Brief Definitive Report

Efficient Major Histocompatibility Complex Class II-restricted Presentation of Measles Virus Relies on Hemagglutinin-mediated Targeting to Its Cellular Receptor Human CD46 Expressed by Murine B Cells

By Denis Gerlier, Marie-Claude Trescol-Biémont, Gayathri Varior-Krishnan, Denise Naniche, Isabelle Fugier-Vivier, and Chantal Rabourdin-Combe

From the Laboratoire d'Immunobiologie Moléculaire, UMR 49, Centre National de la Recherche Scientifique-Ecole Normale Supérieure de Lyon, 69364 Lyon Cedex 07, France

Summary

Measles virus after binding to its cell surface human CD46 receptor fuses with the plasma membrane. This fusion results in envelope hemagglutinin (H) and fusion glycoprotein (F) incorporated into the plasma membrane and injection of the nucleocapsid made of nucleoprotein (NP) into the cytosol. The influence of targeting measles virus (MV) to CD46 in the processing and presentation of MV H and NP to antigen specific MHC class II I-E^d– and I-A^d–restricted T cell hybridomas was explored using murine M12-CD46 B cell transfectants. Parent M12 cells, which lack any MV receptor, were unable to present any of these two viral proteins when incubated with MV particles. Incubating M12.CD46 cells with 200 ng and 10 μg of MV could strongly stimulate H-specific and NP-specific T cells, respectively. Neosynthesis of MV proteins was not necessary since the efficiency of antigen presentation was similar when using ultraviolet-inactivated MV. Similar enhancing effects (more than 1,000-fold) on antigen presentation were also observed when using purified native H soluble or incorporated into liposomes whereas denaturating H glycoprotein resulted in a poor efficiency in T cell stimulation, M12.CD46 being no more potent than the parental M12 counterpart. MV H and NP presentation efficiency did not depend on MV fusion with plasma membrane as revealed by the lack of effect of specific fusion inhibitors. Both MV H and NP presentations were sensitive to chloroquine inhibition indicating that antigens from CD46-mediated captured MV were likely processed in the endosome/lysosome compartment. Altogether these data indicate that (*a*) MV targeting via CD46 has a strong effect on the efficiency of antigen presentation by MHC class II, (*b*) the effect is mediated by the binding of H to CD46, and (*c*) though MV does fuse with plasma membrane, endocytosis, and processing of virus particles are also occurring. Since, in humans, CD46 is expressed in almost every tissue including professional antigen-presenting cells, such a targeting is likely to play a crucial role in the $CD4^+$ T cell–mediated primary immune response against the pathogen in vivo.

Targeting a soluble exogenous antigen to antigen-specific B cells via their cell surface Ig (1, 2) or to macrophages via their FcR after opsonization with specific antibodies (3) result in a strong enhancement of MHC class II–restricted antigen presentation to $CD4^+$ T cells. Therefore, the specific cellular receptor used by an intracellular pathogen to enter hosts' cells should play a crucial role in the induction of the immune response by facilitating the pathogen capture by the APC and the presentation of pathogen-derived antigens by MHC class II molecules to specific T cells. We have recently identified the human CD46 molecule (membrane cofactor protein), a regulator of the complement activation (4), as a receptor for measles virus (MV) (5, 6). MV is a pleiomorphic, enveloped and negative, single strand RNA virus belonging to the Morbillivirus genus and Paramyxovirus family. The envelope is made of two glycoproteins, hemagglutinin (H) and fusion protein (F) responsible for MV attachment and fusion with the plasma membrane of target cells. This fusion results in H and F incorporation into the plasma membrane and in the delivery into the cytosol of the nucleocapsids essentially made of RNA and nucleoprotein (NP). Then viral replication can occur. Thus, MV infection does not require virus particle endocytosis and an acidic pH for fusion as is the case for influenza virus. MV is the causative agent of the measles disease in humans and can only infect human and some simian cells. Expression of CD46 allows mouse M12

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B cell transfectants (*a*) to bind MV, (*b*) to fuse to each other to form syncitium when both measles H and F glycoprotein are expressed after infection with MV or with recombinant vaccinia virus encoding H and F glycoproteins, and (*c*) to replicate MV (6). The aim of the present work was (*a*) to look for a receptor-mediated enhancement of MHC class II–restricted H and NP presentation when MV is targeted to CD46, (*b*) to test the ability of H to bind to CD46, and (*c*) to determine whether after CD46 targeting of MV, H, and/or NP antigen presentation by MHC class II involves an endosomal pathway although MV can fuse at the plasma membrane.

