The haemagglutinin protein is an important determinant of measles virus tropism for dendritic cells in vitro

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Recombinant measles viruses (MV) in which the authentic glycoprotein genes encoding the fusion and the haemagglutinin (H) proteins of the Edmonston (ED) vaccine strains were swapped singly or doubly for the corresponding genes of a lymphotropic MV wild-type virus (strain WTF) were used previously to investigate MV tropism in cell lines in tissue culture. When these recombinants and their parental strains, the molecular ED-based clone (ED-tag) and WTF, were used to infect cotton rats, only viruses expressing the MV WTF H protein replicated in secondary lymphatic tissues and caused significant immunosuppression. In vitro, viruses containing the ED H protein revealed a tropism for human peripheral blood lymphocytes as documented by enhanced binding and virus production, whereas those containing the WTF H protein replicated well in monocyte-derived dendritic cells (Mo-DC). This did not correlate with more efficient binding of these viruses to DC, but with an enhancement of uptake, virus spread, accumulation of viral antigens and virus production. Thus, replacement of the ED H protein with WTF H protein was sufficient to confer the DC tropism of WTF to ED-tag in vitro. This study suggests that the MV H protein plays an important role in determining cell tropism to immune cells and this may play an important role in the induction of immunosuppression in vivo.

Introduction

Although live attenuated vaccines are available, measles is still a major cause of mortality of children in developing countries. During acute measles, an efficient virus-specific immune response is generated that rapidly clears virus from blood and tissues (Griffin, 1995). Paradoxically, immune suppression is observed before the rash and for several weeks after recovery from infection, which favours the establishment of opportunistic infections. As hallmarks of this transient immunosuppression, delayed type hypersensitivity reactions are impaired in vivo, and lymphoproliferative responses to mitogens are strongly reduced ex vivo (Borrow & Oldstone, 1995; Schneider-Schaulies & ter Meulen, 1998). Initial measles virus (MV) infection in the respiratory tract, and the virus replicates in tracheal and bronchial epithelial cells during the incubation period. Thereafter, the infection spreads to the draining lymph nodes (LN), where MV replication results in the appearance of lymphoid or reticuloendothelial giant cells (Warthin–Finkeldy cells), which are thought to be a major source of virus for spread to other tissues.

Dendritic cells (DC) are professional antigen-presenting cells which are also located in the pulmonary airways, pulmonary vessels, alveolar septa and pleura, where they may function as sentinels for invading pathogens. After challenge with stimuli such as bacteria or soluble antigen, DC traffic from the lung to the draining LN where they are able to initiate primary immune responses (Banchereau & Steinman, 1998). It is also evident that targeting DC and interfering with DC maturation and function is a powerful strategy employed by pathogens to avoid immune recognition and to induce immunosuppression (Klagge & Schneider-Schaulies, 1999; Knight & Patterson, 1997). Mainly through the use of vaccine strains, DC were shown to be susceptible to MV infection (Fugier-Vivier et al., 1997; Grosjean et al., 1997; Klagge et al., 1999).
2000; Schnorr et al., 1997; Steineur et al., 1998), although infectious particles were released at only low levels. The interaction of MV-infected DC with T cells enhances virus replication and syncytia formation in a CD40 ligation-dependent manner (Fugier-Vivier et al., 1997; Servet-Delprat et al., 2000a). Interestingly, a lymphotropic MV wild-type strain, WTF, was found to replicate very efficiently in DC cultures (Schnorr et al., 1997). These observations suggest that DC infected with MV in the respiratory tract may transfer to the draining LN, where massive virus replication occurs, and that MV-infected DC may play a role in MV-induced immunosuppression.

