Moesin, and not the murine functional homologue (Crry/p65) of human membrane cofactor protein (CD46), is involved in the entry of measles virus (strain Edmonston) into susceptible murine cell lines

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Membrane cofactor protein (CD46) has been firmly established as the major high affinity receptor for measles virus (MV). In addition, another protein, moesin, has been shown to be linked with the susceptibility of human cells to MV infection. Murine cells are largely resistant to MV infection, although a number of cell types can be productively infected. As murine cells do not express CD46 an additional mechanism for the uptake of MV is likely. Murine cells possess a functional homologue of CD46 (Crry/p65) in addition to murine moesin, which has nucleotide and amino acid homology to human moesin. We report that anti-moesin monoclonal antibodies 119 and 38/87 reduce the number of infectious centres attributed to MV in murine cell lines NS20Y and L929, whereas polyclonal antisera specific for Crry/p65 and CD46 had no effect on MV infection of these cells. We suggest that moesin may be important in the non-CD46-mediated uptake of MV strain Edmonston by susceptible murine cell lines.

Measles virus (MV) is a member of the morbillivirus genus of the family Paramyxoviridae. Its genome is comprised of a single copy of a negative-sense ssRNA and codes for a number of proteins. These include the two major surface glycoproteins, the haemagglutinin (H) and fusion (F) proteins, which are inserted into the envelope of the mature virion. MV H and F proteins serve as virus attachment proteins (VAP) and are involved in the receptor-mediated binding and entry of MV into cells. The initial interaction between the VAP and a specific cell surface receptor largely governs the host range and tissue tropism exhibited by virus (Lentz, 1990).

Recently, a number of groups have identified human membrane cofactor protein (MCP or CD46) as the major high affinity receptor for MV (Naniche et al., 1993; Dörrig et al., 1993). CD46 is a member of the regulators of complement activation (RCA) gene cluster and is responsible primarily for the protection of cells against autologous lysis, acting as cofactor in the Factor I (serine protease)-mediated inactivation of complement components C3b and C4b (Liszewski et al., 1991). A number of different isoforms of CD46 are known to occur naturally and it has been shown that measles virus is able to utilize all isoforms for receptor-mediated uptake into transfected CHO cells (Manchester et al., 1994). CD46 has a very broad tissue distribution in humans and this reflects the largely pantropic nature of natural MV infection, although the virus appears to replicate preferentially in cells of lymphoid tissue (Esolen et al., 1993; Griffin & Ward, 1993). An additional protein has been shown to be important in the infection of cells by MV, namely moesin (membrane-organizing extension spike protein; Dunster et al., 1994). Moesin, like CD46, also exhibits a wide tissue distribution, functions as a receptor for heparin sulphate and is intimately associated with the cell membrane and cytoskeleton (Lankes & Furthmayer, 1991; Schwartz-Albiez et al., 1995). Monoclonal antibodies (MAbs) directed against moesin were able to inhibit MV infection of normally susceptible human cells, and the monkey kidney Vero cell line, by 70–80% depending on the MAb used (Dunster et al., 1994). There is now evidence available to suggest that CD46 and moesin form a physical association as a receptor complex for MV infection of susceptible cells (Schneider-Schaulies et al., 1995).

Cell lines of murine origin, which do not express CD46 and are largely resistant to MV, can be made more susceptible to infection by transfection of cells with
CD46; this may increase virus yield by 50- to 100-fold, thereby confirming the role of CD46 as the major receptor for MV (Naniche et al., 1993; Yanagi et al., 1994). Most notably, following transfection of murine cells with CD46 and subsequent infection with MV, characteristic virus-induced CPE in the form of giant cells was observed. However, a number of native murine cell lines such as the neuroblastoma cell line NS20Y are readily infected with MV, the result of which is the appearance of rapid CPE and high titres of infectious progeny virus (Rager-Zisman et al., 1984). Furthermore, it is also possible to establish persistent MV infections in NS20Y cells (Rager-Zisman et al., 1984). In addition, recent data have shown that native murine L cells and NIH 3T3 cells are able to replicate MV in the absence of CD46 (Yanagi et al., 1994). The above data strongly suggest the existence of a CD46-independent mechanism for the uptake of MV into murine cells.

Whilst no direct murine homologue of CD46 has been detected by Southern blot analysis or direct cloning (Kingsmore et al., 1989; Molina et al., 1992), a putative functional homologue has been identified and its cDNA cloned based on its similarity to human CR1 (an RCA member; Paul et al., 1989). The protein encoded by the gene has been designated Crry/p65 (Li et al., 1993) and regulates complement component C3 deposition on autologous cell membranes of murine origin and, as such, exhibits homology with the functions of CD46 and CD55 (decay accelerating factor or DAF, an RCA family member). Mouse cells also possess a homologue of moesin which has a high degree (98%) of amino acid similarity to the human form, suggesting an essential role for this protein in a number of varied cell types (Sato et al., 1992). We have investigated whether Crry/p65 and murine moesin are involved in the susceptibility of certain murine cells to MV infection.

