JOURNAL OF VIROLOGY, Feb. 1997, p. 1679–1682 Vol. 71, No. 2
0022-538X/97/$04.00+0

Antibody Cross-Reactivity with CD46 and Lack of Cell Surface Expression Suggest that Moesin Might Not Mediate Measles Virus Binding

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Received 9 August 1996/Accepted 8 November 1996

The binding of antimoesin antibodies from ascites fluids to the surfaces of human and rodent cells was found to parallel the level of CD46 expression. No such reactivity was detected with a purified antimoesin antibody which recognized intracellular moesin. In Western blots, antimoesin antibodies were found to react with solubilized CD46 and a recombinant soluble form of CD46. Antimoesin antibodies also reacted with CD46/CD4 molecules containing only the SCR I and II domains required for measles virus (MV) hemagglutinin binding onto CD46. We suggest that the weak cross-reactivity of antimoesin antibodies with CD46 explains the inhibitory effect of these antibodies on MV entry and that moesin is not directly involved in MV binding.

Two molecules have been implicated in measles virus (MV) entry, CD46 (4, 15) and moesin (5, 6). To investigate if a link exists between moesin expression level and MV infection permissiveness for various rodent cell lines, some of which express transfected CD46, the expression of moesin at the surface of the cells was studied by using antimoesin antibodies MoAb38/87 (immunoglobulin G1 [IgG1] isotype) and MoAb70/35, kindly provided by J. Schneider-Schaulies (Wurzburg, Germany). Ascites fluids diluted 1:50 or 1:100 were used to label murine L, ModeK, and 3T3 cells and hamster CHO cells; their transfected counterparts L.CD46, ModeK.CD46 expressing the BC-cyt-2 CD46 isoform, 3T3.CD46gpi, CHO-CD46 expressing the B-cyt-2 CD46 isoform (12), and CHO-CD46gpi expressing a CD46/CD55 chimera anchored by a glycophosphatidylinositol tail; and human HeLa and U937-Tat (expressing the human immunodeficiency virus Tat protein) cells. The antimoesin antibody binding was revealed by treatment with a $F(ab)_2$ anti-mouse IgG (heavy plus light chains) conjugated to phycoerythrin followed by cytofluorometry (3).

Surprisingly, the binding level of antimoesin antibodies was found to correlate with CD46 expression, the human cells being the best-labelled targets (Fig. 1). Moreover, a similar increase in antimoesin antibody binding was observed when CHO cells expressed a CD46/CD4 chimeric molecule containing the SCR I, II, III, and IV domains or only the SCR I and II domains of CD46 linked to Ig-like domains 3 and 4 of CD4 (CHO-I-IV/34 and CHO-I-II/34) (1) (Fig. 1D). Expression of another cell surface protein, CD4 with or without lck (9), has no influence on the binding of antimoesin antibodies to CHO cells (Fig. 1D). Similar results were also observed with ascites fluid moesin-specific monoclonal IgG2a U119, kindly provided by J. Schneider-Schaulies (data not shown). The use of ascites fluid from unrelated B-cell hybridomas secreting IgG resulted in either no significant labelling or a low level of nonspecific background labelling which did not vary as a function of the level of CD46 expression. The experiment was repeated with MoAb38/87 purified at 4°C after binding with protein A in a buffer consisting of 3 M NaCl and 1.5 M glycine (pH 8.9) and elution in 100 mM citric acid (pH 6; kindly provided by R. Schwartz-Albiez, Heidelberg, Germany). Even after incubation with up to 90 μg of purified MoAb38/87 per ml, no surface binding was observed with any cell line (Fig. 2A, histograms a, d, and g; also data not shown). In contrast, when the cells were fixed with 0.3% paraformaldehyde for 10 min and permeabilized with 1% Tween 20, strong intracellular labelling after incubation with 9 μg of MoAb38/87 per ml was observed. All cell lines showed an almost identical labelling profile, and there was no correlation with the level of CD46 expression (Fig. 2B; compare histograms a, d, and g with histograms c, f, and i; also data not shown). If purified MoAb38/87 fails to react with the cell surface, it should not interfere with MV entry. Indeed, and in disagreement with previous reports (5, 16), 100 μg of purified MoAb38/87 per ml failed to inhibit syncytium formation in HeLa cells infected with a recombinant vaccinia virus encoding MV hemagglutinin and fusion protein (data not illustrated).