Monkey kidney cell lines had been commonly used to isolate MV from peripheral blood lymphocytes or respiratory secretions until rapid and reproducible isolation of the virus from clinical specimens was reported using an Epstein–Barr virus (EBV)-transformed marmoset B-lymphocyte line, B95-8, and its derivatives. Importantly, isolates obtained by passage in one of the derivative lines, B95a, retained pathogenicity for monkeys, whereas MV adapted to Vero cells lost pathogenic potential (Kobune et al., 1990). Lymphotropic wild-type strains of MV and tissue culture-adapted MV vaccine strains possess different cell tropisms since lymphotropic strains grow poorly in adherent cells such as HeLa and Vero cells. To study the role of the viral glycoproteins in MV tropism, we generated recombinant vaccine MV based on the Edmonston-tag (ED-tag) molecular clone that contains one or both of the haemagglutinin (H) and fusion (F) proteins from a lymphotropic wild-type strain (WTF) (Johnston et al., 1999). These viruses were viable and grew similarly in a lymphocytic cell line, whereas recombinant viruses expressing the WTF H protein showed a restricted spread in HeLa cells but not in Vero cells.

Using these recombinant viruses and the parental strain WTF to infect cotton rats, we found that the ability to replicate in secondary lymphoid tissues and to cause immunosuppression was restricted to viruses expressing the WTF H protein. Assuming that this could reflect differential interactions of these viruses with particular subpopulations of peripheral blood mononuclear cells (PBMC), we comparatively analysed infection of these recombinant MVs in monocyte-derived dendritic cells (Mo-DC) and in human peripheral blood lymphocytes (PBL). In PBL, recombinant MV containing ED H replicated more efficiently than WTF or recombinant viruses containing the WTF H protein. In Mo-DC, on the other hand, replication of recombinant MV containing ED H was restricted, but WTF and recombinant MV containing WTF H replicated well. This was associated with enhanced virus spread and accumulation of viral antigens as well as an enhancement of cellular fusion in these cultures.

**Methods**

**Cells and viruses.** The human lymphoblastoid B-cell line BJAB was maintained in RPMI 1640 medium containing 10% foetal calf serum (FCS), the Epstein–Barr virus-transformed marmoset B-lymphocytic cell line B95a in RPMI 1640–5% FCS, and Vero cells (derived from African green monkey kidney) in minimal essential medium–5% FCS. Generation and rescue of the recombinant viruses ED-tag (the molecular clone derived based on the sequence of the Edmonston B strain) (Radecke et al., 1995), MV(WTF H)ED (having the H gene of the ED strain replaced by that of the MV wild-type strain WTF), MV(WTF F)ED (having the F gene of the ED strain replaced by that of the WTF strain) and MV(WTF F/WTF H)ED (having both ED-derived glycoproteins replaced by those of the WTF strain) were described previously (Johnston et al., 1999) (schematically depicted in Fig. 1A). The WTFb wild-type virus (WTF), MV(WTF H)ED and MV(WTF F/H)ED were grown on BJAB cells, ED-tag and MV(WTF F)ED on Vero cells. TCID<sub>50</sub> of all viruses were determined on B95a cells. For binding studies, mock preparations from Vero cells or BJAB cells obtained under conditions identical to those of the virus preparations were used.

**Infection of cotton rats and proliferation assay.** For intranasal infection, MV was given in PBS to ether-anaesthetized cotton rats (inbred strain COTTON/Nico, obtained from Iffa Credo). Four days later, animals were sacrificed by administering CO<sub>2</sub> and lungs, mediastinal LN and spleens were removed. LN cells pooled from four infected animals were resuspended in RPMI 1640 and serially (10-fold) diluted for cocultivation with B95a cells. Plates were scored microscopically for cytopathic effect after 7 days (expressed as TCID<sub>50</sub> per 10<sup>7</sup> cells). Virus titre in lung tissues was determined as described previously (Niewiesk et al., 1997). For proliferation assays, spleen cells were plated in triplicate in a 96-well plate (5 x 10<sup>5</sup> cells per well) in RPMI 1640–10% FCS with or without addition of 2.5 µg/ml concanavalin A for 40 h followed by a 16 h pulse labelling with 0.5 µCi of [<sup>3</sup>H]thymidine per well.
Proliferation rates were calculated by dividing the mean of proliferation of mitogen-stimulated cells by that of the medium control.

