JOURNAL OF VIROLOGY, Oct. 1997, p. 7969–7973 Vol. 71, No. 10
0022-538X/97/$04.00+0

CD46 Expression Does Not Overcome the Intracellular Block of Measles Virus Replication in Transgenic Rats

STEFAN NIEWIESK,[1]* JÜRGEN SCHNEIDER-SCHAULIES,[1] HARTMUT OHNIMUS,[1] CHRISTIAN JASSOY,[1] SIBYLLE SCHNEIDER-SCHAULIES,[1] LISA DIAMOND,[2] JOHN S. LOGAN,[2] AND VOLKER TER MEULEN[1]

Institut für Virologie und Immunbiologie, Universität Würzburg, 97078 Würzburg, Germany,[1] *and Nextran, Princeton, New Jersey 08540*[2]

Received 18 April 1997/Accepted 27 June 1997

The study of measles pathogenesis and the testing of improved vaccine candidates is hampered by the lack of a small animal model which is susceptible to infection by the intranasal route. With the identification of CD46 as a measles virus (MV) receptor, it was feasible to generate transgenic rats to overcome this problem. Although there was widespread expression of CD46 in the transgenic Sprague-Dawley rats, no measles-like disease could be induced after various routes of infection. The expressed transgenic protein was functionally intact since it mediated MV fusion and was downregulated by contact with MV hemagglutinin. In vitro studies revealed that CD46-expressing rat fibroblasts take up MV but do not allow viral replication, which explains the nonpermissiveness of the transgenic rats for in vivo infection.

Although the application of a live measles virus (MV) vaccine has reduced the worldwide incidence of MV infection, measles is still the leading cause of infant death in developing countries. The failure to control measles has many reasons: in particular, lack of understanding of the biological properties of MV, the immunologic and immunopathogenic response to both infections and vaccinations, and the epidemiology of measles in different regions of the world in relation to vaccination strategies. In this context, the study of MV pathogenesis and the testing of vaccine candidates has been hampered by the lack of an inexpensive small animal model. In the past it has been shown that in hamsters (2), mice (6, 18) and rats (11) an encephalitis can be induced with a rodent-adapted neurotropic strain of MV, but the infection does not spread to the periphery (6, 15). Recently, the membrane cofactor protein (CD46) has been identified as the receptor for MV (4, 16). CD46, a widely expressed surface glycoprotein, is a member of the complement regulatory protein family and serves to downregulate complement activation on autologous cells by binding to C3b and C4b (12). During MV infection the hemagglutinin binds to CD46 and the process of virus uptake is initiated (3, 13, 14). Depending on the MV strain used, the receptor is downregulated from the cell surface after contact with the agent (9, 17, 22). Although CD46 is expressed in different isoforms in various tissues, all of them are able to function as a receptor for MV (14, 24). Since CD46 or closely related molecules are exclusively expressed on primate cells (for a review, see reference 5), human and monkey cell lines are easily infectable with MV. However, it has also been observed that some rodent cell lines are susceptible to MV infection per se or after transfection with CD46 (4, 16, 19). Based on these observations it has been proposed that the generation of CD46 transgenic animals may result in a small animal model permissive for MV infection which would be useful in the study of MV infection (5).

* Corresponding author. Mailing address: Institut für Virologie und Immunbiologie, Versbacher Str. 7, 97078 Würzburg, Germany. Phone: 49 931 201 3441. Fax: 49 931 201 3934.

Generation of CD46-transgenic rats and MV infection. After intracerebral infection Sprague-Dawley (SD) rats were found to be susceptible to MV-induced encephalitis. The neuropathological changes, the virological findings, weight loss, and mortality observed were as described by us for Lewis rats (11). As brain tissue of young SD rats has the capacity to support virus replication in vivo, we attempted to render SD rats susceptible to peripheral MV infection by expressing the MV receptor CD46 as a transgenic protein.