To ascertain that antimoesin antibodies can cross-react with CD46, these antibodies were also tested for their reactivity to whole-cell lysates from HeLa (Fig. 3A), CHO, and CHO-CD46 cells (Fig. 3B) in Western blots after luminescence staining. After a short exposure, only a double band with a molecular mass of 75 kDa, characteristic of moesin, was detected in all cell extracts after labelling with 9, 0.9, and 0.09 μg of purified MoAb38/87 per ml (Fig. 3A and B, lanes 1, 2, and 3, respectively). Overexposure of the autoradiograph for 30 min revealed an additional faint band in HeLa cell extract (Fig. 3A, lane 1, middle autoradiograph) corresponding to one of the four major CD46 isoforms detected by anti-CD46 antibodies (Fig. 3A, middle and right autoradiographs, lanes 8, 9, and 11). This band was clearly visible after enhanced electrotransfer of the HeLa cell proteins (Fig. 3A, lane 10). The labelling of HeLa cell extract with ascites fluid diluted 1:100 (lanes 4 and 6) or 1:1,000 (lanes 5 and 7) from antimoesin hybridomas MoAb38/87 and MoAb70/35 revealed several bands besides the moesin 75-kDa double band and the CD46-like 57- to 67-kDa double band. The reactivities of ascites fluid antimoesin antibodies were less on a CHO cell extract, but again a 67-kDa band which corresponded to the transfected CD46 isoform detected by anti-CD46 antibodies was labelled on the CHO-CD46 cell extract (Fig. 3B, left autoradiograph; compare

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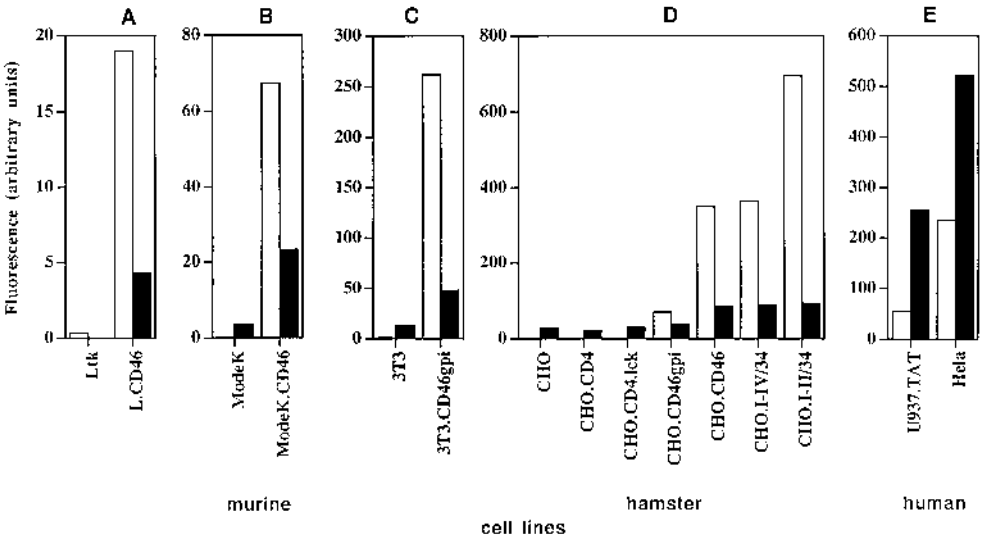

FIG. 1. Binding of ascites fluid antimoesin antibodies to the cell surface in relation to the expression of CD46. Unfixed viable murine cells L (A), ModeK (B), and 3T3 (C); hamster cells CHO (D); and human cells HeLa and U937-Tat (E), either expressing or not expressing CD46, were gently detached with 1 mM EDTA and were incubated with antibodies diluted in tissue culture medium supplemented with 0.1% NaN_3. The level of ascites fluid antimoesin MoAb38/87 binding (solid bars) and that of purified anti-CD46 antibody MCI20.6 (open bars) at the cell surfaces were determined after immunolabelling and flow cytofluorometry.