**Preparation of PBL and generation of Mo-DC.** Human PBMC were isolated by Ficoll–Paque density gradient centrifugation (Amersham Pharmacia) of buffy coats and cultured in RPMI 1640–10% FCS overnight. When indicated, the non-adherent fraction (PBL) was stimulated with phytohaemagglutinin (PHA, 2.5 µg/ml). For generation of Mo-DC, CD14+ monocytes were enriched from PBMC preparations by depletion of T cells by rosetting with AET-treated sheep red blood cells and subsequent removal of B and NK cells using anti-CD19- and anti-CD56-coated magnetic beads (Milteny Biotech). The plastic-adherent fraction of the monocyte-enriched cell population (more than 90% purity) was used to generate Mo-DC in vitro by culture in the presence of 50 ng/ml recombinant human GM-CSF (Novartis) and 30 ng/ml recombinant human IL-4 (Strathmann Biotech) for 7 days with fresh cytokines added on day 4.

**Antibodies and FACS analysis.** The MV H-specific monoclonal antibody (MAb) (L77), the anti-MV-N MAb (F227), the anti-CD46 MAb (11/88) (Schneider-Schaulies et al., 1995) and the anti-signalling lymphocytic activation molecule (SLAM) CD150 MAb (5C6) (Erlenhofer et al., 2001) were produced and purified in our laboratory. The vesicular stomatitis virus (VSV)-G protein-specific MAb was generously provided by Matthias Schnell, Pittsburgh, PA, USA. For intracellular stainings, cells were fixed with 3–7% paraformaldehyde and permeabilized with 0–33% saponin (ICN). Cell stainings were measured using a FACSscan (Becton Dickinson) and the LysisII program and analysed with CELL QUEST software.

**Virus binding and uptake.** For binding studies, virus preparations or, for control, preparations of mock-infected cells (Vero cells or BJAB cell, respectively) or culture medium were used. The amounts of viral glycoproteins in the virus preparations were adjusted by Western blot using antisera against the cytoplasmic domains of H and F proteins. The amounts of mock preparations added to the controls were adjusted to those used for the individual virus preparations. For analysis of virus binding, cells were incubated with viruses (or mock preparations) on ice for 1 h, washed twice with PBS containing 0.4% bovine serum albumin (BSA)–0.02% sodium azide, and stained with a MAb against MV H protein (L77), followed by a fluorescein isothiocyanate (FITC)-labelled goat anti-mouse IgG. For virus uptake studies, Mo-DC were incubated with viruses at an m.o.i. of 2–5 TCID₅₀ per cell at 37 °C for 2 h followed by a 5 min wash with 0.14 M NaCl–8 mM glycine pH 2.5–6% BSA and two subsequent washing steps. Cells were stained for FACS analysis after 14 h cultivation in medium containing 100 µg/ml Z-fFG (Sigma) using the anti-MV-N MAb (F227) and FITC-labelled goat anti-mouse IgG.

**Virus infection and fusion inhibition.** Cells were infected at an m.o.i. of 0.05 TCID₅₀ per cell for 1 h at 37 °C, washed twice with RPMI 1640 and plated at a density of 10⁵ cells per well in 1 ml medium. When indicated, 2.5 µg/ml PHA was added to PBL. Mo-DC were cultured in the cytokine-containing medium. For fusion inhibition studies, infected Mo-DC were plated at a density of 4 x 10⁴ cells per well in 100 µl of RPMI 1640–10% FCS and cytokines (GM-CSF/IL-4) in the presence of antibodies (final concentration, 25 µg/ml). For determination of virus titres, samples (containing both supernatant and cells) were freeze–thawed and assayed by TCID₅₀ titration on B95a cells.

Fig. 2. Cytopathic effects caused by MV WTF and recombinant viruses in human PBL and Mo-DC. Cytopathic effects in PBL (PHA-stimulated after infection) (A) or Mo-DC (B) after 48 h of infection with mock supernatant (a), ED-tag (b), MV(WTF F)ED (c), WTF (d), MV(WTF H)ED (e) or MV(WTF F/WTF H)ED (f) at an m.o.i. of 0.05 TCID₅₀ per cell. Magnification, × 400 (all panels).
Results

Expression of WTF H protein confers enhanced activity to suppress lymphocyte proliferation and targeting to secondary lymphoid tissues in vivo