Materials and Methods

Measles Virus, Viral Proteins, and Antibodies. MV (Halle strain) was produced and purified as previously described (7). In some experiments, MV was UV-irradiated before use by 15 min exposure at 5 cm from a 254-nM UV-lamp. UV-treatment abolished transcription and replication of the viral genome without inhibiting its binding and fusion abilities. Native H glycoprotein was purified from Vero cells infected with MV and incorporated into liposomes as previously described (7). Denaturated H glycoprotein was purified under strong denaturing conditions in the presence of sodium dodecylsulfate and mercaptoethanol from insect cells infected with a recombinant baculovirus encoding H glycoprotein according to a procedure to be described elsewhere (Cretin F., I. Chrétien, I. Fugier-Vivier, D. Gerlier, and C. Rabourdin-Combe, in preparation). It differed from the purified native H by its lack of reactivity with conformational anti-H mAbs such as cl55 and it was no longer in dimeric form due to the use of reducing conditions during purification. A cytosolic extract of High-Five insect cells infected with a recombinant baculovirus encoding measles NP was used as a source of crude NP (BI-NP). High-Five insect cells were infected with 5 PFU/cell of recombinant AcNPVN baculovirus obtained according to a published procedure (8). 3 d later, the cells were washed, pelleted, and disrupted in 1 mM EDTA, 0.5 mM Tris/HCl buffer, pH 7.5, supplemented with a cocktail of antiproteases for 0.5 h on ice. After centrifugation at 10,000 rpm for 1 h, the supernatant was kept frozen at −20°C until use. NP usually represents between 50 and 80% of the total protein present in the cell extract. For some experiments, NP was immunopurified after adsorption on a batch of anti-NP cl25 monoclonal antibody coupled to CNBr activated Sepharose 4B (Pharmacia, Uppsala, Sweden) and elution using 0.2 M HCl-glycine buffer, pH 2.5. The purity of the NP preparation was assessed after SDS-PAGE electrophoresis and silver staining. MV H-, NP-, and F-specific antibodies (cl55, cl25, and B22-F5, respectively) were kindly provided by T. F. Wild and P. Giraudon (Institut Pasteur de Lyon and Université de Lyon, France, respectively) (9, 10).

Cell Lines. M12.CD46 cells expressing human CD46 have been obtained after transfection with the pIRV-CD46 expression vector of the mouse B lymphoma M12.4.1 cells (6). The H-specific and I-E^d–restricted T cell hybridoma TH5.124 has been previously described (11). The NP-specific and I-A^d–restricted TNP.408 T cell hybridoma was isolated using a procedure previously described (11).

T Cell Stimulation Assay. Antigen stimulation of the T cell hybridomas was performed by cocultivating 10^5 hybridoma T cells with 3×10^4 H-2^d M12.4.1 or M12.CD46 B cells in the presence or absence of serial dilutions of antigen in a final volume of 200 μl in 96-well microplates. In some experiments, M12.HELs3 cells that secrete hen egg lysozyme (11) were used instead of M12.4.1 cells. The secretion of lysozyme did not interfere with the bioassay. After incubation for 20 h at 37°C, IL-2 production in supernatants was measured using the MTT colorimetric CTL-L2 bioassay (11). All the experiments were repeated several times with similar results and the standard deviation between replicates was usually well below 5%. Some experiments were done in the presence of 50 μM chloroquine added to APCs before the antigen and maintained throughout the bioassay as described elsewhere (12). These chloroquine concentrations were well tolerated by the cells and did not affect the CTL-L2 bioassay (12). In other experiments, 10 μM of the z-D-Phe-Phe-Gly and Gly-Phe-Phe tripeptides (Neosystèmes, Strasbourg, France) or cl55 anti-H and cl anti-F ascitis fluid in a final dilution of 1:50 were added during the T cell stimulation.