After microinjection of a 35-kb human CD46 genomic clone (3a), three founder animals were obtained and bred further. Line 993 rats expressed the full-length 3.2-kb message, and their peripheral blood lymphocytes expressed the human MCP protein (data not shown). High levels of human CD46 mRNA (Fig. 1a) and protein (Fig. 1b) expression were found in liver, spleen, heart, kidney, lung, intestine, muscle, blood, and brain tissues from transgenic rats at levels comparable to what is seen in human tissues. As expected, nontransgenic rat RNA does not hybridize to the human CD46 probe under the conditions used. The level of CD46 surface expression on peripheral blood lymphocytes of transgenic rats as measured by flow cytometry was comparable to that of humans (data not shown) and was expressed on all cells. Functional activity of human CD46 expressed as transgenic protein was demonstrated by the protection of transgenic white blood cells from complement-mediated lysis upon exposure to human serum (data not shown).

CD46 transgenic protein is fully functional and interacts with MV hemagglutinin. In MV infection, the viral hemagglutinin (H) binds to CD46 which, in the presence of the fusion protein (F), leads to fusion (24). To test whether the transgenic protein interacted with MV H, we incubated spleen and lymph node cells from transgenic animals with Epstein-Barr virus-transformed human B-lymphoblastoid cells expressing MV H and F by means of a vaccinia virus recombinant. After 4 h of contact, fusion between the two cell types (which differ in size) could be seen (data not shown). No fusion was observed with nontransgenic lymphocytes or B-lymphoblastoid cells expressing the reverse transcriptase of the human immunodeficiency virus or MV H or F separately.