As revealed by our previous studies, recombinant MV in which either the F [MV(WTF F)ED] or the H [MV(WTF H)ED] glycoprotein, or both [MV(WTF F/H)ED] (Fig. 1A), were exchanged in the parental molecular clone, ED-tag, for the corresponding genes of an MV wild-type strain, WTF, revealed differences in cellular tropism in tissue cultures with Vero and HeLa cells (Johnston et al., 1999). To investigate the properties of the recombinants in vivo, these and the parental strain, WFT, were used to infect cotton rats. These animals are susceptible to intranasal infection with MV, and infectious virus can be reisolated from lung and LN. Moreover, proliferation of spleen cells isolated from MV-infected cotton rats is impaired after mitogen stimulation (Niewiesk et al., 1997). Cotton rats were infected with 10^3 TCID_{50} of WTF or the recombinant viruses intranasally and after 4 days spleen cultures were harvested for proliferation assays, and lungs and mediastinal LN for titration of infectious virus on B95a cells. In lung tissue, titres of infectious virus after infection with WTF or the various recombinant MV were similar (data not shown). In contrast, virus titres in LN isolated from animals infected with the WTF H-containing viruses significantly exceeded those from animals infected with ED H-containing viruses (Fig. 1B). Similarly, only the WTF H-containing viruses [WTF, MV(WTF H)ED and MV(WTF F/WTF H)ED] were able to induce proliferative inhibition (Fig. 1C), whereas for the ED H-containing viruses [ED-tag and MV(WTF F/ED)], tenfold higher doses were required (not shown). This suggests that WTF and the recombinant viruses containing WTF H protein revealed a higher tropism for secondary lymphatic tissues in vivo and an enhanced ability to suppress lymphocyte proliferation ex vivo than those containing ED H protein.

Interaction of MV recombinants and WTF with human PBL and Mo-DC

The different ability of viruses containing the WTF H or ED H protein to replicate in secondary lymphatic tissues and to cause proliferative inhibition in vivo (Fig. 1) might reflect a differential interaction of these viruses with subpopulations of PBMC. Thus, human PBL (stimulated with PHA following infection) and immature Mo-DC were infected with the MV wild-type strain WTF, molecular cloned vaccine MV ED-tag or the ED-tag-based recombinant viruses MV(WTF F)ED, MV(WTF H)ED or MV(WTF F/WTF H)ED (Fig. 1A) at an m.o.i. of 0.05 TCID_{50} per cell. In PBL, within 2 days of infection only viruses containing the ED H protein, ED-tag and MV(WTF F)ED, induced syncytia (Fig. 2A, panels b and c), whereas only formation of cell clusters, not syncytia, was observed with WTF, MV(WTF H)ED and MV(WTF F/WTF H)ED (Fig. 2A, panels d to f). With the latter viruses, small syncyntia developed on day 3 post-infection (p.i.), which increased in number and size on day 4 (not shown). In contrast, extensive syncytium formation was observed in DC infected with viruses containing the WTF H protein [WTF, MV(WTF H)ED and MV(WTF F/WTF H)ED] within 2 days p.i. (Fig. 2, panels d to f), whereas syncytium formation was barely induced by viruses containing the ED H protein [ED-tag and MV(WTF F)ED] (Fig. 2, panels b and c). These data indicate that the MV H protein has a strong impact on MV tropism for subpopulations of human PBMC.

Differential binding of viruses containing ED H or WTF H proteins to human PBL and Mo-DC

