Pneumoviruses: the Cell Surface of Lytically and Persistently Infected Cells

By J. E. PARRY,* P. V. SHIRODARIA† AND C. R. PRINGLE*‡

* MRC Virology Unit, Institute of Virology, Church Street, Glasgow G11 5JR, Scotland, and † Department of Microbiology and Immunobiology, Grosvenor Road, Queen's University, Belfast BT12 6BN, Northern Ireland

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SUMMARY

Human embryonic lung (MRC-5), feline embryo (FEA), mink lung (MvLu) and monkey kidney (BSC-1) cells infected by respiratory syncytial virus showed characteristic morphological changes when viewed by scanning electron microscopy. The surfaces of respiratory syncytial virus-infected cells developed a profusion of slender filaments after 48 h incubation at 31 °C. Similar changes in surface morphology were observed in BSC-1 cells infected by murine pneumonia virus. Filament production therefore appears to be a common property of pneumoviruses. Filaments were not observed in cells infected with either syncytial and non-syncytial herpes simplex virus, the cytocidal vesicular stomatitis and Batai (Bunyaviridae) viruses, or the focus-inducing rabbit fibroma virus.

Filament production was not observed in cells infected with ts mutants of respiratory syncytial (RS) virus during incubation at the restrictive temperature, or in a persistently infected culture of BSC-1 cells at 37 °C. The persistently infected cells (the RS ts I/BSC-1 line) had some of the characteristics of cells transformed by oncogenic viruses, namely ability to overlap adjacent cells and agglutination by a low concentration of concanavalin A. The pseudo-transformed phenotype was temperature-dependent, however, and suppressed by raising the temperature of incubation to 39 °C. The presence of virus antigen at the cell surface was similarly temperature-dependent in these cells, diminished at high temperature (39 °C) and enhanced at low temperature (31 °C), suggesting that the changes in the host cell were the result of insertion of virus protein into the cell membrane. Evidently, persistent infection by a cytoplasmic virus can produce alterations in the host cell usually associated with transformation by nuclear viruses.

INTRODUCTION

Infection of BSC-1 cells by human respiratory syncytial (RS) virus is accompanied by characteristic changes in the morphology of the cell surface. The surface of infected cells becomes covered by a profusion of slender filamentous processes which can be visualized both by immunofluorescent staining and by scanning electron microscopy. These processes are different in dimensions and aspect from the microvilli which are normal features of the surface of uninfected cells (Faulkner et al. 1976).

We have now examined the surfaces of some other types of cells infected with RS virus and also murine pneumonia virus (PVM), to determine whether these morphological
changes are generally characteristic of infection by pneumoviruses, or specifically of the interaction of RS virus and BSC-1 cells.

Infection of BSC-1 cells with different isolates of RS virus is normally cytocidal; centres of infection develop as foci of heaped cells and only become syncytial late in infection (Faulkner et al. 1976). Under certain conditions, however, RS virus infection of BSC-1 cells can be non-cytocidal and can result in virus persistence (Pringle et al. 1978a, b). These persistently infected cells were resistant to superinfection by RS virus and some other viruses, showed enhanced agglutination by concanavalin A, and had undergone a change in karyotype. All cells exhibited intracytoplasmic antigen, but surface antigen was reduced and processes were absent. Examination of these cells by scanning electron microscopy showed that, in addition to concanavalin A sensitivity, they had acquired an ability to overlap adjacent cells. Thus persistent infection of cultured cells with a non-oncogenic cytoplasmic virus can produce surface alterations usually associated with transformation of cells by nuclear viruses. The appearance of surface antigen and processes was temperature-dependent, however, and this pseudo-transformed phenotype was suppressed when the temperature of incubation was raised.

METHODS

Cells. The BSC-1 line of African green monkey kidney cells, human embryonic lung (MRC-5) cells, secondary feline embryo (FEA) cells, mink lung (Mv1Lu) cells and HeLa cells were propagated in 2-litre roller bottles in medium consisting of Eagle's medium supplemented with twice the normal concentration of amino acids (Glasgow modification) and 10% foetal bovine serum (virus and mycoplasma screened; Gibco Biocult, Glasgow). The MRC-5 cells were obtained from the National Institute for Biological Standards, Hampstead, U.K., the FEA cells from Dr O. Jarrett, Department of Veterinary Pathology, University of Glasgow, the Mv1Lu cells from Dr Janet Cameron of the Institute of Virology and the HeLa cells from Dr V. Mautner of the Institute of Virology, Glasgow.