Another consequence of contact between CD46 and the H

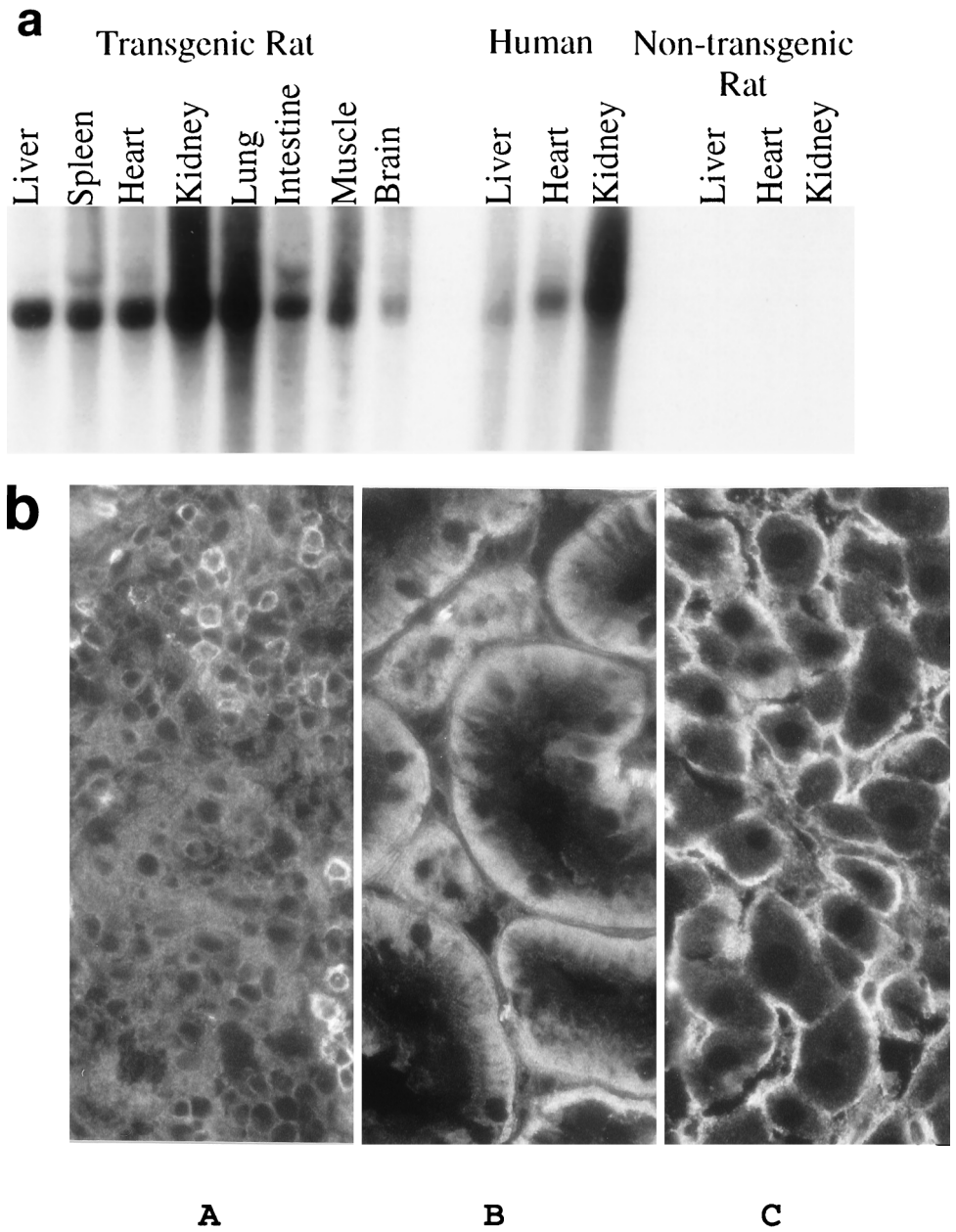

FIG. 1. Expression of CD46 mRNA and protein in various tissues of transgenic rats. (a) CD46 mRNA expression was demonstrated in organs from transgenic rats and humans but not in tissues from nontransgenic animals. Twenty micrograms of total RNA was electrophoresed on a 1% agarose-formaldehyde gel and transferred to a nylon filter. The filter was hybridized with an (α-^{32}P)dCTP-labeled probe. The probe was a 1.3-kb DNA fragment corresponding to the human CD46 cDNA, with the 5′ end beginning 40 bp upstream of the first ATG of the CD46 gene. (b) Paraffin-embedded sections of spleen (A), kidney (B) and liver (C) tissues from transgenic SD rats were stained with anti-CD46 monoclonal antibody (clone 122-2 from Biodesign) Magnification, × 40.

of the Edmonston virus strain is downregulation of the CD46 receptor molecule, which is not seen with the lymphotropic MV WTF strain (1, 10, 20). Spleen cells from transgenic rats were incubated with BJAB cells persistently infected with the Edmonston (BJAB-pED) or WTF (BJAB-pWTF) virus strain at a ratio of 1 to 1 for 2 h. Contact of BJAB-pED with CD46 transgenic lymphocytes led to rapid downregulation of CD46 on the rat cells, whereas contact with BJAB-pWTF did not (Fig. 2). These experiments demonstrate that CD46 expressed on transgenic cells interacts with the MV envelope protein as found for human cells and is fully functional.

Infection of CD46 transgenic rats does not lead to virus replication. To test the susceptibility of transgenic rats to MV infection, animals were infected intratracheally and intraperitoneally with MV isolates (Edmonston, wild-type isolate Chicago 1, and the rat-adapted MV CAM/RBH) and lung and spleen tissues were examined by cocultivation for infectious virus and reverse transcription-PCR (RT-PCR) for viral RNA. Between 1×10^6 and 2×10^7 PFU per inoculum was used for intraperitoneal infection and 1×10^6 to 3×10^6 was used for intratracheal infection. Animals were sacrificed on days 4, 7, and 10 after infection. Up to 7 days after inoculation, lung as