Results

Expression of CD46 by Murine APC Is Required for Efficient Presentation of Virus Proteins by MHC Class II Molecules. When incubated with infectious MV, M12.CD46 cells were very efficient in presenting H glycoprotein to the I-E^d–restricted TH5124, whereas M12 parent cells were unable to stimulate these T cells (Fig. 1 *a*). Incubating M12.CD46 cells with as few as 200 ng of virus was sufficient to stimulate TH5124 T cells. Viral replication and neosynthesis of H glycoprotein

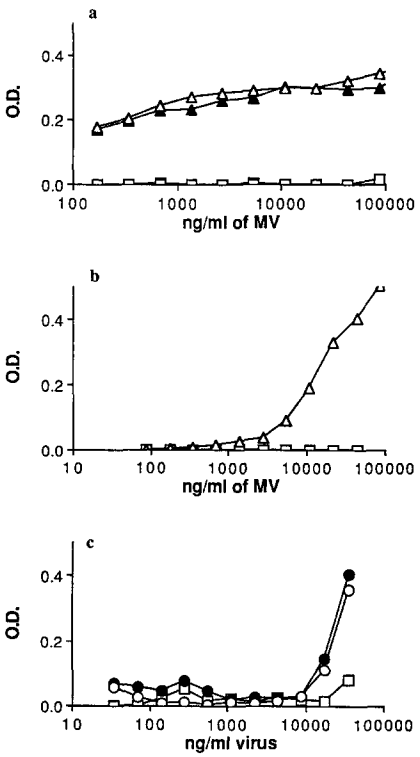

Figure 1. Presentation of CD46-targeted MV proteins to H-specific and NP-specific T cell hybridomas. Ability to M12 (*squares*) and M12.CD46 (*triangles*) cells to stimulate I-E^d–restricted and H-specific TH5.124 (*a* and *c*) or I-A^d restricted NP-specific TNP.408 (*b*) when fed with (*a* and *b*) infectious MV (*solid squares* and *triangles*) or UV-inactivated MV (*open squares* and *triangles*) or (*c*) UV-inactivated MV in the presence of anti-H (*solid circles*) or anti-F (*open circles*) antibodies. The T cell stimulation was measured by quantitating the IL-2 production using the CTLL-2 and MTT bioassay.

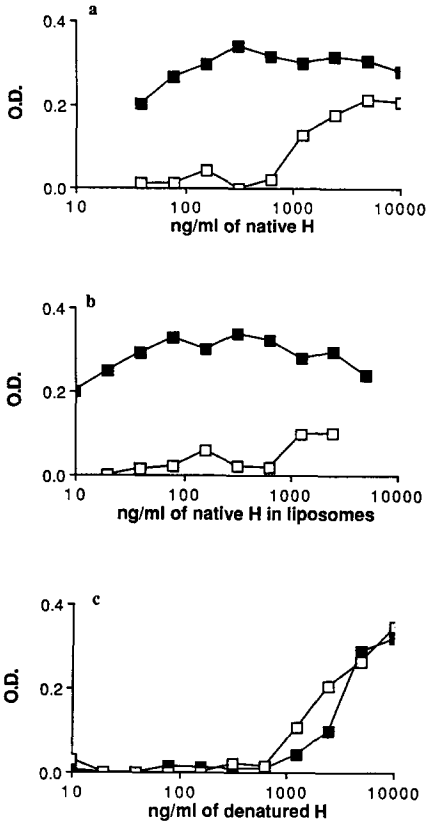

Figure 2. Efficiency of the CD46-mediated presentation to TH5.124 T cells of (*a*) purified native H, (*b*) purified native H in liposomes, or (*c*) purified denatured H by M12 (*open squares*) and M12.CD46 (*solid squares*).