Murine cell lines NS20Y (neuroblastoma), L929 (fibroblast), NIH 3T3 (fibroblast) and VLM (fibroblast) and human HeLa cells were propagated in Eagle's MEM supplemented with 5% fetal calf serum and antibiotics (penicillin 0·1 g/litre and streptomycin 0·1 g/litre). Stocks of MV strain Edmonston (MV-Ed) were prepared according to Dunster et al. (1994) and stored at −70 °C. The initial investigation concentrated on the ability of MV-Ed to infect the cell lines used in this study. Cells were seeded at a density of 5 × 10⁴ per ml in 12-well cluster plates, infected when 80% confluent with MV-Ed at an m.o.i. of 1·0 and observed daily for the appearance of CPE. Not surprisingly, CPE, in the form of characteristic multinucleated giant cells, was evident 24 h post-infection (p.i.) on HeLa cell monolayers and progressed rapidly to involve the entire monolayer (data not shown). At approximately 36 h p.i., MV-Ed-induced CPE was also evident in murine NS20Y cells. The remaining murine cell lines (L929, NIH 3T3 and VLM) showed no evidence of virus-induced CPE (even after prolonged incubation times of up to 5 days). Indirect immunofluorescence (IIF) studies with L929 cells, using human MV-hyperimmune serum derived from subacute sclerosing panencephalitis patients, showed a high proportion (approximately 60%) of cells to be infected (data not shown). Using a similar technique it was found that there was little or no evidence of MV infection of NIH 3T3 and VLM cells (data not shown). It has been reported that CD46 may be indispensable for syncytium formation and the evidence that vaccinia virus constructs expressing MV H and F proteins do not cause giant cell formation in CD46-negative murine cells enhances this claim (Wild et al., 1991; Yanagi et al., 1994). Moreover, MV infection of L cells transfected with CD46 exhibited CPE in comparison to native cells (Yanagi et al., 1994) suggesting that CD46 was important in the fusion process and spread of MV. However, CD46-negative NS20Y cells exhibit extensive giant cell formation when infected with MV-Ed, and hence alternative mechanisms for the formation of giant cells and spread of MV infection in NS20Y cells appears to be necessary and the absolute requirement of CD46 for the fusion process can be questioned.
At the time points indicated in Fig. 1, production of infectious MV-Ed for each cell line was titrated on Vero cell monolayers following a freeze–thaw step to release cell-bound virus. The titration curves for infectious MV-Ed (Fig. 1) show disparity with highest yields recorded for HeLa cells (which express CD46) with a peak level of 8.7 × 10⁵ p.f.u./ml after 48 h incubation. However, MV also replicated in the murine cell lines NS20Y and L929 (CD46-negative). Most notably, NS20Y cells generated titres of infectious MV-Ed of 4.6 × 10⁴ p.f.u./ml at 72 h p.i. (Fig. 1). Although no virus-induced CPE was observed in L929 cells, there was nonetheless evidence of infectious virus production by Vero cell plaque assay, albeit of low titre (Fig. 1). The yield of MV-Ed from NIH 3T3 cells was much lower than that described by Yanagi et al. (1994) and may represent differences in cell lines and also virus strains used. The observation that the titre of MV by Vero cell plaque assay showed a rapid reduction following infection of NIH 3T3 cells suggests that the ability of this cell line to support MV replication is lower than that of NS20Y and L929 cells. MV was not detected in VLM cells, with no evidence of MV nucleocapsid (N) present even at 24 h p.i. by IIF using MV anti-N-specific antibody. It is possible that MV may not have bound with sufficient avidity to initiate infection of VLM cells or that conversely, MV-Ed may have not have bound with sufficient avidity to initiate infection of VLM cells but was unable to replicate because of cell type-specific barriers to transcription and/or translation of the virus genome. It is interesting to note that transfection of certain murine cell lines with CD46 will allow effective binding and uptake of MV although no virus replication is subsequently observed (Naniche et al., 1993).

