Antibody-induced endocytosis of viral glycoproteins and major histocompatibility complex class I on pseudorabies virus-infected monocytes

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Purified porcine monocytes, the natural carrier cells of pseudorabies virus (PrV) in the pig, were inoculated in vitro with PrV. At different time-points post-inoculation (p.i.) (from 7 to 17 h p.i.), the cells were washed and incubated with fluorescein isothiocyanate-labelled porcine PrV-specific polyclonal antibodies (IgG) at 37 °C. At all time-points tested p.i., 1 h of antibody incubation induced passive patching and subsequent internalization of the plasma membrane-anchored viral glycoproteins in approximately 65% of the infected monocytes. This endocytosis process is antibody-dependent, since biotinylated glycoproteins did not undergo spontaneous endocytosis. The process is fast and efficient, since only very low amounts of viral glycoproteins on the plasma membrane (7 h p.i.) and a minimal concentration of antibodies (0.04 mg IgG/ml) were needed to induce endocytosis. Experiments with PrV strains carrying deletions in the genes encoding the 11 different viral glycoproteins showed that viral glycoproteins gB and gD play a very important role in endocytosis (80% reduction with deletion mutants, P < 0.001), while the gE:gl Fc receptor complex, but not gE or gl alone, has a significant but lesser effect (45% reduction, P < 0.05). Double staining of viral glycoproteins and major histocompatibility complex class I (MHC I) showed a clear co-localization and co-endocytosis of MHC I with the viral glycoproteins, suggesting a possible role of the process in immune evasion of the virus.

Introduction

Pseudorabies virus (PrV) is an alphaherpesvirus which causes Aujeszky’s disease in its natural host, the pig. The disease is characterized by nervous symptoms, respiratory disorders and reproductive failures, mainly abortions. Virus replication in the respiratory tract, viraemia and transplacental spread may still occur in the presence of an immunity raised by vaccination (Nauwynck & Pensaert, 1992; Wittmann et al., 1980). Nauwynck & Pensaert (1992) showed that injection of in vitro-infected monocytes into the arteria uterina of vaccinated sows induced abortion, whereas injection of cell-free virus did not. These observations stressed the importance of infected monocytes in PrV immune evasion.

Two major adaptive immune effector mechanisms are involved in elimination of PrV-infected cells (Kimman, 1994). First, newly synthesized viral proteins may be processed and coupled to the major histocompatibility complex class I (MHC I) in the endoplasmic reticulum of the infected monocytes. These MHC I–viral peptide complexes are then transported to the plasma membrane, where they may be recognized by cytotoxic T-lymphocytes (CTLs). Secondly, specific antibodies are capable of binding to newly synthesized viral envelope glycoproteins which are exposed on the plasma membrane of the infected cell. Antibody, in association with complement or phagocytes, may then result in lysis of the infected cell.

Earlier experiments in our laboratory showed that the addition of virus-specific antibodies to PrV-infected swine kidney (SK) cells in vitro induced a redistribution of the plasma membrane-anchored viral glycoproteins (Favoreel et al., 1997). They became clustered and moved towards one side of the cell,
forming a cap. This cap detached from the cell and was finally released into the surrounding medium, leaving a PrV-infected, viable cell with no visually detectable levels of viral glycoproteins on its plasma membrane.

In order to investigate whether the observed mechanism in SK cells might reflect a potential new immune-evasive strategy used by the virus, we now studied in detail the effect of virus-specific antibodies on the monocyte, the natural carrier of the virus in porcine blood. We report on antibody-induced ‘endocytosis’ (different from ‘release’ in SK cells) of viral glycoproteins, which is a highly efficient and fast process, again resulting in the occurrence of PrV-infected cells without visually detectable levels of viral glycoproteins on their plasma membrane. Moreover, co-endocytosis of MHC I was demonstrated. Viral glycoproteins gB and gD are important for the endocytosis process, while the gE:gl complex (but not gE or gl alone) has a lesser effect, enhancing the efficiency of the process.