The origin and characteristics of the RS ts 1/BSC-1 line of persistently-infected BSC-1 cells has been described previously (Pringle et al. 1978a, b). These cells were maintained in plastic tissue culture flasks at 37 °C and subcultured twice weekly by scraping cells into suspension and re-seeding at a dilution of 1:4.

Monolayers for scanning electron microscopy or immunofluorescent staining were prepared by transferring 10^6 cells into glass Petri dishes (50 mm diam.) containing four glass coverslips (13 mm diam.) and incubating at 37 °C for 24 h. Virus infection of these cells was carried out by inoculating a 0.4 ml vol. of RS virus or PVM to give an approximate m.o.i. of 1 p.f.u./cell. The infected coverslips were incubated at 31 °C for 30 min, washed with one change of medium, and then incubated at 31 °C for 48 h in the case of RS virus, or at 31 °C for up to 12 days in the case of PVM.

Virus. The origin of the RSN-2 strain of RS virus has been described previously (Faulkner et al. 1976) and the A2 strain was obtained from Dr Linda Richardson, National Institutes of Health, Bethesda, U.S.A. PVM (strain 15) was obtained from the American Type Culture Collection. All three viruses were cloned by three sequential isolations from single focal plaques on BSC-1 monolayers to obtain genetically homogeneous stocks.

Syn (syncytial) and syn + (non-syncytial) mutants of herpes simplex virus (HSV) types 1 and 2 were obtained from Dr J. H. Subak-Sharpe and Dr Morag Timbury of the Institute of Virology, Glasgow, U.K. Rabbit fibroma virus (RFV) was obtained from Dr W. C. Russell, N.I.M.R., Mill Hill, London, U.K. and cloned in BSC-1 cells. Batai virus (Bunyaviridae) was obtained from the Center for Disease Control, Fort Collins, Colorado, U.S.A., and cloned in BSC-1 cells. The vesicular stomatitis virus (VSV) was the New Jersey M strain.
Immunofluorescent staining. The coverslips for immunofluorescent staining were examined with and without prior acetone fixation. Bovine anti-bovine RS virus serum produced in a gnotobiotic calf was generously provided by Dr J. Stott, Animal Diseases Research Institute, Compton, U.K., and rabbit anti-bovine globulin conjugated with fluorescein isothiocyanate (FITC) was obtained from Nordic Immunology, Maidenhead, U.K. The indirect immunofluorescence staining procedure has been described in detail previously (Faulkner et al. 1976; Pringle et al. 1978b).

Scanning electron microscopy. Infected or uninfected monolayers on coverslips were fixed in 2.5% glutaraldehyde with phosphate buffer for 1 to 2 h. The monolayers were then post-fixed for 1 h in 1% osmium tetroxide with phosphate buffer. The fixed monolayers were dehydrated by transfer through a gradient of 30, 50, 70, 90 and 100% ethanol (5 min in each solution) and placed in a solution of 50% acetone in ethanol for 15 min. This was replaced with 100% acetone (two changes) before drying at the critical point of liquid CO₂ using a Polaran Critical Point Drying Apparatus (Polaron Equipment Ltd., Watford, Herts, U.K.). The dried coverslips were mounted on aluminium stubs using 'Electrodag 915' (Acheson Colloids Company, Plymouth, U.K.) and coated with gold in a Polaron E5000 diode sputtering apparatus. They were examined in a Philips PSEM 500 scanning electron microscope (Philips Electronic Instruments, Eindhoven, Netherlands) at 25 kV. A total of 780 photographs were taken and the 24 illustrations in Fig. 1, 3, 4 and 6 are judged to be representative of the phenomena observed.

RESULTS

The morphology of the surface of cells lytically infected with pneumoviruses

Filament production may be characteristic of pneumovirus infection in general or solely of the interaction of the RSN-2 strain of RS virus and BSC-1 cells. To resolve this question the surface morphology of three other types of susceptible cells has been examined by scanning electron microscopy 48 h p.i. with the RSN-2 strain of RS virus. Uninfected and infected human embryonic lung (MRC-5) cells are illustrated in Fig. 1(a), (b), uninfected and infected secondary feline embryo (FEA) cells in Fig. 1(c), (d) and uninfected and infected mink lung (MvILu) cells in Fig. 1(e), (f).