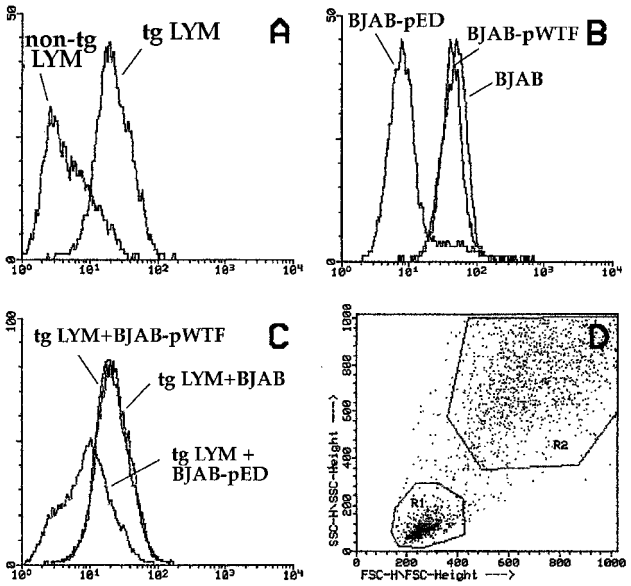

FIG. 2. CD46 expressed on transgenic lymphocytes is downregulated by contact with MV strain Edmonston-infected BJAB cells. (A) The level of expression of CD46 on spleen cells from transgenic rats (tg LYM) and spleen cells from nontransgenic rats (non-tg LYM) is shown by staining with the 13/42 monoclonal antibody (21) and subsequent analysis by cytometry. (B) Uninfected BJAB cells (immortalized human B-cell line) and BJAB cells persistently infected with the wild-type MV(WTF) (BJAB-pWTF) express normal levels of CD46, whereas BJAB cells persistently infected with the MV vaccine strain Edmonston (BJAB-pED) downregulate CD46. (C) Spleen cells from transgenic and nontransgenic spleen cells show a similar pattern of CD46 expression after 2 h of incubation with BJAB-pWTF and BJAB-pED. (D) Rat lymphocytes can be distinguished as small cells in the dot blot (lower left) and from the bigger BJAB (upper right).

well as spleen tissue was found to be positive on RT-PCR (data not shown). In spite of the presence of viral RNA, no viable virus could be recovered by cocultivation (data not shown). In CD46 transgenic rats, no clinical signs of disease, weight loss, or mortality were observed.

In brain tissue, high levels of CD46 mRNA were seen by Northern blotting (Fig. 1a) and RT-PCR (data not shown) and protein expression was seen by immunocytochemistry (data not shown). However, after intracerebral infection transgenic animals developed no enhanced encephalitis (assessed by clin-