known to occur in M12.CD46 cells (6) were not necessary for efficient presentation because T cells were stimulated as efficiently when MV was UV-inactivated (Fig. 1 *a*). Efficient presentation was not limited to the viral envelope H glycoprotein since NP-specific and I-A^d-restricted TNP.408 T cell hybridomas were stimulated in the presence of M12.CD46 but not M12 fed with UV-MV (Fig. 1 *b*). Allowing MV genome transcription and replication in M12.CD46 did not result in further enhancement of NP presentation (data not shown). These results indicated that MV captured via CD46 provided an optimal amount of both virions H and NP proteins for antigen processing and presentation by MHC class II molecules. In an attempt to mimic such a capture effect, M12 cells that express FcRγ (13) were incubated with MV in the presence of anti-H or anti-F mAbs.

Indeed, the opsonization of MV particles by antibodies resulted in some presentation of both viral structural proteins H (Fig. 1 *c*) and NP (data not shown) by MHC class II molecules. The efficiency of antibody-mediated capture of MV was however far lower than the CD46 mediated capture (compare Fig. 1 *a* and *c*) in agreement with the poor internalization of the FcRγIIb1 receptor isoform expressed in B cells (14).

CD46 Mediates Efficient Presentation of Purified Native But Not Denatured H Glycoprotein. The MV H envelope glycoprotein has long been thought to be the binding structure used by MV to attach to its target cells. To verify that MV H can directly bind to CD46, the efficiency of M12.CD46 to present purified native H glycoprotein to TH5.124 T cells was tested. Indeed, M12.CD46 requires ~100-fold less purified soluble H than its parental M12 counterpart to stimulate TH5.124 T cells with the same efficiency (Fig. 2 *a*). The difference was even higher when purified H were given to the APC as liposomes (Fig. 2 *b*). This was due both to the higher efficiency of M12.CD46 cells and to the poor ability of M12 cells to present H in a particulate form as observed with MV particles. M12.CD46 was so efficient at capturing H-liposomes that as little as 10 ng/ml of H glycoprotein (i.e., an H concentration around 0.06 pM since the M_r of H homodimer is ~160) was sufficient to give a strong stimulatory signal to TH5.124 T cells. This antigen presentation efficiency was comparable to the high efficiency of endogenous H presentation by M12.H transfectants (data not shown and 11). This was >100 times less than the amount of purified native H required by parental M12 cells to stimulate these T cells with the same efficiency (compare Fig. 2, *a* and *b*). In contrast, M12.CD46 and M12 cells were equally (and rel-