The uninfected cells show quantitative differences in the distribution and number of microvilli, but otherwise exhibit no exceptional surface features. Morphologically the three cell types are distinguishable from one another, and from uninfected BSC-1 cells (Fig. 4a). The infected cells in all cases exhibit the long slender processes, which are characteristic of RS virus infection of BSC-1 cells (Fig. 4b) after 48 h incubation at 31 °C. The abundance of the filaments differed, however, being most extensive in RS virus-infected FEA (Fig. 1d) and MvILu cells (Fig. 1f), and least in MRC-5 cells (Fig. 1b). The ultrastructural changes observed on the surface of RS virus-infected FEA and MvILu cells are very similar in degree and kind to those observed in BSC-1 cells (Faulkner et al. 1976; Fig. 4b). RS virus infection of HeLa cells also induced filaments, but the effect was masked slightly by the unusual abundance of microvilli on the surface of uninfected HeLa cells (J. E. Parry & C. R. Pringle, unpublished observations).

Filament production can also be revealed by indirect immunofluorescent staining and light microscopy, although reproduction by monochromatic photography is more difficult. Fig 2(a) shows filaments on the surface of unfixed RS virus-infected BSC-1 cells and Fig. 2(b) surface filaments and intracytoplasmic fluorescence in acetone-fixed RS virus-infected BSC-1 cells after 48 h at 31 °C. Fig. 2(c, e and g) show similar filament production in acetone-fixed RS virus-infected FEA, MRC-5 and MvILu cells respectively. As was observed by scanning electron-microscopy the filaments are less numerous in infected
Fig. 1. Scanning electron micrographs of RS virus-infected cells. (a) Uninfected and (b) RS virus-infected MRC-5-cells after 48 h at 31 °C; (c) uninfected and (d) RS virus-infected FEA cells after 48 h at 31 °C; (e) uninfected and (f) RS virus-infected Mv1Lu cells after 48 h at 31 °C.
Fig. 2. Indirect immunofluorescence with bovine anti-RS virus and FITC-conjugated anti-bovine globulin. (a) Unfixed BSC-1 cells infected with wild-type RS virus at 31 °C; (b) acetone-fixed BSC-1 cells infected with wild type RS virus at 31 °C; (c) acetone-fixed FEA cells infected with ts 19 (RSN-2) at 31 °C; (d) as (c) at 39 °C; (e) acetone-fixed MRC-5 cells infected with ts 19 (RSN-2) at 31 °C; (f) as (e) at 39 °C; (g) MvILu cells infected with wild-type RS virus at 31 °C; (h) HeLa cells infected with ts 19 (RSN-2) at 31 °C.
Fig. 3. Scanning electron micrographs of pneumovirus-infected cells. (a) Uninfected BSC-1 cells after incubation for 12 days at 31 °C; (b) PVM-infected BSC-1 cells after 6 days incubation at 31 °C; (c) PVM-infected BSC-1 cells after 9 days at 31 °C; (d) PVM-infected BSC-1 cells after 12 days at 31 °C; (e) persistently infected RS ts1/BSC-1 cells after incubation for 48 h at 37 °C, showing overlapping of adjacent cells; (f) persistently infected RS ts1/BSC-1 cells after exposure to 30 μg/ml concanavalin A for 2 h at 37 °C.
Fig. 4. Scanning electron micrographs of uninfected and RS virus-infected BSC-1 cells, and persistently infected RS ts 1/BSC-1 cells. (a) Uninfected BSC-1 cells after incubation for 48 h at 31 °C; (b) BSC-1 cells infected with mutant ts 1 after 48 h incubation at 31 °C, showing development of filamentous processes; (c) persistently infected RS ts 1/BSC-1 cells after incubation for 48 h at 37 °C; (d) persistently infected RS ts 1/BSC-1 cells after incubation for 48 h at 39 °C; (e) persistently infected RS ts 1/BSC-1 cells after incubation for 48 h at 31 °C; (f) enlargement of another area from (e) to show filamentous processes (fp) and normal microvilli (mv).
MRC-5 cells than BSC-1 or MvI Lu cells. Filaments were fewer, shorter, slimmer and more difficult to resolve in HeLa cells (Fig. 2b).