lanes 4 and 6 with lanes 8 and 9), whereas this band was absent from the CHO cell extract (Fig. 3B, right autoradiograph, lanes 4 and 6 for antimoesin labelling [note that the lower bands were artifacts identified as such on an autoradiograph exposed for a shorter time] and lanes 8 and 9 for anti-CD46 labelling). Finally, Western blotting was performed on a recombinant soluble CD46 (sCD46) purified to homogeneity (kindly provided by B. Loveland, Heidelberg, Australia) (2, 3). Whereas up to 9 μg of purified antimoesin MoAb38/87 per ml did not show any reactivity with purified sCD46 (Fig. 3C, lanes 1, 2, and 3), clear evidence of reactivity was observed with ascites fluid antimoesin antibodies (Fig. 3C, lanes 4 and 6).

Altogether, these data clearly show that antimoesin antibodies exhibit some cross-reactivity with CD46. But why did purified antimoesin antibodies have a lower reactivity with CD46? It is possible that ascites fluid contained moesin-antibody complexes which could bind to CD46 because of some direct moesin-CD46 interactions (16) or that it contained antibody complexed with an as yet unknown natural ligand for CD46; these complexes would have been dissociated during the purification process. Alternatively, the exposure of antimoesin antibodies to harsh elution conditions during their purification could have resulted in some minor conformational change weakening their cross-reactivity with CD46 such that the cross-reactivity could no longer be detected when using a technique with limited sensitivity such as immunocytofluorometry. Consistent with the latter hypothesis, we were unable to detect any moesin-antibody complexes in ascites fluids, and when purified antimoesin antibodies were mixed with a moesin-rich cell extract, no enhancement of their reactivity with CD46 was observed.

The detection of some cross-reactivity of antimoesin antibodies with CD46 and the lack of cell surface expression of moesin led us to reconsider our view of the respective roles of CD46 and moesin in MV entry. On one hand, the ability of CD46 to act as a receptor for MV has been confirmed by many studies (see reference 7 for a review) and direct binding between the ectodomains of the virus hemagglutinin and CD46 has been demonstrated (3). On the other hand, the role of moesin in MV entry has been mainly, if not solely, inferred from the observation that antimoesin antibodies can inhibit MV binding and entry into human and rodent cells (5, 6, 16). Moesin, or membrane-organizing external spike protein, belongs to the talin-ezrin-radixin-moesin family. These proteins are part of the cytoskeleton and are normally expressed in the cytoplasm. Although there is no obvious signal sequence or transmembrane hydrophobic domain in moesin cDNA (11), moesin had been thought to be localized partly on the cell surface because exogenous antimoesin antibodies can have biological effects such as the inhibition of smooth-muscle cell proliferation (10). Can our data shed some light on these apparent discrepancies? First, antimoesin antibodies, as antibodies or immune complexes, clearly have the ability to bind to CD46 molecules and other cell proteins (Fig. 3A, lanes 4 and 6). As such, they might be able to inhibit nonspecifically several interactions with the cell surface and have biologically inhibitory effects on MV entry mediated either by CD46 (5) or by another unknown cell surface receptor expressed on mouse cells (6) and on smooth-muscle cell proliferation (10). It is striking that the cross-reactivity with CD46 mapped to the MV hemagglutinin binding domains SCR I and II (1, 8, 14). Second, moesin and CD46 have been found to colocalize in the microvilli of the cells and to be coimmunoprecipitated (16). These data might reflect, at least partly, the cross-reactivity of antimoesin antibodies with CD46. Alternatively, moesin could have the ability to bind to CD46. It would be interesting to investigate if moesin can bind to the extracellular domain of CD46 and affect its function in the presence of antimoesin antibodies. Indeed, during in vitro infection experiments, some moesin is likely to be shed in the surrounding medium since we could easily detect moesin in a cell tissue culture supernatant (unpublished data).