Methods

**Viruses.** PrV strains 89V87 and Kaplan [PrV (Ka)], and PrV (Ka) deletion mutants gB, gC, gD’, gE, gF, gE:gl’, gG, gH’, gK, gL, gM’ and gN were used. All strains have been described earlier (Dijkstra et al., 1996; Jöns et al., 1996; Kaplan & Vatter, 1959; Klupp et al., 1992, 1997, 1998; Mettenleiter et al., 1987a, 1987b, 1988; Rauh & Mettenleiter, 1991; Rauh et al., 1991). Strains carrying deletions in the genes encoding essential glycoproteins were grown on complementing cell lines.

**Antibodies.** Three weeks after challenge of pigs which had been vaccinated several times with a subunit vaccine (Geskypur, Merial, France) with PrV (89V87), serum was collected (Nauwyck & Pennaert, 1995) and IgG antibodies were protein A-purified and fluorescein isothiocyanate (FITC)-conjugated. In some experiments, protein G-purified IgG antibodies, derived from convalescent sera obtained from pigs surviving at 21 days after inoculation with virulent PrV (Ka or 75V19 respectively), were used. Both sera contained approximately 5 mg IgG/ml. Dilutions of the antibodies were made in RPMI medium, supplemented with glutamine (0.3 mg/ml), penicillin (100 U/ml), streptomycin (0.1 mg/ml) and kanamycin (0.1 mg/ml).

**Isolation of blood monocytes.** PrV-negative pigs were used as blood donors at the age of 2 to 6 months. Blood was collected from the vena jugularis on heparin (15 U/ml) (Leo, Zaventem, Belgium) and blood mononuclear cells were separated on Ficoll-Paque (Pharmacia Biotech) following the manufacturer’s instructions. Mononuclear cells were resuspended in medium A, based on RPMI 1640 (Gibco BRL) and supplemented with 10% foetal calf serum (FCS), 0.3 mg/ml glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.1 mg/ml kanamycin, 10 U/ml heparin, 1 mM sodium pyruvate and 1% non-essential amino acids 100 x (Gibco BRL). Afterwards, cells were plated on 28 mm Petri dishes with cell culture coating (Nunc) at 2 x 10^6 cells/ml and cultivated at 37 °C with 5% CO₂. After 48 h, non-adherent cells (lymphocytes) were removed by washing the Petri dishes three times in RPMI 1640. Purity of the monocytes was assessed by flow cytometric analysis after indirect immunofluorescence labelling of the monocytes by incubating the cells in 1/300 74.22.15 (Pescovitz et al., 1984) mouse monoclonal antibody in medium A and 1/100 FITC-labelled goat anti-mouse antibody (Molecular Probes) in medium A. Percentage of monocytes that was always ≥ 75%.

**Inoculation of the monocytes.** After 48 h of cultivation of the mononuclear cells in Petri dishes, lymphocytes were removed as described above. Different virus strains were added at an m.o.i. of 10 in 1.5 ml medium A without heparin. One-and-a-half hours p.i., another 3-5 ml medium A without heparin was added to the Petri dishes. Cells were further incubated at 37 °C with 5% CO₂.

**Incubation of PrV-infected monocytes with porcine anti-PrV polyclonal antibodies.** In general, cells inoculated for 13 h were centrifuged at 500 x for 10 min, washed and resuspended in medium A without FCS and heparin. The cells were incubated at 0.6 x 10^7 cells/ml with FITC-conjugated PrV polyclonal antibodies (0.25 mg IgG/ml, except where indicated otherwise) for 1 h at 37 °C. Every 10 min, the cells were shaken gently to avoid sedimentation. At different times (0, 10, 30 and 60 min) during antibody incubation, the cells were fixed with 0.4% formaldehyde. Finally, the cells were washed thoroughly, mounted in a glycerin–PBS solution (0.9:0.1, v/v) with 25% L4-diazabicyclo(2.2.2)octane (Janssen Chimica), excited with an Osram HBO 50 W bulb and an L3 filter, and observed with a Leitz DM RBE microscope (Wild Leitz).

**Definition of different viral glycoprotein distributions.** The viral glycoprotein distribution was scored as a rim when the fluorescence label exhibited a homogeneous cell surface cover. The cells were scored as ‘patched’ when the labelled viral glycoproteins formed randomly distributed aggregates on the cellular surface. The viral glycoproteins were considered ‘endocytosed’ when all visible viral glycoproteins were located in vesicles inside the cell, without any remaining on the plasma membrane.