Infection of BSC-1 cells with two early ts mutants of the RSN-2 strain of RS virus, classified in complementation groups B and E (Gimenez & Pringle, 1978), showed that filament production was a virus-specific phenomenon, since filaments were only observed in cultures incubated at the permissive temperature of 31 °C. Fig. 2(c and d) show BSC-1 cells infected with ts 19 of complementation group E at 31 and 39 °C, and Fig. 2(e, f) MRC-5 cells infected with the same mutant at 31 and 39 °C. At 39 °C few cells show any fluorescence and those which do lack surface filaments; in these particular cells nuclear antigen is visible (Faulkner et al. 1976). Similar morphological changes were observed in BSC-1 and FEA cells infected with the A2 strain of RS virus. Filament production, therefore, is a regular consequence of RS virus infection of susceptible cells.

BSC-1 cells infected with PVM, the only other recognized member of the pneumovirus genus of the paramyxoviridae (Fenner, 1976), were examined to determine whether the induction of filaments was an attribute of pneumoviruses and not just RS virus alone. These observations extended over a period of 12 days, because the c.p.e. induced by PVM in susceptible cells (BSC-1 and BHK-21) at 31 °C develops slowly. Comparison of Fig. 4(a) (uninfected BSC-1 cells after 48 h at 31 °C) with Fig. 3(a) (the same cells after 12 days at 31 °C) shows that little change is discernible in the surface morphology of uninfected BSC-1 cells over the 10-day interval. PVM-infected cells, on the other hand exhibit drastic changes. Filaments are abundant after 6 days incubation at 31 °C (Fig. 3b), becoming very extensive by 9 days (Fig. 3c). At 12 days the filaments appeared to be disintegrating and the cells are rounded, blebbed and fused (Fig. 3d). Examination of cells at intermediate times showed that the filaments are prominent by the third day and that there is a gradual progression of the c.p.e. The rate of progression of the c.p.e. was influenced to some extent by the composition of the medium, but the same sequence of changes was always observed. Although ts mutants of PVM are not available to confirm unequivocally that these changes are virus-specific, it is reasonable to conclude that the modification of the cell surface by production of filaments is a common property of pneumoviruses.

The morphology of the surface of BSC-1 cells persistently infected with RS virus

A persistent infection of BSC-1 cells was initiated by propagation of ts mutant-infected cells at the restrictive temperature of 39 °C (Pringle et al. 1978a, b) and one of these cultures (RS ts 1/BSC-1) has been propagated for more than 100 sequential transfers. All the cells in the RS ts 1/BSC-1 culture carry infectious RS virus and from passage 21 onwards the cultures could be maintained routinely at 37 °C.

The surface morphology of RS ts 1/BSC-1 cells was examined by scanning electron microscopy between passages 80 and 90. The filaments normally associated with RS virus infection are absent. Fig. 4(b) shows filaments induced by mutant ts 1 in susceptible BSC-1 cells at permissive temperature and Fig. 3(e) a typical area from a monolayer of RS ts 1/BSC-1 persistently infected cells. Cells infected by mutant ts 1 at restrictive temperature (not shown) resemble the uninfected BSC-1 cells illustrated in Fig. 4(a), whereas the persistently infected cells are more elongate and frequently overlap adjacent cells in a manner reminiscent of transformed cells (Fig. 3e). The blebs on the surface of the RS ts 1/BSC-1 cells visible in Fig. 3(e) and Fig. 4(c) may be artifacts of glutaraldehyde fixation (Hasty & Day, 1978). Exposure of RS ts 1/BSC-1 cells to 30 μg/ml concanavalin A for 2 h at 37 °C produced rounding and agglutination (Fig. 3f), whereas uninfected BSC-1 cells were unaffected (not shown) and indistinguishable from untreated cells apart from a greater protrusion of nucleus and nucleoli.

The appearance of the cell surface of RS ts/BSC-1 cells was dependent on the temperature.
Table 1. Immunofluorescence of RS ts I/BSC-1 cells after temperature shift

<table>
<thead>
<tr>
<th>Temperature shift at 0 h</th>
<th>Preparation</th>
<th>Total</th>
<th>+ve</th>
<th>%</th>
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<tr>
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<td></td>
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<td>111</td>
<td>23.3</td>
<td>162</td>
<td>144</td>
<td>27.2</td>
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<tr>
<td>37 → 31 °C (shift-down)</td>
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<td>400</td>
<td>400</td>
<td>100</td>
<td>491</td>
<td>491</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Unfixed</td>
<td>228</td>
<td>93</td>
<td>40.3</td>
<td>213</td>
<td>100</td>
<td>47.0†</td>
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<tr>
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<td>362</td>
<td>98.1</td>
<td>608</td>
<td>595</td>
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<tr>
<td></td>
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<td>37</td>
<td>8.2</td>
<td>420</td>
<td>10</td>
<td>2.4</td>
</tr>
</tbody>
</table>

* RS ts I/BSC-1 cells at passage number 82 at 37 °C were re-spread in 50 mm Petri dishes with glass coverslips at 1 × 10⁶ cells/dish and incubated at 31, 37 or 39 °C. The number of fluorescing cells in fixed and unfixed preparations was counted after 24 and 48 h.
† Underestimated because cells were beginning to detach.

