The ectodomain of measles virus envelope glycoprotein does not gain access to the cytosol and MHC class I presentation pathway following virus–cell fusion

Alicia I. Cardoso,1 Denis Gerlier,2 T. Fabian Wild3 and Chantal Rabourdin-Combe1

1 Immunobiologie Moléculaire, CNRS, UMR 49, École Normale Supérieure de Lyon, 46 Allée d’Italie, 69364 Lyon Cedex 07, France
2 Immunité et Infections Virales, IVMC, CNRS-UCBL, UMR 5537, Faculté de Médecine Lyon R. T. H. Laennec, 69372 Lyon Cedex 08, France
3 INSERM U 404 ‘Immunity and Vaccination’, Institut Pasteur de Lyon, Avenue Tony-Garnier, 69365 Lyon Cedex 07, France

To unravel the intracellular fate of measles virus (MV) haemagglutinin (H) following fusion of the virus envelope with the cell membrane, its presentation by MHC molecules to T cells was explored. After MV infection, murine cells expressing CD46 were lysed by MHC class I-restricted CD8 CTLs specific for the ectodomain of H. In contrast, when sensitized with UV-inactivated MV, they were not lysed by these effectors, but were recognized by H-specific and class II-restricted CD4 CTLs. Thus, after MV binding and fusion, H becomes associated with plasma membrane and its ectodomain can reach the endosomal MHC-II but not the cytosolic MHC-I antigen presentation pathway. From these data and a reappraisal of previous reports, it appears that the ectodomains of both MV haemagglutinin fusion proteins, having undergone the fusion step, are not translocated into the cytosol and end up in the endosomes.

Measles virus (MV) attaches to human host cells by binding to the CD46 receptor (Naniche et al., 1993) and the virus envelope fuses with the cell membrane at neutral pH (see Gerlier et al., 1995, for review). The haemagglutinin (H) binds to CD46 and the fusion protein (F) triggers the fusion process. This requires a precise scaffolding between H, F and CD46 so that the nucleocapsid can be injected into the cell without extracellular leakage of cytosol. An appropriate H and F structural pairing governs the efficiency of fusion (Wild et al., 1994) (see Gerlier et al., 1995, for review), and the distance between the H binding site on CD46 and its membrane anchor must be kept within an optimum interval (Buchholz et al., 1996). Moreover, fusion probably involves insertion of a hydrophobic fusion peptide implying a conformational change in the F protein (Lamb, 1993), as modelled from the acid-dependent fusion induced by influenza virus haemagglutinin (White, 1994). Little is known as to the fate of H and F glycoproteins after fusion. Together with other viral proteins H and F are targets of the CD4+ and CD8-dependent immune response. Knowledge of how the individual MV antigens are routed intracellularly in order to be presented by MHC class I and II molecules to CD8 and CD4 T cells would help in defining the requirements a vaccine must meet in order to stimulate the corresponding T cells. Indeed, MHC class I-restricted presentation involves antigen processing in the cytosol and/or endoplasmic reticulum (ER) whereas MHC-II-restricted presentation uses an endosomal pathway (Germain, 1994). Failure to stimulate CD8 CTL and/or CD4 Th1/Th2 subsets may result in a severe immunopathology after infection with the wild-type virus, as documented with formalin-inactivated vaccine against MV and respiratory syncytial virus (Cardoso et al., 1995; Connors et al., 1994; Fulginiti et al., 1967). To follow the intracellular fate of the ectodomain of MV H glycoprotein after CD46-mediated MV binding and fusion, the efficiency of H antigen presentation by MHC-I and MHC-II molecules was investigated.

Polyclonal H-specific MHC class I-restricted CTLs were obtained by immunization of H-2d mice with a recombinant vaccinia virus encoding MV H (vvV-H) (Wild et al., 1992) and in vitro secondary stimulation with MHC-I+II+ P815-H cells, which constitutively express MV H (see Beauverger et al., 1994, for details). The polyclonal CTLs were Ld-restricted and recognized two H epitopes, amino acid sequence 343–351 and 544–552, which both belong to the ectodomain of the protein (Beauverger et al., 1994). Murine MHC-I+II+ P815 cells expressing (Cardoso et al., 1995) or not expressing CD46 were incubated overnight with infectious MV (Hallé strain) at 0·45 p.f.u. per cell, and then tested for their sensitivity to lysis by the polyclonal CTLs. When infected with MV, P815-CD46, but not parental P815 cells, were readily lysed by H-specific
These CTLs were able to lyse class I" II" M12-CD46 cells that were either infected with MV or sensitized with UV-inactivated MV (Fig. 2a, b). The class II restriction of this CTL activity was ascertained by showing that (i) these effectors were unable to kill MHC class I"II" P815-H or P815-CD46 cells infected with MV or incubated with UV-inactivated MV (Fig. 2c, d); (ii) killing of M12-CD46 cells sensitized with MV was abolished in the presence of anti-CD4 but not anti-CD8 antibodies (Fig. 3b, c); (iii) killing was also inhibited when antigen processing was blocked by treatment (50 μM) with the lysosomotropic agent chloroquine during antigen loading of the targets and the CTL assay according to a procedure reported by Lombard-Platet et al. (1993) (Fig. 3c); and (iv) they lack any detectable NK activity since no killing of YAC cells was observed (Fig. 2c). These results show that UV-inactivated MV can provide the CD46-expressing antigen-presenting cells (APC) with H antigen available for class II- but not for class I-restricted presentation to T cells.