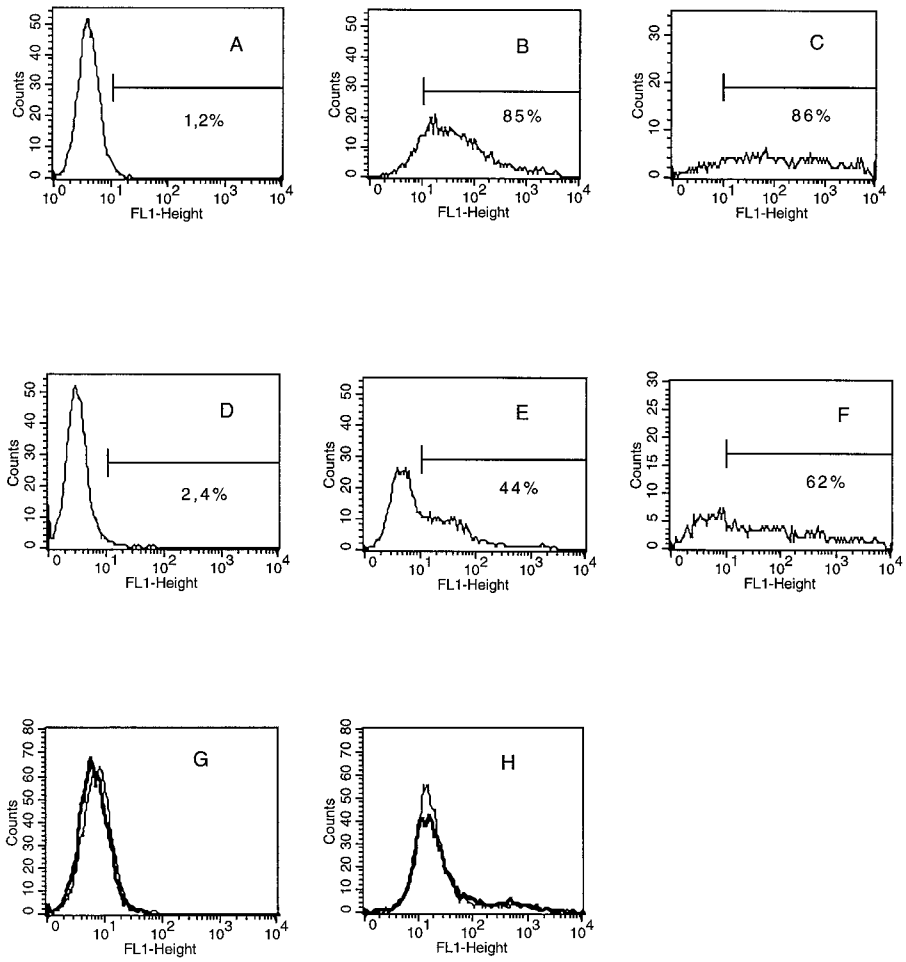

FIG. 3. Binding and internalization of MV by CD46-expressing fibroblasts. CD46-expressing (A to C) and CD46-negative (D to F) rat fibroblasts were infected for 1 h with MV strain Edmonston (multiplicity of infection [MOI] of 5), fixed, and stained for the surface expression of hemagglutinin (H) (B and E). After permeabilization, the cells were stained for the presence of the nucleocapsid (N) protein (C and F). Uninfected cells served as controls (A and D). CD46-expressing (thin line) and CD46-negative (thick line) rat fibroblasts were infected for 1 h with a recombinant MV in which the H and F glycoproteins are replaced by the G protein of VSV(MGV) (23) (MOI of 1), fixed, permeabilized, and stained for N (H). Uninfected cells served as a control (G).

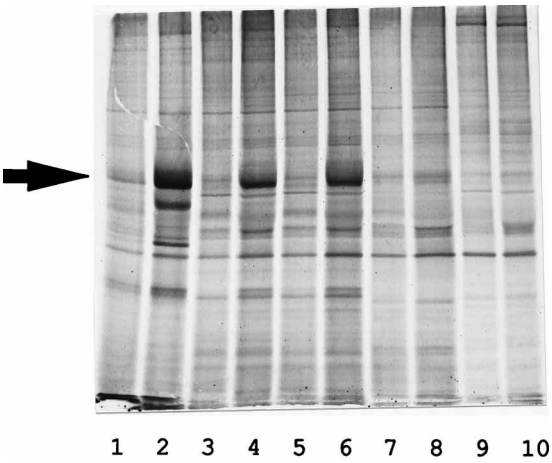

FIG. 4. Immunoprecipitation of MV nucleocapsid protein from infected CD46 transgenic fibroblasts. Cells were infected overnight with the recombinant MGV in which the H and F glycoproteins are replaced by the G protein of VSV (23) and labeled with [^{35}S]methionine for 6 h. The N protein (arrow) was precipitated from cell lysates with a human MV-hyperimmune serum. Immunoprecipitations of infected cells are shown in lanes 2, 4, 6, 8, and 10, and uninfected controls are in lanes 1, 3, 5, 7, and 9. Vero cells (lanes 1 and 2), CHO cells expressing CD46 (lanes 3 and 4), and normal (CD46-negative) CHO cells (lanes 5 and 6) were expected to replicate MGV. SD fibroblasts either expressing CD46 (lanes 7 and 8) or not (lanes 9 and 10) did not allow the replication of MGV.

ical signs, neuropathological changes, virological findings, weight loss, and mortality).