Fig. 5. Staining by indirect immunofluorescence of persistently infected RS ts I/BSC-1 cells at passage 82 with bovine anti-bovine RS virus serum and FITC-conjugated anti-bovine serum. (a) Unfixed RS ts I/BSC-1 cells after 48 h at 31 °C to show surface antigen; (b) unfixed RS ts I/BSC-1 cells 48 h at 39 °C to show surface antigen; (c) acetone-fixed RS ts I/BSC-1 cells after 48 h at 31 °C to show intracytoplasmic antigen; (d) acetone-fixed RS ts I/BSC-1 cells after 48 h at 39 °C to show intracytoplasmic antigen.

of incubation. If the temperature of incubation was raised to 39 °C for 48 h, the RS ts I/BSC-1 cells became flatter in aspect, more similar to normal BSC-1 cells, with their adjacent borders in close contact (Fig. 4d). If the temperature of incubation was lowered to 31 °C for 48 h, on the other hand, the appearance of the cells was more irregular and
Fig. 6. Scanning electron micrographs of cells infected with HSV, RFV, VSV and Batai virus. 
(a) HeLa cells 24 h p.i. with a syncytial mutant of HSV, type 2; 
(b) HeLa cells 24 h p.i. with a non-syncytial mutant of HSV, type 2; 
(c) BSC-1 cells 24 h p.i. with RFV; 
(d) BSC-1 cells 96 h p.i. with RFV; 
(e) BSC-1 cells 24 h p.i. with Batai virus; 
(f) a BSC-1 cell 24 h p.i. with VSV, New Jersey.
filaments were present on some of the cells (Fig. 4e, f). And, in fact, RS ts I/BSC-1 cultures became degenerate at 31 °C and could not be serially propagated at this temperature.

The presence of virus antigen at the cell surface followed a similar pattern. Table I gives the results of examination of fixed and unfixed cells stained with anti-RS virus serum and FITC-conjugated anti-bovine serum, which provide a measure of surface and intracytoplasmic antigen. At 37 °C antigen is detectable at the surface of 23% of cells in the culture at 24 h, and 27% of the cells at 48 h. After 48 h at 39 °C, however, the number of cells which had detectable surface antigen had declined to 2.4%. On the other hand, the number of cells with surface antigen had increased to at least 47% after 48 h at 31 °C. The presence of virus antigen at the cell surface, therefore, was correlated with the morphological changes observed, since virtually all the cells exhibited intracytoplasmic antigen at all three temperatures.

Fig. 5(c) and (d) show that there is no qualitative difference in the distribution of intracytoplasmic antigen at 31 and 39 °C. However, Fig. 5(a) and (b) show that there is a qualitative difference in surface antigen at 39 °C, in addition to the quantitative difference.

The morphology of the surface of cells infected with some other viruses

We have examined the surface morphology of cells infected with some other viruses which produce different types of c.p.e. Fig. 6(a) and (b) illustrate the surface of HeLa cells 24 h p.i. with syncytial and non-syncytial mutants of HSV type 2 and similar pictures were obtained with infection of BSC-1 cells and with HSV type I (not shown). Fig. 6(c) and (d) show a developing focus of RFV-infected BSC-1 cells at 24 and 96 h p.i. respectively. These RFV-induced foci resemble RS virus foci macroscopically but there is no filament production in the RFV-infected cells. Fig. 6(e) and (f) show BSC-1 cells 24 h p.i. with Batai virus and VSV respectively.

In none of these infections is there any filament production resembling that associated with pneumoviruses.

DISCUSSION

Filamentous processes are regularly produced following infection of susceptible BSC-1, MRC-5, FEA and Mv1Lu cells by RS virus, and BSC-1 cells by PVM. Filament production was also observed after RS virus infection of HeLa cells, but it was less distinctive because of the abundance of microvilli on the surface of uninfected cells. The absence of filaments on BSC-1 cells infected with ts mutants of RS virus in two different complementation groups, when incubated at the restrictive temperature, showed that the filaments were induced by the virus and not by an extraneous agent in the inoculum. Fuchs & Bächi (1975) also observed filaments associated with RS virus infection of Vero cells and Panem & Kirsten (1974) suggested that microvilli present on the surface of RS virus infected HEp-2 cells had a diam. similar to that of the RS virion.