Since the MV H protein mediates attachment to the MV receptor on the host cell, we examined whether the differential tropism of the recombinants for PBL and Mo-DC was reflected by differential binding affinities to these cell populations. Binding studies were performed with equal concentrations of viral glycoproteins of the individual viruses as determined by Western Blot analyses using antisera against the cytoplasmic domains of H and F protein (data not shown). Human PBL, stimulated with PHA for 3 days or left unstimulated, or Mo-DC were incubated with WTF and the recombinant MV for 1 h on ice and subsequently stained using an anti-MV-H MAb. Mock preparations of Vero cells, BJAB cells or culture medium were used to determine the background binding to all cell populations (Fig. 3). Viruses containing the ED H protein, ED-tag and MV(WTF F)ED, bound efficiently to unstimulated PBL, and this was further enhanced when PBL were preactivated (Fig. 3A, B). Binding efficiencies of viruses containing the WTF H protein [WTF, MV(WTF H)ED and MV(WTF F/WTF H)ED] to unstimulated PBL were generally lower as compared to ED-tag and MV(WTF F/ED); however, a proportional increase (on average by a factor of 2) in binding was observed with preactivated PBL (Fig. 3A, B). Thus, in PBL, enhanced syncytium formation observed with viruses containing the ED H protein apparently correlates with the binding affinity of these viruses, whereas the F protein does not seem to influence MV tropism in these cells. Binding of all viruses to Mo-DC could be detected, but was generally low, and major differences relating to the expression of the respective F or H proteins were reproducibly not observed (Fig. 3C). Thus, on Mo-DC, enhanced syncytium formation induced by viruses containing the WTF H protein was not reflected by enhanced binding of these viruses to the cell surface.

Inhibition of syncytium formation in MV-infected Mo-DC by anti-SLAM/CD150 and anti-CD46 MAb

Since binding of all viruses used to the surface of Mo-DC was low, yet WTF H-containing but not ED H-containing strains efficiently induced syncytium formation, the expression levels of both MV receptors, CD46 and SLAM/CD150, on the
surface of Mo-DC and their role in membrane fusion in these cells were investigated. Mo-DC were found to express both CD46 (Fig. 4A, left panel) and SLAM (Fig. 4A, right panel), albeit at significantly lower levels than PBL (Fig. 4A, left and right panel). To study the role of these molecules in syncytium formation in MV-infected Mo-DC, blocking of either SLAM (by the antibody 5C6) or CD46 (by the antibody 11/88) was done at a concentration previously found to completely abolish MV binding to B95a cells (Erlenhofer et al., 2001). For control, isotype-matched unrelated antibodies (directed against VSV-G protein or against CD1a) were included, which had only a minimal effect on syncytium formation in all cultures (exemplified for the VSV-G-specific antibody in Fig. 4B, panels a to d). For these studies, only viruses containing the WTF H protein, which efficiently caused formation of syncytia in Mo-DC cultures (Fig. 2B), were used. 5C6 completely inhibited syncytium formation induced by WTF H-containing viruses [WTF, MV(WTF H)ED or MV(WTF F/WTF H)ED] (Fig. 4B, panels e to h). Although less efficient, 11/88 also inhibited syncytium formation induced by these viruses (Fig. 4B, panels i to l), indicating that WTF H protein-containing viruses may preferentially, but not exclusively, depend on SLAM for membrane fusion in DC. Taken together, these findings suggest that WTF H protein-containing viruses can interact with CD46.

Fig. 3. Binding of MV WTF and recombinant viruses to human PBL and Mo-DC. Resting PBL (A), PBL preactivated with PHA for 3 days (B) or day 7 Mo-DC (C) were incubated with equal amounts of glycoproteins of viruses (solid profiles) or, for control, equivalent amounts of medium (empty profiles) for 1 h on ice. After washing, cells were stained using an MV H-specific MAb followed by an FITC-labelled anti-mouse IgG, and subsequently analysed by FACSan.
and SLAM on DC for membrane fusion; however, the interaction with SLAM seems to be more important.

Replication of the recombinant viruses in PBL and Mo-DC

To determine how the recombinant viruses spread and replicate in PBL and Mo-DC, infected PBL stimulated with PHA or Mo-DC were harvested at various intervals p.i. and stained with an anti-MV-H MAb, or used for titration of infectivity on B95a cells. In PBL, the percentage of MV H-positive cells increased more quickly, indicating virus spread (Fig. 5A). Differences in virus spread between MV ED H- and MV WTF H-containing viruses in PBL (and also in Mo-DC, see below) may actually be greater than indicated in the figure considering the loss of infected cells already recruited in syncytia during FACS analysis. MV H protein accumulated both more quickly and to higher levels in PBL cultures infected with viruses containing the ED H protein [ED-tag and MV(WTF F)ED] compared to cultures infected with WTF H protein-containing viruses [WTF, MV(WTF H)ED and MV(WTF F/WTF H)ED] (Fig. 5B). Similarly, production of infectious virus in these cultures was more efficient with viruses containing the ED H protein [ED-tag and MV(WTF F)ED] than with those viruses containing WTF H protein [WTF, MV(WTF H)ED or MV(WTF F/WTF H)ED] (Fig. 5C).