Viral replication, not uptake, is inhibited. To ascertain whether the block of viral replication was due to impaired viral entry or to inhibiting factors within the cell, we infected rat fibroblasts isolated from transgenic and nontransgenic animals with MV for 1 h and then stained the cells with an H-specific antibody. FACScan analysis demonstrated clearly binding of MV to transgenic fibroblasts (Fig. 3B). Another portion of infected cells was permeabilized and subsequently internally stained with an MV nucleocapsid (N)-specific antibody. Again, the presence of MV could be demonstrated (Fig. 3C). This indicates that MV does not only bind to transgenic rat fibroblasts but is also taken up by the cells. In comparison, nontransgenic fibroblasts also take up MV, but to a lesser extent (Fig. 3D to F), and antibodies to CD46 do not completely abolish uptake (data not shown), suggesting that binding and uptake are enhanced by the expression of CD46, but do not solely depend on it. As both transgenic and nontrangenic fibroblasts permit the replication of vesicular stomatitis virus (VSV) equally well (data not shown), we used a recombinant MV in which the H and F glycoproteins are replaced by the G protein of VSV(MGV) (23). MGV is replication competent, and a broad tissue specificity is conferred by the G protein. Therefore, viral uptake is independent of CD46 as demonstrated by CHO cells which lack the CD46 receptor molecule and cannot be infected with MV (4) but allow replication of MGV (Fig. 4, lanes 5 and 6). Fibroblasts expressing CD46 as well as CD46-negative fibroblasts were shown to be infected to the same extent with MGV (Fig. 3G and H).

To confirm that there is no block of viral uptake we used MGV to infect Vero cells, which are susceptible to MV infection, and CHO cells, which replicate MV only after transfection with CD46 (4). MGV was shown to replicate independently of CD46 expression in all three cell types (Vero, CHO, and CHO transfected with CD46) (Fig. 4, lanes 1 to 6). In contrast, no replication of MGV is seen by immune precipitation in SD transgenic or nontransgenic fibroblasts (Fig. 4, lanes 7, 8, 9, and 10).

In summary, our data demonstrate that the expression of a functional CD46 molecule is not sufficient to render rats susceptible to MV infection. Although MV is taken up by the cells it does not replicate, indicating a block of replication by intracellular factors. Our data also indicate that in rat cells there is an additional mode of entry for MV. These data are in agreement with those of Horvat and colleagues (7, 8) who have reported that the expression of CD46 in transgenic mice does not render them susceptible to MV infection and that an alternative receptor in mice seems to be implicated in viral uptake.

We thank Martin Billeter and Hussein Y. Naim for providing VSV(MGV) and information about it prior to publication.

S.N. was supported by Stipendienprogramm Infektionsforschung (Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie). This work was supported in part by Deutsche Forschungsgemeinschaft and Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie.

REFERENCES

1. **Bartz, R., U. Brinckmann, L. M. Dunster, B. Rima, V. ter Meulen, and J. Schneider-Schaulies.** 1996. Mapping amino acids of the measles hemagglutinin responsible for receptor (CD46) downregulation. Virology **224:**334–337.
2. **Burnstein, T., J. H. Jensen, and B. H. Waksman.** 1963. The development of a neurotropic strain of measles virus in hamsters and mice. J. Infect. Dis. **113:**265–272.
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.

3a. **Diamond, L.** Unpublished data.