In conclusion, the lack of cell surface expression of moesin, the failure to demonstrate direct binding of MV envelope glycoproteins to moesin (13), the fortuitous weak cross-reactivity of antimoesin antibodies with CD46, and the failure of purified antimoesin antibody to block MV-induced fusion suggest that moesin is unable to directly mediate the binding of MV to the plasma cell membrane. It remains possible that moesin may interfere with a postbinding event. The study of

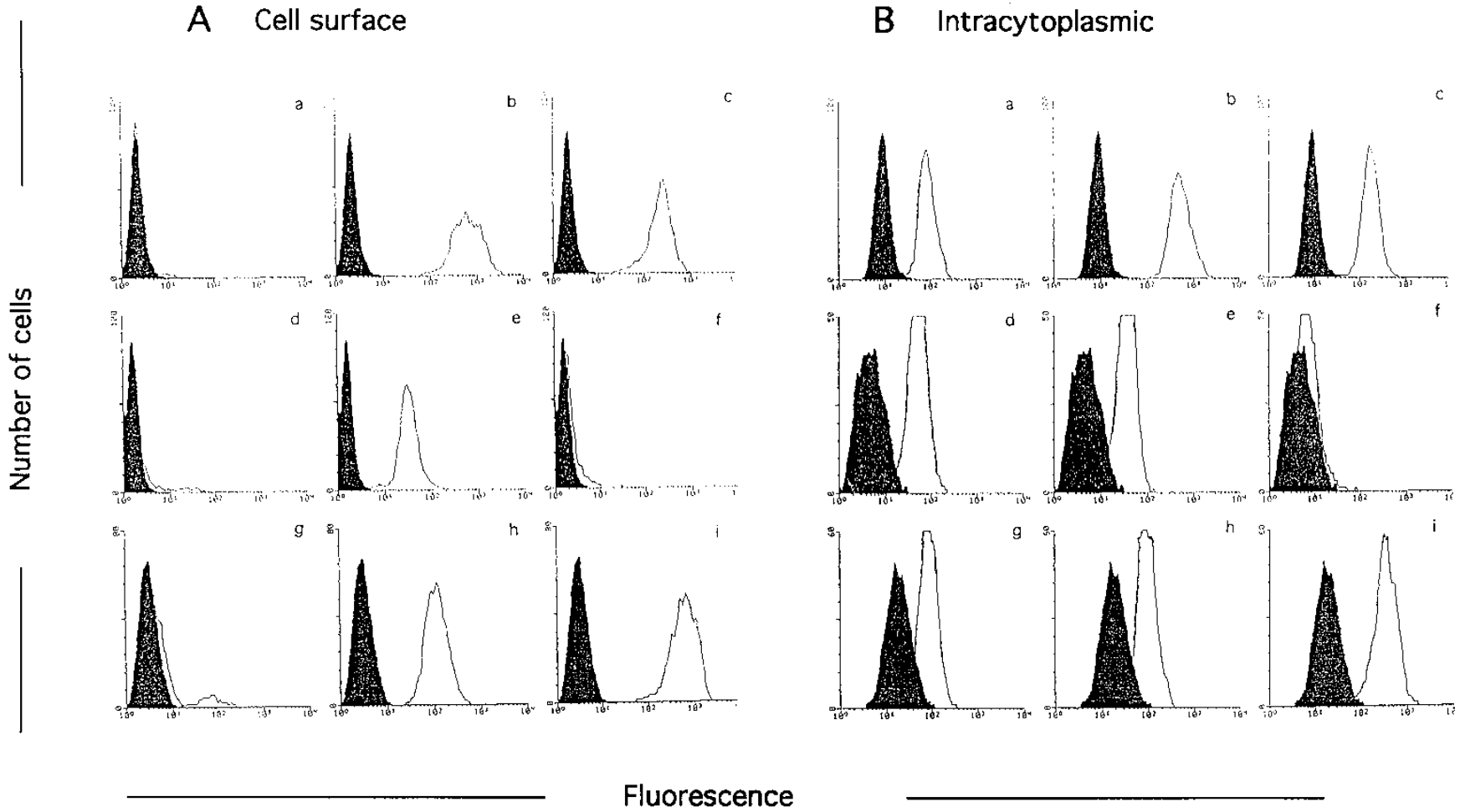

FIG. 2. Cell surface and intracytoplasmic labelling with antimoesin antibodies. Unfixed viable (A) or permeabilized and paraformaldehyde-fixed (B) human HeLa (histograms a to c), hamster CHO (histograms d to f), and CHO-CD46 cells (histograms g to i) were labelled with purified MoAb38/87 (histograms a, d, and g), ascites fluid antimoesin MoAb38/87 (histograms b, e, and h), and purified anti-CD46 antibody MCI20.6 (histograms c, f, and i), then analyzed by flow cytofluorometry. The specific labelling is shown by the shift of the white histograms relative to the black histograms (fluorescence after incubation with the fluorescent conjugate only).

