Expression of the Respiratory Syncytial Virus 22K Protein on the Surface of Infected HeLa Cells

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SUMMARY

Immunofluorescent staining of unfixed respiratory syncytial virus-infected HeLa cells with monoclonal antibodies (MAbs) demonstrated that the 22K protein is expressed on the cell membrane along with the fusion (F) protein and large glycoprotein (G). All three proteins were detected in the cytoplasm at 17 h post-infection and in the case of the F and G proteins this coincided with their appearance on the cell surface. However, the 22K protein could not be detected on the surface until approximately 16 h after its detection in the cytoplasm, when cytopathic effect was extensive. No evidence for the surface expression of the phosphoprotein (P), matrix (M) or nucleocapsid (N) proteins was found. Trypsin treatment of infected cells prior to unfixed immunofluorescent staining and Western blot analysis indicated that, unlike the G protein, the quantity of 22K protein detected on the cell surface constituted only a small proportion of the total present in the cell. A comparison of the patterns of immunofluorescent staining produced by MAbs on acetone-fixed infected cells suggested that the N, P and 22K proteins, but not the M protein, may be associated with the same intracellular structures.

Respiratory syncytial (RS) virus, a major cause of bronchiolitis in infants, is classified as a member of the Paramyxoviridae. Unlike other paramyxoviruses, RS virus possesses two non-glycosylated proteins associated with the virion membrane: the matrix (M) protein and the 22K protein (Wunner & Pringle, 1976; Peeples & Levine, 1979; Huang et al., 1984, 1985). The 22K protein (which migrates with a relative molecular weight of 23000 in gels run in our laboratory; Routledge et al., 1985) is relatively hydrophilic (Collins & Wertz, 1985) and is the most basic of the proteins produced in cells as a result of RS virus infection (Dubovi, 1982), but its function is unknown. Immunofluorescent staining with monoclonal antibodies (MAbs) indicates that in addition to the RS virus fusion (F) protein and large glycoprotein (G), the 22K protein is expressed on the surface of infected cells (Routledge et al., 1985). However, it is not known when the 22K protein appears on the cell membrane and thus whether infected cells could be recognized in vitro by the immune system via the 22K protein early in the virus replicative cycle. We report here experiments which indicate that surface expression of the 22K protein only occurs late in the replicative cycle.

Using indirect immunofluorescent staining, we have compared the expression of the 22K protein on the surface of RS virus (A2 strain)-infected HeLa cells with that of the F, G, M, phosphoprotein (P) and nucleocapsid (N) proteins. Cells were stained at approximately 8 h intervals post-infection (p.i.) with MAbs specific for RS virus proteins (Table I, a). Staining was performed either with prior acetone fixation to render the cell membranes permeable to the staining antibodies, or without fixation to limit staining to cell surface components.

Anti-F, G and 22K MAbs produced a low intensity coarse random speckling on the surface of unfixed cells immediately post-infection, presumably due to the newly adsorbed virus inoculum. This speckling, which had faded by 8 h p.i., was most obvious with anti-22K protein MAb but...
Fig. 1. Immunofluorescent staining of acetone-fixed RS virus-infected HeLa cells. HeLa cell monolayers infected with a clarified suspension (2500 g, 15 min) of RS virus at a m.o.i. of approx. 3 were maintained for 17 h in medium 199 supplemented with 2% (v/v) heat-inactivated foetal calf serum, penicillin and streptomycin. Single cell suspensions were prepared using EDTA, fixed in acetone and stained by the indirect immunofluorescence technique (Scott et al., 1976; Gardner & McQuillin, 1980). Anti-RS virus MAbs (Table 1, a) directed against F (a), G (b), the 22K protein (c), P (d), M (e) and N (f) were used as detector antibodies. FITC-conjugated rabbit anti-mouse IgG was obtained from Miles Research Laboratories.

Table 1. Anti-RS virus MAbs used in immunofluorescent staining

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was not seen in fixed cells (not shown). At 17 h p.i. (Fig. 1) cytopathic effect was not apparent but the synthesis of all six virus proteins was detected in the cytoplasm of all (fixed) cells. Only the F and G proteins were simultaneously detected on the surface of all (unfixed) cells (Fig. 2a to f). The 22K protein, although seen in a small number of unfixed cells at 17 to 24 h p.i., was not
Fig. 2. Immunofluorescent staining of unfixed RS virus-infected HeLa cells. Single cell suspensions of HeLa cells infected 17 (a to f) or 40 (g) h previously were prepared as described in Fig. 1. Cells were then stained by the indirect immunofluorescence technique without prior acetone fixation using anti-RS virus MAbs (Table 1, a) directed against F (a), G (b), the 22K protein (c, g), P (d), M (e) and N (f).