Thus, expression of the MV receptor, human CD46, in mouse cells is an absolute requirement for efficient presentation of MV H to MHC class I-restricted specific CTLs. This effect is a result of the ability of CD46-transfected cells to capture MV particles, allow fusion, genome replication and de novo synthesis of viral proteins (Gerlier et al., 1994; Naniche et al., 1993; D. Gerlier, unpublished). Indeed, H synthesized in the target cells after infection with MV was presented by class I molecules, since P815-CD46 cells infected with MV were lysed by H-specific CTLs as were the P815-H cells (transfected with H gene). With UV-inactivated MV, H glycoprotein could not be presented by MHC class I molecules since P815-CD46 cells, which expressed only class I molecules, were not recognized by H-specific CTL when sensitized with UV-inactivated MV. The lack of class I-restricted presentation of H from UV-MV was not due to the lack of H association with the target cells because H can be processed and presented by MHC class II molecules, as shown by the recognition of UV-MV-sensitized M12-CD46 cells by H-specific CD4 CTLs (see Fig. 2a, b) and by a class II I-Eα-restricted CD4 T cell hybridoma (Gerlier et al., 1994). It should be stressed that the UV-MV was still able to fuse with the cell plasma membrane as the NP component was presented by the cytosolic MHC class I antigen presentation pathway (Cardoso et al., 1995).

Why is there class II- but not class I-restricted presentation of H from UV-inactivated MV? In H-2d mice, both class I- (Beauverger et al., 1994) and class II- (P. Devaux & D. Gerlier, unpublished) restricted epitopes mapped to the ectodomain (luminal domain) of H. On the one hand, the lack of class I-restricted presentation of the luminal part of H provided to the targets and the CTL assay according to a procedure reported by Lombard-Platet et al. (1993) (Fig. 3c); and (iv) they lack any detectable NK activity since no killing of YAC cells was observed (Fig. 2c). These results show that UV-inactivated MV can provide the CD46-expressing antigen-presenting cells (APC) with H antigen available for class II- but not for class I-restricted presentation to T cells.

Thus, expression of the MV receptor, human CD46, in mouse cells is an absolute requirement for efficient presentation of MV H to MHC class I-restricted specific CTLs. This effect is a result of the ability of CD46-transfected cells to capture MV particles, allow fusion, genome replication and de novo synthesis of viral proteins (Gerlier et al., 1994; Naniche et al., 1993; D. Gerlier, unpublished). Indeed, H synthesized in the target cells after infection with MV was presented by class I molecules, since P815-CD46 cells infected with MV were lysed by H-specific CTLs as were the P815-H cells (transfected with H gene). With UV-inactivated MV, H glycoprotein could not be presented by MHC class I molecules since P815-CD46 cells, which expressed only class I molecules, were not recognized by H-specific CTL when sensitized with UV-inactivated MV. The lack of class I-restricted presentation of H from UV-MV was not due to the lack of H association with the target cells because H can be processed and presented by MHC class II molecules, as shown by the recognition of UV-MV-sensitized M12-CD46 cells by H-specific CD4 CTLs (see Fig. 2a, b) and by a class II I-Eα-restricted CD4 T cell hybridoma (Gerlier et al., 1994). It should be stressed that the UV-MV was still able to fuse with the cell plasma membrane as the NP component was presented by the cytosolic MHC class I antigen presentation pathway (Cardoso et al., 1995).

Why is there class II- but not class I-restricted presentation of H from UV-inactivated MV? In H-2d mice, both class I- (Beauverger et al., 1994) and class II- (P. Devaux & D. Gerlier, unpublished) restricted epitopes mapped to the ectodomain (luminal domain) of H. On the one hand, the lack of class I-restricted presentation of the luminal part of H provided to the APC by fusion of the envelope from UV-inactivated MV indicates that it cannot gain access to any cellular compartment relevant for appropriate MHC-I-restricted antigen processing, including the cytosol. Indeed, four pathways leading to the formation of peptide-class I complexes from transmembrane antigens have been identified so far (see Siliciano & Soloski, 1994; Cardoso et al., 1995).
Fig. 2. H from infectious or UV-inactivated MV is presented by M12-CD46 but not by P815-CD46 cells to CD4+CD8- class I-restricted CTLs. Specific cytolysis (%) by H-specific CTLs induced after in vitro stimulation with UV-MV of: (a) MV-infected M12-CD46 cells (●) and unloaded M12-CD46 (□); (b) UV-MV-loaded M12-CD46 cells (●) and unloaded M12-CD46 (□); (c) P815-H cells (▲), P815-NP cells (○) and YAC-1 cells (□); (d) MV-infected P815-CD46 cells (●), UV-MV-loaded P815-CD46 cells (■) and unloaded P815-CD46 cells (□).