In Mo-DC, however, WTF H-containing viruses spread significantly faster (Fig. 5D) than those containing the ED H protein. In addition, only low levels of MV H accumulated on DC infected with ED H-containing viruses [ED-tag and MV(WTF F)ED], whereas after infection with viruses containing the WTF H protein [WTF, MV(WTF H)ED and MV(WTF F/WTF H)ED] this antigen was detected at high levels over time on the cell surface (Fig. 5E). This indicates that in DC WTF H rather than ED H proteins favour virus entry and
MV tropism for dendritic cells

Fig. 5. Growth kinetics of MV WTF and recombinant viruses in human PBL and Mo-DC cultures. PBL (A to C) and Mo-DC (D to F) were infected with an m.o.i. of 0.05 TCID$_{50}$ per cell, and each 10$^5$ cells were incubated at 37 $^\circ$C in medium supplemented with PHA (for PBL) or cytokines (GM-CSF/IL-4 for Mo-DC). At the time intervals indicated, samples were stained for the expression of MV H protein and analysed by FACScan for the percentage of H-positive cells (A and D) and the mean fluorescence intensity of this antigen (B and E). As a control, an isotype-matched antibody was used. (C, F) Samples were harvested at the time intervals indicated and stored at −80 $^\circ$C. Cell lysates were titrated on B95a cells, and virus titres were determined as log$_{10}$ TCID$_{50}$/ml. (G) Mo-DC were infected with an m.o.i. of 2.5 TCID$_{50}$ per cell for 2 h, washed in glycine-containing buffer extensively, kept in medium containing 100 µg/ml Z-fFG and stained for MV N protein expression after 14 h.

spread. Production of infectious virus particles was low in DC infected with ED-tag and MV(WTF F)ED, and higher, but only transient, in DC infected with MV(WTF H)ED or MV(WTF F/WTF H)ED (Fig. 5F). Virus production was, however, reproducibly higher after infection with WTF when compared to the WTF H-containing recombinants [MV(WTF H)ED and MV(WTF F/WTF H)ED] (Fig. 5F). This suggests that, in contrast to PBL (Fig. 5C), in DC the efficiency to replicate is also determined by virus components other than the glycoproteins. Since all recombinants had the ED-tag backbone, we reasoned that the WTF H protein may provide a better fusion helper function on DC than ED H protein, which would lead to a more efficient uptake of infectious virus. To address this aspect, DC were infected with an m.o.i. of 2.5 of either virus and stained for MV N protein expression within the first replication cycle (14 h). To prevent virus spread, the fusion inhibitory peptide Z-fFG was added. Using these conditions, MV N protein accumulated to significantly higher levels after infection with viruses containing WTF H protein (Fig. 5G), indicating that the uptake of replication competent virus was indeed enhanced.

Discussion

Immunosuppression induced by MV has been attributed to disturbances of T cell polarization, function and proliferation (Griffin et al., 1994; Karp et al., 1996; McChesney et al., 1988; Naniche et al., 1999; Schlender et al., 1996; Schneider-Schaulies & ter Meulen, 1998; Yanagi et al., 1992) and, for each, T cell interaction with professional antigen presenting cells as DC is a major determinant. MV infection of DC during acute measles has not been substantiated as yet in humans. There is, however, evidence that it does occur in experimentally infected animals (Mrkic et al., 2000). MV interaction with DC has to occur in order to explain the efficient induction of a primary antiviral immune response as established in the course of acute measles. In the induction of immunosuppression, DC have been suggested to play an important role based on observations made in vitro where MV infection caused functional maturation of these cells, yet rendered them unable to stimulate T cell proliferation and to interfere with terminal maturation and IL-12 release (Fugier-Vivier et al., 1997; Grosjean et al., 1997; Karp et al., 1996; Klagge et al., 2000; Schnorr et al., 1997; Servet-Delprat et al., 2000 b). In our study we aimed to investigate how MV interacts with subpopulations of PBMC, which virus determinants confer MV tropism for these cells, and how this would influence virus spread and immunosuppressive activity in vivo. Using recombinant MV expressing one or both MV glycoproteins derived from the lymphotropic MV WTF strain within the ED vaccine strain backbone we found that the MV H, but not the F, protein is an important determinant of MV tropism for both lymphocytes and DC, and it is likely that differences observed with respect to the viral glycoproteins directly reflect the interaction of the respective H proteins with the cognate MV receptors CD46 and SLAM.