found on the surface of all cells until 32 to 48 h p.i. (Fig. 2g), when c.p.e. was extensive. Nevertheless, the surface expression of the 22K protein at 32 to 48 h p.i. supports previous detergent-salt dissociation studies (Huang et al., 1984) which have identified it as a fourth membrane-associated protein. The N, P and M proteins were detected in only a small number of unfixed cells at any time p.i. (10% at 40 h) suggesting that these proteins are not normally expressed on the cell surface. As the number of fluorescing cells correlated well with the total number (10%) of infected cells which absorbed the vital stain trypan blue, the fluorescence observed here, and with the 22K protein at 17 to 24 h p.i. was probably indicative of cell damage.
Fig. 3. Trypsin treatment of RS virus-infected cells. (a) Suspensions of HeLa cells infected with RS virus
40 h previously were prepared as described in Fig. 1. The cells were then pelleted by gentle
centrifugation and resuspended in Hanks' balanced salt solution (HBSS) (1,2) or HBSS containing 2.5
mg/ml trypsin (Flow Laboratories) (3,4) and incubated at 37 °C for 15 min. Cells were then rinsed with
cold Eagle's MEM containing 10% (v/v) foetal calf serum and stained at 4 °C without prior acetone
fixation as described in Fig. 1. MAbs against G (1,3) and the 22K protein (2,4) (MAbs 3F4 and 1C1
respectively) were used. (b) Uninfected (1,2) or RS virus-infected (3,4) suspensions of HeLa cells were
prepared and treated with HBSS (1,3) or HBSS and trypsin (2,4) as described above. Cells were then
taken up in boiling SDS-PAGE non-reducing sample buffer and electrophoresed on a 10%
polyacrylamide gel using the buffer system of Laemmli (1970). Proteins in the gel were electrophoblotted on
to nitrocellulose paper (Burnette, 1981) and stained using the immunoperoxidase method (Samson
et al., 1986). A pool of MAbs 3F4, 2B9 and 1C1 (Table 1) was used as the detector antibody. [In non-
reducing SDS-PAGE the 23000 22K protein is resolved as two bands. These represent conformational
variants of the 22K protein which arise due to sulphydryl–disulphide bond interchange reactions,
caused by solubilization of the protein in SDS (Routledge et al., 1987).]

rather than surface antigen expression. This conclusion was supported by an experiment in
which infected cells (40 h p.i.) stained with the anti-P protein MAAb 2B9 were subsequently co-
stained with trypan blue and then viewed alternately under blue or white light. The presence of
the dye was observed in all fluorescing cells detected. When cells stained with the anti-22K
protein MAAb 1C1 were similarly co-stained only 10% of the total number of cells absorbed the
dye despite fluorescence in 100% (not shown).

The use of an additional panel of anti-F, G, 22K, N and M protein MAAbs of different epitopic
specificity (Table 1, b) and mouse polyclonal sera raised against immunoaffinity-purified 22K
and P proteins produced no alteration in the fluorescence picture described above (not shown).
To assess the extent of 22K protein surface expression at 40 h p.i., living infected cells were exposed to trypsin and subsequently stained by the immunofluorescence method or analysed by Western blotting. The behaviour of the G and P proteins under the same conditions was also monitored to demonstrate that only cell surface components were affected by the trypsin treatment. Immunofluorescent staining of unfixed trypsinized cells (Fig. 3a) indicated that the 22K protein expressed on the surface was trypsin-sensitive. When trypsinized cells were examined by staining after fixation (not shown) or by Western blotting (Fig. 3b) the level of 22K protein appeared unaffected, suggesting that even at 40 h p.i. most 22K protein was inaccessible to trypsin. This observation, in conjunction with the presence of the 22K protein in the cell and absence from the surface at 17 h p.i., suggests that most 22K protein molecules are normally situated below the cell surface even late in infection. However, we cannot rule out the possibility that a small part of each molecule, undetectable due to an absence of antibody binding sites and trypsin sensitivity, is exposed on the cell surface throughout the replicative cycle.

The appearance of the 22K protein on the surface, as detected by immunofluorescence and trypsin sensitivity, may be an incidental event caused by a generalized deterioration of the cell membrane due to advancing c.p.e. Alternatively it may be an important stage in the progression of the replicative cycle. Which of these is the true explanation is unknown. However, if the appearance of the 22K protein on the surface is due to membrane damage, then it must be different in nature from that accounting for the staining of occasional cells by anti-M, P and N protein MAbs, as the 22K protein is seen on all cells late in infection and only 10% of these cells absorb trypan blue.

A clue to the function of the 22K protein may be obtained by a comparison of the staining patterns produced in acetone-fixed cells by MAbs specific for the various RS virus proteins (Fig. 1). Anti-22K protein MAb produced a pattern of staining which was broadly similar, particularly in the early stages of infection, to that seen with anti-N and P protein MAbs, suggesting that a proportion of all three proteins may be associated with the same intracellular structures. This, in addition to its association with the cell membrane and inclusion in the virion, raises the possibility that the 22K protein may act as a link between the nucleocapsid and membrane at some point in the replicative cycle. The difference in staining between the anti-22K and M MAbs suggests that whatever the function of the 22K protein is, it may be distinct from that of the M protein.

These studies confirm that the 22K protein is expressed on the cell surface and therefore is likely to be available to the immune system during infection. An immune response to this antigen is less likely to play a significant role in protection than that directed against the glycoproteins (F and G proteins) which are expressed earlier on the cell surface. However, a response to the 22K protein may contribute to the clearance of syncytia and thus assist in recovery from infection.

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REFERENCES


Short communication


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