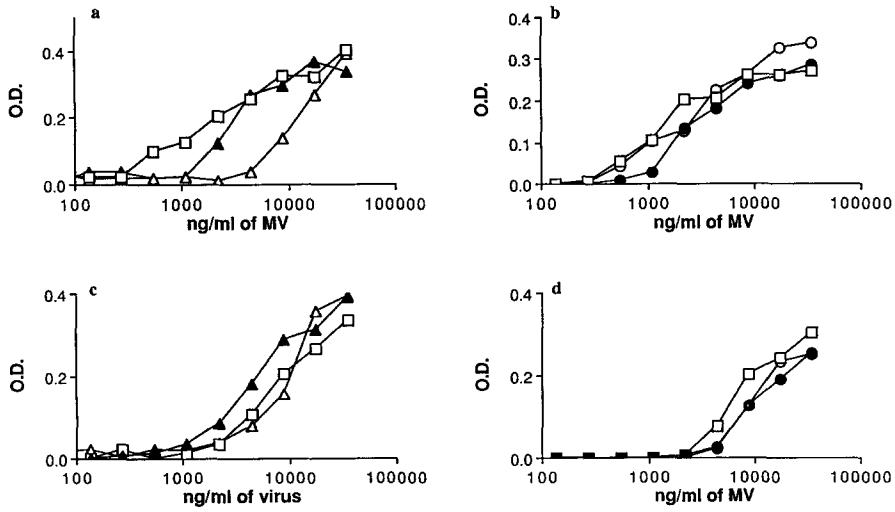

Figure 3. CD46-mediated efficient presentation of MV H (*a* and *b*) and NP (*c* and *d*) proteins is not sensitive to specific fusion inhibiting agents. M12.CD46 cells were incubated with various amounts of UV-MV alone (*open squares*), or (*a* and *c*) in the presence of anti-H (*open triangles*) and anti-F (*solid triangles*) antibodies, or (*b* and *d*) 10 μM of z-D-Phe-Phe-Gly (*solid circles*) and Gly-Phe-Phe (*open circles*) tripeptides, and used to stimulate TH5.124 (*a* and *b*) and TNP.408 (*c* and *d*) T cells.

atively poorly) efficient in presenting purified denatured H glycoprotein. In addition, the dose-response curves were similar to that observed for purified native H presented by M12 cells (compare Fig. 2, *a* and *c*). This indicates that native H glycoprotein has the ability to bind directly to CD46.

Efficient Presentation of MV Antigens Targeted to CD46 Does Not Require MV Fusion at the Cell Membrane but Involves a Chloroquine Sensitive Pathway. For cell infection, MV enters its target by fusing with the plasma cell membrane and does not require endocytosis. This fusion process involves both H and F glycoproteins, F glycoprotein having the fusogenic properties (6, 15). To test the role of the fusion, anti-F mAbs (10) and the tripeptide z-D-Phe-Phe-Gly (16) were used as specific fusion inhibitors. Whereas both anti-F antibodies and z-D-Phe-Phe-Gly tripeptide completely abrogated the H+F induced cell–cell and MV–cell fusion (data not shown) they left intact the efficiency of MV H and NP presentation by M12.CD46 to TH5.124 and TNP.408 T cell hybridomas (Fig. 3). The tripeptide Gly-Phe-Phe, which has no effect on MV-induced fusion, was used as a negative control. The lack of inhibition by the anti-F antibody could have been compensated for by the opsonizing effect and targeting to the M12 FcRγ. This is unlikely, since the efficiency of MV capture in the presence of anti-F is very limited (see Fig. 1 *c*). Surprisingly, when anti-H antibodies were used as a control, they were found to significantly decrease the efficiency of MV H presentation but not that of MV NP presentation (Fig. 3, *a* and *c*). This suggests that anti-H antibodies could specifically protect H glycoproteins from proteolytic degradation as previously observed by others (17).

Endosomal processing is inhibited by lysosomotropic agents, and the effect of chloroquine on the ability of M12.CD46 cells to present MV H and NP proteins was then tested. Presentations of H and NP from MV particles to TH5.124 and TNP.408 T cells, respectively, were almost completely abolished in the presence of 50 μM of chloroquine (Fig. 4). Similar results were obtained when using soluble NP, purified H in liposomes, or antibody-opsonized MV targeted to FcRγ of M12 cells (data not shown).

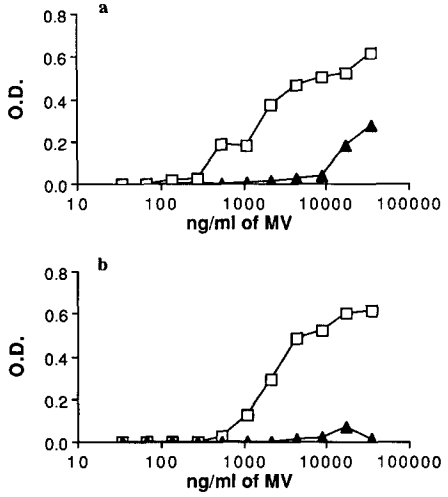

Figure 4. Chloroquine sensitivity of MV H and MV NP presentation by M12.CD46 cells. Purified native MV (*a* and *b*) were used to stimulate TH5.124 (*a*) or TNP.408 (*b*) T cells in the absence (*open squares*) or in the presence of 50-μM (*solid triangles*) chloroquine.