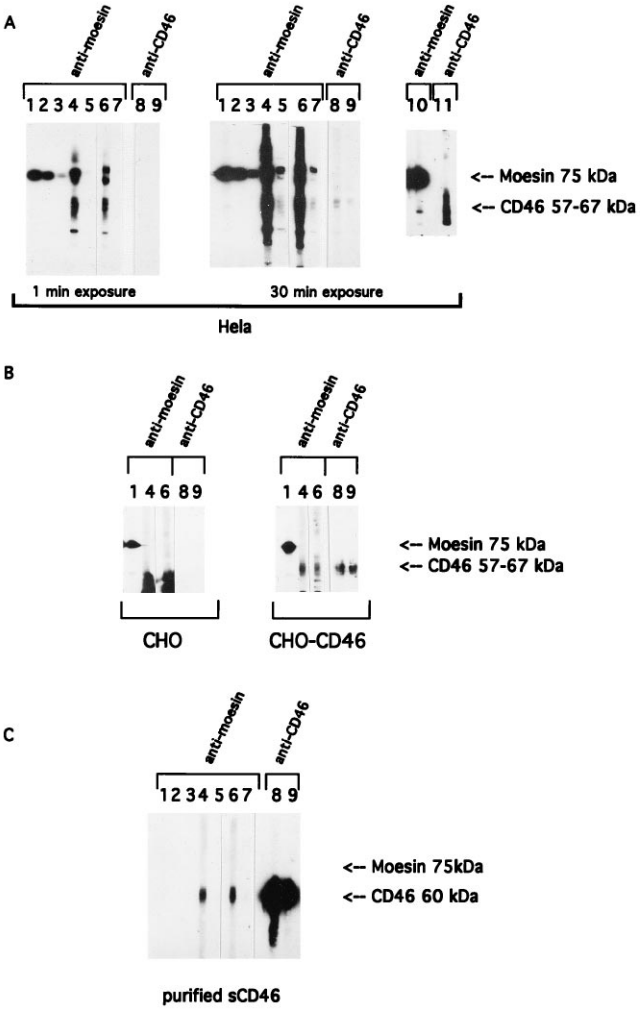

FIG. 3. Western blot analysis of cellular extract from 10^5 human HeLa cells (A), 2×10^5 hamster CHO cells (B, left autoradiograph), 2×10^5 CHO-CD46 cells (B, right autoradiograph), and 500 ng of purified sCD46 (C) after 10% polyacrylamide gel electrophoresis in nonreducing conditions (panel A, lanes 1 to 9, and panel B) or 8% polyacrylamide gel electrophoresis in reducing conditions (panel A, lanes 10 and 11). The blots were labelled with serial dilutions of purified antimoesin MoAb38/87 (lanes 1 to 3 and 10), ascites fluid antimoesin MoAb38/87 (lanes 4 and 5), ascites fluid antimoesin MoAb70/35 (lanes 6 and 7), purified anti-CD46 MoAbJ4.48 (lanes 8 and 9), or MoAbGB24 (lane 11). In panel A the length of exposure is indicated below each autoradiograph.

how moesin could be involved in the entry of MV into target cells will await the availability of cells devoid of the expression of moesin in which CD46 and moesin can be separately expressed.

We thank J. Schneider-Schaulies, R. Schwartz-Albiez, C. Doyle, B. Loveland, and R. Cattaneo for providing some reagents.

This work was supported in part by grants from CNRS (ATIPE, DG) and MENESR (ACC.SV). P.D. is supported by a fellowship from the Fondation Marcel Mérieux.