The data obtained from the above experiments implicated non-CD46-mediated entry of MV-Ed into mouse NS20Y and L929 cells. The cell lines were then studied for the expression of epitopes specific for Crry/p65, CD46 and moesin by fluorescence-activated cell sorting (FACS) analysis. MAbs for mouse Crry/p65 were prepared by immunizing rats with recombinant baculovirus-expressed protein. In addition, a polyclonal antiserum (PAb) was produced in a similar manner in rabbits. The characteristics of the above MAbs and PAb have been described in detail (Li et al., 1993). A CD46-specific PAb was the kind gift of Dr G. Yeh (CytoMed, Cambridge, Mass., USA) and the origins of anti-moesin MAbs 119 and 38/87 has been described previously by Dunster et al. (1994). For FACS analysis, 1 × 10⁵ cells were removed non-enzymatically from culture flasks using PBS and 5 mM-EDTA. After washing in FACS buffer (PBS, 0.5 % BSA, 0.2 % sodium azide) cells were fixed in PBS containing 3 % paraformaldehyde, washed and the primary antibody added for 45 min at 4 °C. The cells were washed and FITC-conjugated secondary antibody was added for 45 min at 4 °C. Following a final wash, fluorescence was measured by FACS analysis using a FACSscan machine (Becton Dickinson).

Table 1 details the MAb and PAb reactivities with murine NS20Y, L929, NIH 3T3 and VLM cells and human HeLa cells. The Crry/p65 antibodies reacted predominantly with cells of murine origin, although there was a positive reaction of MAb 5D5 and the PAb with HeLa cells. This may represent a non-specific interaction with respect to the PAb, although when considered in conjunction with the positive result obtained for MAb 5D5, it could also be a genuine cross-reaction. This is not an unreasonable assumption given that Crry/p65 shares similar functions with CD46. In a similar manner the CD46 PAb, whilst detecting epitopes on HeLa cells, was also able to react with an epitope on all mouse cell lines tested. To determine the specificity of the cross-reactions observed in the FACS experiments, immunoprecipitations were made with the MAb and PAbs with HeLa cells and the murine cell lines. In all cases, no proteins were immunoprecipitated by the Crry/p65-specific MAb with human HeLa cells and conversely the anti-CD46 PAb did not precipitate proteins from murine cells (data not shown). All four murine cell lines and HeLa cells gave a positive reaction for moesin when detected by MAbs 119 and 38/87. This

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Anti-Crry/p65 antibody</th>
<th>Anti-CD46 antibody</th>
<th>Anti-moesin antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7E8 5D5 1F2 10A2 7G1 12A7 PAb</td>
<td>13/42 J4/48 PAb</td>
<td>38/87 119</td>
</tr>
<tr>
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<td></td>
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<tr>
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<tr>
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<tr>
<td>NIH 3T3</td>
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<td>HeLa</td>
<td>- - - - ND - ND + + + + + + + + + + + +</td>
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</tbody>
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* Antibody reactivities were scored on the following scale of fluorescence intensity: 0–10, −; 11–100, +; 101–201, + +; ≥ 202, + + +.

ND, Not done.
points to a very specific conservation of the protein between cell types and species (man and mouse) especially given that there is 98% similarity at the amino acid level between human and mouse forms of this protein (Sato et al., 1992). Interestingly, the surface reactivity of anti-moesin MAb 119 is higher than that obtained for MAb 38/87 in the case of human and murine cell lines. MAbs 119 and 38/87 recognize distinct epitopes; this is based on data obtained by Western blotting, in which MAb 38/87 alone reacts, and the ability of MAb 119 to inhibit MV infection of human cells to a greater extent.

The use of CD46 as the major receptor component in MV infection was originally established using a MAb directed against a protein designated gp57/67 (later identified as CD46) which was able to inhibit infection of normally susceptible human cells with MV (Naniche et al., 1992). In addition, antibodies directed against moesin (119 and 38/87) were also shown to have an inhibitory effect on the ability of MV to infect susceptible cells (Dunster et al., 1994). There is now evidence to show an additive effect between anti-CD46 and anti-moesin antibodies when added in suboptimum concentrations (a concentration of MAb at which MV infection is inhibited by 30–40% in comparison to controls) to the point at which MV is completely inhibited from binding to cells (Schneider-Schaulies et al., 1995). This, in conjunction with co-immunoprecipitation and colocalization data, suggests the close proximity of CD46 and moesin which may form a receptor complex for the uptake of MV (Schneider-Schaulies et al., 1995). Whether or not Crry/p65, as a functional homologue of CD46, or moesin, is used as receptor by MV was determined using an infectious centre assay. For this study NS20Y, L929 and HeLa cells were seeded at a density of 5 x 10^4 cells per ml into 12-well plastic dishes containing coverslips. The cells were then treated with PBS (as control) or antibody prior to infection with MV-Ed at an m.o.i. of 1.0 (for the murine cells) or 0.1 (for HeLa cells; higher m.o.i. caused rapid destruction of the monolayer). Cells were then incubated for 48 h after which infectious centres were visualized by IIF using MV anti-N antibody. The ability of antibody directed against Crry/p65 (PAb), CD46 (PAs) or moesin (MAb 38/87) was determined by infectious centre assay. Cells (HeLa, NS20Y and L929) were seeded (5 x 10^4 cells per ml) in 12-well plastic dishes containing poly-L-lysine-coated coverslips. MV was inoculated at an m.o.i. of 1.0 for HeLa cells as CPE is too rapid at higher m.o.i. leading to complete destruction of the monolayer) and incubated for 48 h. The coverslips were then removed and fixed in ice-cold acetone for 5 min prior to the addition of anti-N MAb diluted in PBS and incubation at room temperature for 45 min. After washing in PBS, FITC-conjugated secondary antibody was added and incubated at room temperature for a further 45 min before analysis by IIF using a Leitz Labovert FS microscope. For each cell line the number of infectious centres per control well (PBS-treated) was set as standard (100%) to which the results for antibody-treated test wells were compared. Data are based on the average of five sets of experiments. 1, virus control (PBS-treated monolayer); 2, antibody control (W6/32, anti-MHC class I); 3, anti-Crry/p65 PAb; 4, anti-CD46 PAb; 5, anti-moesin MAb 119 at 75 µg/ml; 6, anti-moesin MAb 38/87 at 75 µg/ml; 7, combination of both anti-moesin MAbs at 75 µg/ml each.