4. **Doerig, 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. **Doerig, R. E., A. Marcil, and C. D. Richardson.** 1994. CD46, a primate-specific receptor for measles virus. Trends Microbiol. **2:**312–318.
6. **Griffin, D. E., J. Mullinx, O. Narayan, and R. T. Johnson.** 1974. Age dependence of viral expression: comparative pathogenesis of two rodent-adapted strains of measles virus in mice. Infect. Immun. **9:**690–695.
7. **Horvat, B., P. Rivailler, G. Varior-Krishnan, A. Cardoso, D. Gerlier, and C. Rabourdin-Combe.** 1996. Transgenic mice expressing human measles virus (MV) receptor CD46 provide cells exhibiting different permissivities to MV infection. J. Virol. **70:**6673–6681.
8. **Horvat, B., G. Varior-Krishnan, P. Rivailler, A. Cardoso, M. C. Trescol-Biemont, F. Wild, D. Gerlier, and C. Rabourdin-Combe.** 1995. Characterization of transgenic mice expressing human measles virus receptor, p. 364. *In* Abstracts of the 9th International Congress of Immunology. San Francisco, Calif.
9. **Krantic, S., C. Gimenez, and C. Rabourdin-Combe.** 1995. Cell-to-cell contact via measles virus haemagglutinin-CD46 interaction triggers CD46 downregulation. J. Gen. Virol. **76:**2793–2800.
10. **Lecouturier, V., J. Fayolle, M. Caballero, J. Carabana, M. L. Celma, R. Fernandez-Munoz, T. F. Wild, and R. Buckland.** 1996. Identification of two amino acids in the hemagglutinin glycoprotein of measles virus (MV) that govern hemadsorption, HeLa cell fusion, and CD46 downregulation: phenotypic markers that differentiate vaccine and wild-type MV strains. J. Virol. **70:**4200–4204.
11. **Liebert, U. G., and V. ter Meulen.** 1987. Virological aspects of measles virus-induced encephalomyelitis in Lewis and BN rats. J. Gen. Virol. **68:** 1715–1722.
12. **Liszewski, M. K., T. W. Post, and J. P. Atkinson.** 1991. Membrane cofactor protein (MCP or CD46): newest member of the regulators of complement activation gene cluster. Annu. Rev. Immunol. **9:**431–455.
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 determinant. J. Virol. **68:**6299–6304.
14. **Manchester, M., M. K. Liszweski, J. P. Atkinson, and M. B. Oldstone.** 1994. Multiple isoforms of CD46 (membrane cofactor protein) serve as receptors for measles virus. Proc. Natl. Acad. Sci. USA **91:**2161–2165.
15. **McFarland, H. F.** 1974. The effect of measles virus infection on T and B lymphocytes in the mouse. J. Immunol. **113:**1978–1983.
16. **Naniche, D., G. Varior-Krishnan, G. Cervoni, T. F. Wild, B. Rossi, C. Rabourdin-Combe, and D. Gerlier.** 1993. Human membrane cofactor pro-

tein (CD46) acts as a cellular receptor for measles virus. J. Virol. **67:**6025–6032.
17. **Naniche, D., T. F. Wild, C. Rabourdin-Combe, and D. Gerlier.** 1993. Measles virus haemagglutinin induces down-regulation of gp57/67, a molecule involved in virus binding. J. Gen. Virol. **74:**1073–1079.
18. **Niewiesk, S., U. Brinckmann, B. Bankamp, S. Sirak, U. G. Liebert, and V. ter Meulen.** 1993. Susceptibility to measles virus-induced encephalitis in mice correlates with impaired antigen presentation to cytotoxic T lymphocytes. J. Virol. **67:**75–81.
19. **Nussbaum, O., C. C. Broder, B. Moss, L. B. Stern, S. Rozenblatt, and E. A. Berger.** 1995. Functional and structural interactions between measles virus heamagglutinin and CD46. J. Virol. **69:**3341–3349.
20. **Schneider-Schaulies, J., L. M. Dunster, F. Kobune, B. Rima, and V. ter Meulen.** 1995. Differential downregulation of CD46 by measles virus strains. J. Virol. **69:**7257–7259.
21. **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.
22. **Schneider-Schaulies, J., J.-J. Schnorr, U. Brinckmann, L. M. Dunster, K. Baczko, U. G. Liebert, S. Schneider-Schaulies, and V. ter Meulen.** 1995. Receptor usage and differential downregulation of CD46 by measles virus wild type and vaccine strain. Proc. Natl. Acad. Sci. USA **92:**3943–3947.
23. **Spielhofer, P., T. Bächi, G. Christiansen, R. Cattaneo, K. Kaelin, M. Billeter, and H. Y. Naim.** Engineering and characterization of a chimeric measles virus containing the vesicular stomatitis virus G protein instead of its own envelope glycoproteins. Submitted for publication.
24. **Varior-Krishnan, G., M.-C. Trescol-Biemont, D. Naniche, C. Rabourdin-Combe, and D. Gerlier.** 1994. Glycosyl-phosphatidylinositol-anchored and transmembrane forms of CD46 display similar measles virus receptor properties: virus binding, fusion, and replication; down-regulation by hemagglutinin; and virus uptake and endocytosis for antigen presentation by major histocompatibility complex class II molecules. J. Virol. **68:**7891–7899.