REFERENCES

1. **Buchholz, C. J., U. Schneider, P. Devaux, D. Gerlier, and R. Cattaneo.** 1996. Cell entry by measles virus: long hybrid receptors uncouple binding from membrane fusion. J. Virol. **70:**3716–3723.
2. **Christiansen, D., J. Milland, B. R. Thorley, I. F. C. Mckenzie, P. L. Mottram, L. J. Purcell, and B. E. Loveland.** 1996. Engineering of recombinant soluble CD46: an inhibitor of complement activation. Immunology **87:**348–354.
3. **Devaux, P., B. Loveland, D. Christiansen, J. Milland, and D. Gerlier.** 1996. Interactions between the ectodomains of haemagglutinin and CD46 as a primary step in measles virus entry. J. Gen. Virol. **77:**1477–1481.
4. **Dörig, R. E., A. Marcil, A. Chopra, and C. D. Richardson.** 1993. The human CD46 molecule is a receptor for measles virus (Edmonston strain). Cell **75:** 295–305.
5. **Dunster, L. M., J. Schneider-Schaulies, S. Löffler, W. Lankes, R. Schwartz-Albiez, F. Lottspeich, and V. ter Meulen.** 1994. Moesin: a cell membrane protein linked with susceptibility to measles virus infection. Virology **198:** 265–274.
6. **Dunster, L. M., J. Schneider-Schaulies, M. H. Dehoff, V. M. Holers, R. Schwartz-Albiez, and V. ter Meulen.** 1995. 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. J. Gen. Virol. **76:**2085–2089.
7. **Gerlier, D., V. Varior-Krishnan, and P. Devaux.** 1995. CD46-mediated measles virus entry: a first key to host-range specificity. Trends Microbiol. **3:**338–345.
8. **Iwata, K., T. Seya, Y. Yanagi, J. M. Pesando, P. M. Johnson, M. Okabe, S. Ueda, H. Ariga, and S. Nagasawa.** 1995. Diversity of sites for measles virus binding and for inactivation of complement c3b and c4b on membrane cofactor protein CD46. J. Biol. Chem. **270:**15148–15152.
9. **Kinch, M. S., A. Sanfridson, and C. Doyle.** 1994. The protein tyrosine kinase p56lck regulates cell adhesion mediated by CD4 and major histocompatibility complex class II proteins. J. Exp. Med. **180:**1729–1739.
10. **Lankes, W., A. Grioeshmacher, J. Grünwald, R. Schwartz-Albiez, and R. Keller.** 1988. A heparin-binding protein involved in inhibition of smooth-muscle cell proliferation. Biochem. J. **251:**831–842.
11. **Lankes, W. T., and H. Furthmayr.** 1991. Moesin: a member of the protein 4.1-talin-ezrin family of proteins. Proc. Natl. Acad. Sci. USA **88:**8297–8301.
12. **Loveland, B. E., R. W. Johnstone, S. M. Russell, B. R. Thorley, and I. F. C. McKenzie.** 1993. Different membrane cofactor protein (CD46) isoforms protect transfected cells against antibody and complement mediated lysis. Transplant. Clin. Immunol. **1:**101–108.
13. **Maisner, A., J. Schneider-Schaulies, M. K. Liszewski, J. P. Atkinson, and G. Herrler.** 1994. Binding of measles virus to membrane cofactor protein (CD46): importance of disulfide bonds and *N*-glycans for the receptor function. J. Virol. **68:**6299–6304.
14. **Manchester, M., A. Valsamakis, R. Kaufman, M. K. Liszewski, J. Alvarez, J. P. Atkinson, D. M. Lublin, and M. B. A. Oldstone.** 1995. Measles virus and c3 binding sites are distinct on membrane cofactor protein (CD46). Proc. Natl. Acad. Sci. USA **92:**2303–2307.
15. **Naniche, D., G. Varior-Krishnan, F. Cervoni, T. F. Wild, B. Rossi, C. Rabourdin-Combe, and D. Gerlier.** 1993. Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. J. Virol. **67:**6025–6032.
16. **Schneider-Schaulies, J., L. M. Dunster, R. Schwartz-Albiez, G. Krohne, and V. ter Meulen.** 1995. Physical association of moesin and CD46 as a receptor complex for measles virus. J. Virol. **69:**2248–2256.