![Graph](image-url)

**Fig. 2.** Inhibition of MV-Ed infection of human and murine cells by antibody. The ability of antibody directed against Crry/p65 (PAb), CD46 (PAs) or moesin (MAb 38/87) was determined by infectious centre assay. Cells (HeLa, NS20Y and L929) were seeded (5 x 10^4 cells per ml) in 12-well plastic dishes containing poly-L-lysine-coated coverslips. MV was inoculated at an m.o.i. of 1.0 for HeLa cells as CPE is too rapid at higher m.o.i. leading to complete destruction of the monolayer) and incubated for 48 h. The coverslips were then removed and fixed in ice-cold acetone for 5 min prior to the addition of anti-N MAb diluted in PBS and incubation at room temperature for 45 min. After washing in PBS, FITC-conjugated secondary antibody was added and incubated at room temperature for a further 45 min before analysis by IIF using a Leitz Labovert FS microscope. For each cell line the number of infectious centres per control well (PBS-treated) was set as standard (100%) to which the results for antibody-treated test wells were compared. Data are based on the average of five sets of experiments. 1, virus control (PBS-treated monolayer); 2, antibody control (W6/32, anti-MHC class I); 3, anti-Crry/p65 PAb; 4, anti-CD46 PAb; 5, anti-moesin MAb 119 at 75 µg/ml; 6, anti-moesin MAb 38/87 at 75 µg/ml; 7, combination of both anti-moesin MAbs at 75 µg/ml each.

murine NS20Y and L929 cells, addition of anti-Crry/p65 or -CD46 PAs did not reduce the number of infectious centres attributed to MV-Ed (Fig. 2). However, the addition of anti-moesin MAbs 119 and 38/87 reduced the number of MV-Ed infectious centres by 78% and 43% for NS20Y and 85% and 40% for L929 cells, respectively (Fig. 2). In light of these results it seems clear that antibodies directed against moesin are able to interfere with the infection process of MV in murine cells. At no point was there complete inhibition of MV-Ed infection in NS20Y or L929 cells by anti-moesin MAbs and this may suggest the presence of additional
mechanisms for the uptake of MV; alternatively it may indicate that the epitopes recognized by MAbs 119 and 38/87 on murine cells do not interfere as efficiently with MV binding as on human cell lines.

In human cells CD46 represents the major receptor for MV and moesin is a cofactor in a receptor complex with CD46 (Schneider-Schaulies et al., 1995); it remains to be established whether moesin is acting as a low avidity receptor for the uptake of MV into murine cells alone or in combination with a murine cell surface protein. As murine cells are on the whole resistant to MV infection, detailed analysis of receptor-mediated uptake of MV has not been made. However, it is interesting to find that moesin, a protein which exhibits a high degree of conservation between species, appears to be important for the entry of MV into a number of different cells types of different origins, a point which has important ramifications for MV pathogenesis and research. Whether or not moesin interacts closely with CD46 in murine cells transfected with cDNA encoding the CD46 protein, in a manner analogous to human cells, remains to be established. With a number of researchers pursuing the goal of generating transgenic CD46 mouse models for MV infection, it must be taken into account that certain tissues may have alternative mechanisms for MV uptake and, in addition, only a small number of those CD46-transfected cells may support MV replication. Data will therefore require careful interpretation.

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References


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