The differential binding of ED H- and WTF H-containing viruses to unstimulated PBL probably represents their affinity for the constitutively expressed CD46, which is high for ED H and low for H proteins of most wild-type viruses propagated...
on PBMC, B95-8 or BJAB cells, including WTF (Bartz et al., 1998; Lecouturier et al., 1996; Manchester et al., 2000). A tyrosine residue at position 481 confers high, and asparagine confers low, affinity binding of MV H proteins to PBMC, which is in agreement with our observations that viruses containing the ED H protein (481Y) do interact with CD46-positive cells more efficiently than those containing WTF H (481N) (Johnston et al., 1999) (Fig. 3). Upon stimulation, the surface expression levels of CD150 increased as expected (not shown), which could explain the enhanced binding of both ED H- and WTF H-containing viruses (Fig. 3). Since for the infection experiments (Fig. 5A to C), PBL were only stimulated with PHA after infection, viruses with higher affinity for CD46 are predominantly taken up and amplified rather than those mainly interacting with SLAM. Upregulation of this molecule after PHA stimulation may account for spread of and fusion by WTF H-containing viruses that occurred later than that with ED H protein-containing viruses in PBL (Fig. 5A to C).

On Mo-DC, both CD46 and SLAM were constitutively expressed (Fig. 4A). SLAM in particular was, however, detected only at low levels with 5C6, and barely with a commercially available anti-SLAM antibody (A12, Pharmingen) (Fig. 4A and not shown). Since our Mo-DC were generated from CD14+ monocytes which were reported to be SLAM-negative (Cocks et al., 1995; Sidorenko & Clark, 1993), it would be interesting to determine when and by what mechanisms during DC maturation from these precursors this molecule is upregulated and how this correlates with susceptibility to infection with MV. Our finding that MV H proteins are important determinants for the differential tropism of MV strains for DC is in agreement with a recent study in which a single amino acid exchange within glycoprotein 1 conferred both DC tropism and immunosuppressive activity to lymphocytic choriomeningitis virus (LCMV; Sevilla et al., 2000). Similar to our findings with PBL and ED H-containing viruses, a clear correlation was found in this study between the binding affinity of the immunosuppressive LCMV strains and their receptor, α-dystroglycan. It is unclear why, in contrast to PBL, we did not detect differential binding of MV containing ED H or WTF H proteins to DC (Fig. 3C). Due to the low expression levels of SLAM, but also CD46 (Fig. 4A), binding sites for all MV strains may not be very abundant and thus bound particles might not be detected. Specific binding to the less abundant SLAM in particular may additionally be masked by nonspecific binding of the MV H proteins to C-type lectin receptors such as mannose receptors, which are abundant on DC (Banchereau & Steinman, 1998; Sallusto & Lanzavecchia, 1995). Since formation of syncytia does occur in DC cultures infected with WTF H-containing viruses and this is efficiently prevented by anti-SLAM antibodies, it is clear that this molecule is expressed on DC and is functional in providing at least fusion helper function for lymphotropic MV strains (Fig. 4B).