Discussion

By comparing mouse B cells devoid of cell surface expression of MV receptor with their counterparts expressing the human CD46, it is shown that targeting the virus to the APC via its cellular receptor dramatically increases (>1,000-fold) the efficiency of viral antigen presentation by MHC class II molecules. This effect is due to the efficient uptake of virus particle following the interaction of the viral envelope H glycoprotein and CD46 molecules as firstly reported here. It does not depend on the presentation of neosynthesized viral proteins since the UV-inactivation of MV did not change the efficiency of antigen presentation to $CD4^+$ T cells.

The coexpression of MV H, MV F, and CD46 glycoproteins is sufficient to induce fusion between two cell membranes leading to the formation of syncitia (6). This fusion process is likely to be similar to the fusion between MV envelope and cell membrane allowing the MV nucleocapsids to enter the cytosol where viral replication occurs (6). This raises the possibility that MV fusion at the cell surface of M12.CD46 might be involved in the efficiency of MV antigen presentation. All the experimental data that have been obtained so far argue against this hypothesis. (*a*) Potent and specific inhibitors of MV H–and F–mediated fusion after binding to CD46 virus receptor such as anti-F antibodies (10) and z-D-Phe-Phe-Gly tripeptide which is homologous to the hydrophobic NH_2 terminus of the F1 subunit of F glycoprotein (16) has no effect whatsoever on the ability of M12.CD46 cells to present MV proteins. (*b*) The CD46-mediated enhancing effect on MV antigen presentation was observed using purified soluble native H glycoprotein or H liposomes that, as shown previously (7), do not contain any detectable F glycoprotein strictly required for the fusion. Moreover, the chloroquine sensitivity of MV H and MV NP antigen presentation strongly suggests that the processing of these viral structural antigens occurs in an acidic endosomal/lysosomal compartment after receptor-mediated endocytosis of some MV particles although MV can fuse with plasma membrane. So, CD46-mediated enhancement of MV antigen presentation is likely to involve an endosomal processing pathway as observed for antigen targeted to a plasma membrane protein such as cell surface Ig of specific B cells or FcR of macrophages (1–3, 14, 17).

Comparison between soluble native H and H liposomes shows that the latter was more efficient in delivering H antigen for processing by M12.CD46 cells. A likely explanation is that the liposome vector allows the capture by a single APC of several H glycoproteins contained in one vesicle as soon as a single or few H glycoproteins have bound to CD46 molecules. This may partly explain the strong enhancing effect of liposome vector on the immunogenicity of a cell surface murine retrovirus glycoprotein previously reported by one of us (18). It has been previously reported that, in vitro, macrophages but not B cells can mediate presentation of (untar-

geted) liposome-associated antigen because B cells lack phagocytic activity (19, 20). Accordingly, M12 B cells were found to be almost unable to present MV antigens when in particulate form (virions, H liposome). In contrast, when H liposomes (and virions) were targeted to the cell surface CD46, M12.CD46 B cells became able to present H to MHC class II–restricted T cells with a high efficiency in agreement with the recent work of Grivel et al. (21) showing that B cells can endocytose targeted liposome-associated antigen. As a consequence, phagocytic activity of APCs may not be required for efficient presentation of particulate antigens by MHC class II provided that receptor-mediated binding and internalization can occur.

We hypothesize that, in vivo, efficient priming of $CD4^+$ T cell–mediated immune response could mainly rely on peptides derived from exogenous antigens targeted to the APC (1–3, 14, 17, this paper) and/or endogenous antigens synthesized and translocated into the endoplasmic reticulum within the APC (11). Human CD46 is expressed on every cell type except on erythrocytes (4, 6). Thus, human $CD46^+$ dendritic cells and macrophages that are likely to be the main APC involved in the in vivo primary stimulation of MHC class II–restricted $CD4^+$ T cells should be able to capture efficiently MV leading to a strong primary immune stimulation even if the amount of virus and/or its infectivity is limited.

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Address correspondence to Denis Gerlier, CNRS-ENS Lyon, UMR 49, 46 Allée d'Italie, 69364 Lyon CEDEX 07, France.

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