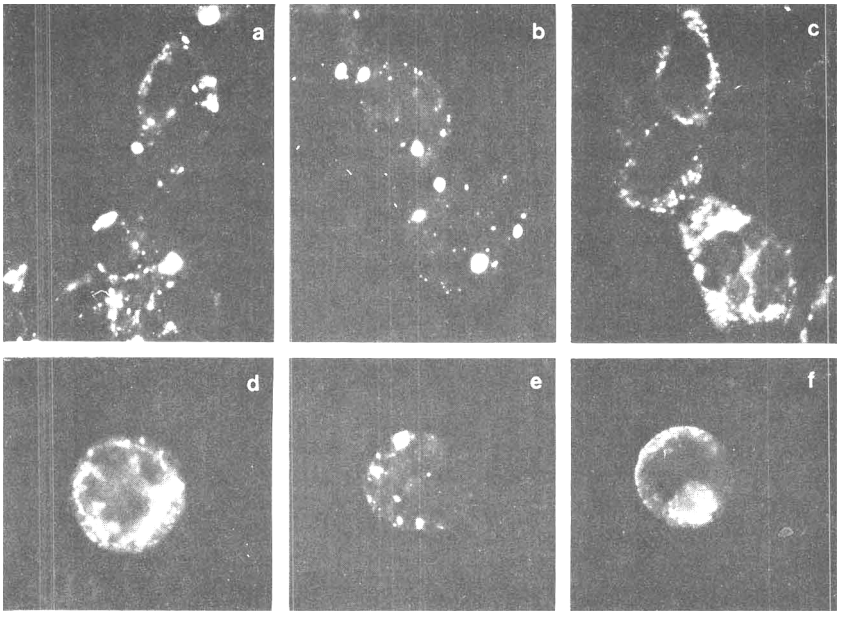


FIG. 2. BCH4 cells at passage level 38 were grown as in Fig. 1. Suspension cultures of HeLa Ohio cells were infected at MOI of 1 with RS virus. Eighteen hours after infection, the cells were fixed with acetone and stained with the indicated antiserum. BCH4 cells stained with: (a) mouse anti-RS/HO, (b) rabbit anti-RNP, (c) rabbit anti-"spike." RS infected HeLa cells stained with: (d) mouse anti-RS/HO, (e) rabbit anti-RNP, (f) rabbit anti-"spike." ×640.

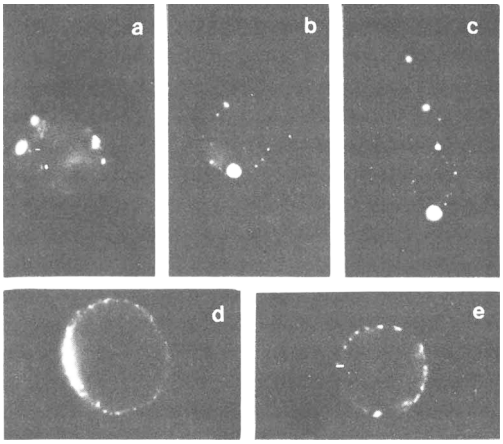


FIG. 3. BCH4 cells were grown as in Fig. 1. HeLa cells were infected as in Fig. 2. Live cells were stained for surface fluorescence as described[3] (12). BCH4 cells stained with: (a) mouse anti-RS/HO, (b) rabbit anti-RNP, (c) rabbit anti-"spike." Infected HeLa cells stained with: (d) mouse anti-RS/HO, (e) rabbit anti-"spike." Live, infected HeLa cells were negative when stained with rabbit anti-RNP. ×640.

systems for RS antibody. A solid-phase radioassay was developed using methanol-fixed BCH4 cells[3] and has greatly facilitated the screening of RS virus-specific hybridoma clones. Since RS virus is normally grown in cells of primate or bovine origin, the possibility of host cell cross-reactivity is greatly diminished by using mouse cells in these assay systems. Although hybridomas specific for RS viral antigens have been successfully raised using RS infected HeLa cells as the immunogen,[3] the use of BCH4 cells should enhance the development of hybridomas to minor RS antigens since the mice will not react with the Balb/c cell components as they do with HeLa cell components. In fact, Balb/c cells were selected for their utility in a Balb/c hybridoma system, without regard for intrinsic sensitivity or resistence to RS infection (16). The mechanism of RS virus persistence in BCH4 cells has not been studied. The development of these cells differs from the development of persistently infected HEp-2 cells (17) in that the level of infectious virus increased with passage in Balb/c cells while it decreased with passage in HEp-2 cells. The presence of an RNP component on the cell surface and the lack of syncytia formation indicates the maturation process of RS in these cells is quite different from that of infected HeLa cells. While the presence of RNP is unusual, it is not without precedent as viral matrix (18) and RNP (19) have been reported on the surface of influenza-infected cells. Further study of these cells should provide a better insight into the maturation process of RS virus and the presence or absence of a distinct fusion protein.

1. Jacobs, J. W., Peacock, D. B., Corner, B. D., Caul, E. O., and Clarke, S. K. R., Lancet 1, 871 (1971).
2. Kingsbury, D. W., Bratt, M. A., Choppin, P. W., Hanson, R. P., Hosaka, Y., ter Meulen, V., Norrby, E., Plowright, W., Rott, R., and Wunner, W. H., Intervirology 10, 137 (1978).
3. Chanock, R., Roizman, B., and Myers, R., Amer. J. Hyg. 66, 281 (1957).
4. Scheid, A. and Choppin, P. W., Virology 57, 475 (1974).
5. Merz, D. C., Scheid, A., and Choppin, P. W., J. Exp. Med. 151, 275 (1980).
6. Kim, H. W., Canchola, J. G., Brandt, C. D., Pyles, G., Chanock, R. M., Jensen, K., and Parrott, R. H., Amer. J. Epidemiol. 89, 422 (1969).
7. Norrby, E., Enders-Ruckle, G., and ter Meulen, V., J. Infect. Dis. 132, 262 (1975).
8. Levine, S., and Hamilton, R., Arch. Gesamte Virusforsch. 28, 122 (1969).
9. Fernie, B. F., and Gerin, J. L., Virology 106, 141 (1980).
10. Hoffman, E. J., Ford, E. C., and Gharpure, M. A., Abstract, ASM Annual Meeting (1974).
11. Hoffman, E. J., Ford, E. C., and Gerin, J. L., Abstract, ASM Annual Meeting (1975).
12. Sternberger, L. A., "Immunochemistry," Prentice-Hall, Englewood Cliffs, N.J. (1974).
13. Tevethia, S. S., Katz, M., and Rapp, F., Proc. Soc. Exp. Biol. Med. 119, 896 (1965).
14. Cavallaro, J. J., and Maassab, H. F., Proc. Soc. Exp. Biol. Med. 121, 37 (1966).
15. Hoffman, E. J., Ford, E. C., and Gerin, J. L., Abstract, ASM Annual Meeting (1977).
16. Prince, G. A., Horswood, R. L., Berndt, J., Suffin, S. C., and Chanock, R. M., Infect. Immunity 26, 764 (1979).
17. Baldridge, P., and Senterfit, L. B., Proc. Soc. Exp. Biol. Med. 151, 684 (1976).
18. Ada, G. L., and Yap, K. L., Immunochemistry 14, 643 (1977).
19. Virelizier, J. L., Allison, A. C., Oxford, J. S., and Schild, G. C., Nature (London) 266, 52 (1977).

Received November 17, 1980. P.S.E.B.M. 1981. Vol. 167.