Although WTF H protein was found to determine the tropism for DC to a large extent by improving membrane fusion, virus uptake and spread, replication of the authentic parental WTF strain was more efficient in DC, indicating that other MV components may also be important (Fig. 5F). At present, the MV gene products involved and the underlying mechanisms are unclear. Experimental evidence has been provided that amino acid exchanges within the F gene relate to MV attenuation and to replication efficiencies in lymphoid cells in vitro and in vivo (Takeda et al., 1998; Takeuchi et al., 2000). Moreover, disruption of the C reading frame, which is also encoded within the P gene, was associated with attenuation of MV replication in PBMC (Escoffier et al., 1999), and both C and V proteins were found to determine MV virulence after intracerebral infection of mice (Patterson et al., 2000). It is currently unknown by which mechanisms these proteins may affect MV replication in lymphoid cells. C and/or V proteins were linked to modulation of either induction or action of type I IFN in related virus systems (Goodbourne et al., 2000). Differential induction of type I IFN as suggested by a recent study for MV wild-type strains (Naniche et al., 2000) is, however, not likely to play a role in our system, since both the MV Edenston strain B (which is the parental strain of the molecular clone ED-tag) and the WTF strain induce type I IFN after infection of DC cultures to similar levels (Klagge et al., 2000; I. M. Klagge, unpublished). Induction of type I IFN as a result of CD46 ligation as described in mouse macrophages (Katayama et al., 2000) is also unlikely to be important in our system, since at least in PBL ED H protein-containing viruses strongly interacting with CD46 replicated well (Fig. 5A to C). In addition, expression levels of CD46 on DC are much lower (Fig. 4A), and firm binding to DC was not observed with either virus strain (Fig. 3C). If WTF were able to evade type I IFN efficiently by whatever mechanism, it would be difficult to explain why it should fail to do so in PHA-stimulated PBL (Fig. 5C). Implying that the antiviral activity of type I IFN can be cell type-specific, we have documented that MxA protein does not interfere with MV replication in Vero cells, but inhibits virus transcription in neural cells and accumulation of MV glycoproteins in a monocytic cell line (Schneider-Schaulies et al., 1994; Schnorr et al., 1993). Since WTF readily replicates on DC, at least MxA-mediated restrictions obviously do not occur in these cells (Fig. 5D).

Importantly, the tropism of WTF H-containing viruses in vitro correlated both with their enhanced spread to mediastinal LN in vivo and with the induction of immunosuppression indicated by impaired proliferative responses of lymphocytes to mitogen stimulation ex vivo (Fig. 1C). Whether a SLAM orthologue is expressed in these animals is not known as yet. It is of interest to note that the source of the F protein did not influence either the tropism of the recombinant viruses for PBL or DC in vitro or the virus spread to the LN and the induction of immunosuppression in vivo. Based on our observations made in vitro, these findings suggest that expression of H proteins of lymphotropic MV strains may facilitate entry of
these viruses into DC also in vivo. As indicated by our findings
with WTF (Fig. 5E), as yet unknown virus determinants allow
these viruses to replicate efficiently in DC. Ongoing virus
replication of particularly MV WTF in immature DC was
associated with a more rapid maturation of these cells (Schnorr
et al., 1997) which can thereby be recruited more quickly into
secondary lymphatic tissues. By processing and presenting
MV antigens, MV infected DC may initially stimulate a
primary antiviral immune response, but also efficiently transmit
infectious virus to uninfected DC. Moreover, virus replication
may be additionally stimulated by CD40 ligation (Servet-
Delprat et al., 2000b). MV-infected DC are likely to undergo
rapid cell death either by fusion or by apoptosis (Fugier-Vivier
et al., 1997; Grosjean et al., 1997; Servet-Delprat et al., 2000a).
In agreement with previous findings (Fugier-Vivier et al.,
1997), transmission of virus from DC to T cells occurred only
to a very limited extent irrespective of the recombinant MV
strain used (not shown). Inhibition of T cell proliferation may,
however, have been largely brought about by the MV F/H
proteins which accumulate to high levels at the surface of DC
infected with WTF H-containing viruses (Fig. 5E; Schnorr
et al., 1997), and which were previously found to confer T cell
unresponsiveness in a contact-dependent manner both in vitro
and in vivo (Klagge et al., 2000; Niewiesk et al., 1997; Schlander
et al., 1996). Thus, MV H proteins allowing specific targeting
of DC are likely to enhance the immunosuppressive activity of
their corresponding viruses by a faster and more efficient
transport of virus into secondary lymphoid tissues.

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