Furthermore, Fuchs & Bächi (1975) observed that polymethyl methacrylic spheres coated with RS virus antibody adhered to the filaments as well as the cell body of infected Vero cells. Similarly, staining of BSC-1 cells with fluorescent antibody shows that virus antigen is present throughout the length of the filaments (Fig. 2 and Faulkner et al. 1976; Pringle et al. 1978b). It is not clear whether the filaments represent processes actively extruded from the cell surface, or whether they are produced as a result of localized adhesion of membrane to a substrate followed by retraction of the cell body. Filament production does not appear to be related, however, to the focal c.p.e. observed on monolayers of BSC-1 cells under agar overlay (Wunner et al. 1975; Faulkner et al. 1976) since RFV induced similar foci on monolayers of BSC-1 cells, but not filaments (Fig. 6c, d).

The abundant production of filaments in several types of cells, including lung-derived...
cells, suggests that this phenomenon may be a factor in the pathogenesis of RS virus infection, since there is evidence that mechanical obstruction may play a role in bronchiolitis in infants (Simpson et al. 1974a, b).

Filament production was not observed with four other viruses (HSV, RFV, VSV and Batai virus) which we have examined and it has not been reported in other studies of virus-cell interactions by scanning electron microscopy. These have included herpes-transformed cells (Glaser et al. 1977), herpes virus in HEp-2 cells (Panem & Kirsten, 1974), foot-and-mouth disease virus in bovine kidney cells (Yilma et al. 1978), a porcine enterovirus in PK-15 cells (Sulochana & Derbyshire, 1977), murine leukaemia virus in Friend erythroleukaemia cells (DeHarven et al. 1973); rabies virus in chick embryo cells (Minamoto et al. 1978) and VSV Indiana in L-2 cells (Holmes, 1975). The situation with regard to other paramyxoviruses is unclear, because Panem & Kirsten (1974) could only differentiate parainfluenza virus type 3-infected cells from RS virus-infected cells on minor features*. However the HEp-2 cells used by these workers did not show extensive filament production and we have observed previously major differences between measles virus and RS virus infection of BSC-1 cells (E. A. C. Follett & C. R. Pringle, unpublished data).

Persistent infection of BSC-1 cells is associated with phenotypic changes, namely overlapping and concanavalin sensitivity, which are reminiscent of transformation induced by oncogenic viruses. Inoculation of RS ts 1/BSC-1 cells into nude mice or newborn hamsters, however, has not induced overt tumours. Because of the change in karyotype of the RS ts 1/BSC-1 culture, it is not possible to ascribe this phenotype to the virus genome directly, since virus infection may have brought about karyotypic change by selection of a sub-population of semi-refractory cells. An attempt is now being made to resolve this question by study of RS virus persistent infection of another cell type. In the case of the RS ts 1/BSC-1 culture, the temperature-dependence of the surface antigen and the pseudo-transformed phenotype suggest that the latter is a consequence of the appearance of virus protein in the cell membrane. The temperature-dependence of the surface changes is also consistent with the hypothesis that the persistent infection is a consequence of selection of a variant of RS virus which is partially defective in maturation at normal incubation temperature (Pringle et al. 1978b).

Transmission electron microscopy of various types of cells infected with human RS virus (Bloth et al. 1963; Norrby et al. 1970; Bäch & Howe, 1973; Kalica et al. 1973; Berthiaume et al. 1974; J. E. Parry, unpublished data), bovine RS virus (Ito et al. 1973); and PVM (Compans et al. 1967; Berthiaume et al. 1974) has demonstrated also that surface filaments are abundant at late stages of infection. Scanning electron microscopy has clear advantages, however, in speed of specimen preparation, the minimal disruption of the cell surface in comparison with plastic replica preparation and the three-dimensional image which requires no reconstruction for interpretation.

We thank Karen Brunton and Sandra Barr for able assistance.

* Note added in proof. Recently, we have examined BSC-1 cells infected with bovine parainfluenza virus type 3. Extensive syncytia with nuclear fusion were observed, but no process formation.

REFERENCES

Pneumoviruses: surface antigen and filaments


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