Fig. 3. Characterization of CTL effectors. (a) H-specific polyclonal CTLs from mice immunized with rVV-H and restimulated in vitro with P815-H cells are CD8+CD4-. Spleen cells were tested for their cytolytic activity at an E/T ratio of 3:1 in the presence or absence of anti-CD8 H35.17 or anti-CD4 H129.19 MAbs added to the effectors prior to incubation of the P815-CD46 targets with MV. Results are expressed as percentage inhibition of cytolysis. (b, c) H-specific polyclonal CTLs from mice immunized with rVV-H and restimulated in vitro with UV-irradiated MV are CD4+CD8-. Spleen cells were tested for their cytolytic activity at E/T = 3/1 in the presence or absence of anti-CD8 or anti-CD4 MAbs added to the effectors prior to loading of the M12-CD46 targets with (b) infectious or (c) UV-irradiated MV. Other tests were done in the presence of 50 μM-chloroquine added to the APC prior to the virus and maintained throughout the bioassay (CHQ column).

1995, for review). One involves antigen degradation into the ER and peptide association with the nascent heavy chain of MHC-I. In the second, some misfolded proteins fail to be translocated into the ER and are degraded into peptides by cytosolic proteasomes. The peptides are then transported through the TAP1/TAP2 heterodimers to the ER where they can associate with nascent MHC-I molecules. Alternatively, the transmembrane antigen is degraded within the ER into long peptides that are able to cross the ER membrane and reach the cytosol. Here, they can be further degraded into smaller peptides able to return to the ER via the TAP1/TAP2 transporter. In a fourth and unusual pathway, the transmembrane antigen is internalized by endocytosis where it is degraded. The peptides became available (by 'regurgitation'? for association with (recycling?) cell-surface MHC-I molecules.

On the other hand, the class II-restricted presentation of the luminal part of H indicates that it has access to the cellular compartment relevant for appropriate MHC-II-restricted antigen processing, i.e. the endosomal compartment (Germain, 1994). As a newly incorporated plasma membrane glycoprotein, H from the fused viral envelope has easy access to the endosomes as it follows the usual endocytic fate of plasma membrane proteins. In order to reach the lumen of the endosomes, the extracellular domain of H will have to remain oriented towards the outside of the cell, as demonstrated in the case of the human immunodeficiency virus glycoprotein (Callahan et al., 1993). From these data, we infer that during fusion of the MV envelope with the plasma membrane of target cells, the ectodomain of H (or at least the domain of H encompassing the H 343-351 and 544-552 class I epitopes)
does not cross the plasma membrane and remains oriented towards the outside of the cell.

What is the orientation of the F glycoprotein during fusion?
A partial answer come from our reappraisal of class I-restricted presentation of F in humans as reported by Van Binnendijk et al. (1992, 1993). F provided to the APC as UV-inactivated MV was also found not to be presented by MHC class I whereas F from infectious MV was processed and presented by MHC class I molecules. One of the CTL clones (clone F40) recognized the extracellular domain of the F1 subunit. These data imply that during the fusion process, the extracellular domain of F1 (at least the domain encompassing the F438–446 epitope) remains oriented outside the cell and is not translocated to the cytosolic side, although its C-terminal part, which contains the fusion peptide (F116–147), is thought to be inserted into the plasma membrane during fusion (Paterson & Lamb, 1987). Altogether, this favours the possibility that during MV-to-cell fusion, both envelope H and F glycoproteins keep their extracellular domain oriented towards the cell exterior (i.e., they are not translocated into the cytosol) and end up into endosomal compartments. We predict that this topology of the envelope glycoproteins after fusion will be confirmed by other means such as immunoelectron microscopy. Indeed, during membrane fusion with the influenza virus envelope there is no merger of the inner and outer leaflets of the two fusing membranes, so that transverse membrane asymmetry is maintained. In contrast, when H and F are endogenously synthesized by cells, their luminal portion can be presented by MHC class I molecules but it has to be processed prior to its expression at the cell surface. These observations should be taken into account when engineering and evaluating new vaccines against MV in order to obtain an adequate stimulation of CD8+ and/ or CD4 Th1/Th2 subsets and to avoid the imbalance of the immune response which may be responsible for the exacerbated atypical disease as seen with inactivated vaccines (Cardoso et al., 1995; Fulginiti et al., 1967).

The authors are indebted to K. Belorizky, I. Fugier-Vivier, C. Gimenez, B. Horvat, S. Kntics-Moyse, J. Marvel, P. Rivaller, M.-C. Trescol-Biémont and G. Varier-Krishnan for helpful discussions and comments. This work was supported in part by grants from the Association pour la Recherche contre le Cancer (CRC 6108) and the World Health Organization/UNPP Programme for Vaccine Development (PVD) (B.H.). A. Cardoso was supported by Fondation Mérieux and WHO.

References
White, J. (1994). Fusion of influenza virus in endosomes: role of the


Received 17 April 1996; Accepted 16 July 1996