Quantitative results were obtained by examining the fluorescence distribution on at least 200 cells. The number of cells displaying endocytosis was expressed as a percentage of viral antigen-positive cells. All assays were run independently at least three times.

**Biotinylation of plasma membrane proteins.** At 13 h p.i., monocytes were washed twice with cold PBS and incubated with 40 µg biotinylation reagent per 10⁵ monocytes (Amersham International) on ice for 1 h. Afterwards, cells were washed with cold PBS, resuspended in medium A without FCS and heparin, and incubated at 37 °C in the absence or presence of 0.25 mg IgG/ml virus-specific antibodies for 1 h before fixation with formaldehyde as described above. To visualize internalized biotinylated proteins, cells were permeabilized in 0.1% Triton X-100 in PBS (PBS containing 0.5% FCS) for 1 min. Afterwards, cells were washed in PBS and incubated for 1 h with 1/100 Texas red-X-conjugated streptavidin (Molecular Probes) in PBS at room temperature. Finally, cells were washed in PBS, mounted in a glycerin–DABCO solution as described above and observed by confocal microscopy.

**Double staining of viral glycoproteins and MHC I.** Cells, inoculated with PrV (89V87) were incubated with FITC-labelled virus-specific antibodies at 13 h p.i. as described above. At 0 and 60 min post-infection, cells were formaldehyde-fixed and permeabilized as described above. After washing in PBS, cells were incubated with 1/100 mouse anti-porcine MHC I monoclonal antibody (VMRD, Pullman, Washington) in PBS for 1 h at room temperature, washed twice in PBS with 10% goat serum, and resuspended in 1/100 Texas red-X-conjugated goat anti-mouse antibodies (Molecular Probes) in PBS with 10% goat serum for 1 h at room temperature. Finally, cells were washed twice in PBS, mounted in a glycerin–DABCO solution as described above and observed by confocal microscopy.

**Flow cytometric analysis.** Flow cytometric analysis was conducted with a Becton Dickinson FACScan/F aquipped with a 15 mW air-cooled argon ion laser and interfaced to a Macintosh Quadra 650 computer (Apple Computer) using BD Cellquest software. Acquisition
rates were maintained at 300 to 500 cells/s. At least 5000 cells were analysed for each sample and forward-scattered light versus side-scattered light dot plots were used to identify monocyte populations.

**Confocal laser scanning microscopy.** Fluorescent samples were examined on a Bio-Rad MRC 1024 confocal laser scanning system linked to a Nikon Diaphot 300 microscope and interfaced to a Compaq Prosignia 300 computer. Krypton–argon laser light was used to excite FITC (488 nm line) and Texas red (568 nm line) fluorochromes. In order to avoid signal spillover, FITC and Texas red signals were recorded consecutively with respectively the 488 nm excitation line and the T1 dichroic beam splitter and the 568 nm excitation line and the T1/E2 beam splitter. Extended focus images were obtained with Bio-Rad COMOS and Lasersharp software.

**Results**

**Expression of viral glycoproteins on the plasma membrane of PrV-infected monocytes**

Fig. 1 shows that the expression of viral glycoproteins on the plasma membrane of PrV-infected monocytes starts between 5 and 7 h p.i. and increases less rapidly at 11 h p.i.

**Antibody-induced redistribution and endocytosis of viral glycoproteins, expressed on PrV-infected monocytes**

During earlier studies on the antibody-induced redistribution of viral glycoproteins on SK cells (Favoreel et al., 1997), cells were incubated with antibodies at 13 h p.i. Since monocytes show a similar kinetic pattern of viral antigen expression as that observed in SK cells, they were also incubated at 13 h p.i. with 0.25 mg FITC-labelled IgG/ml derived from PrV-infected pigs and incubated at 37 °C.

Shortly after antibody addition, viral glycoproteins became clustered into aggregates (patches), which were randomly distributed on the plasma membrane (Fig. 2a). Starting 10 min post-antibody addition, the patched viral glycoproteins were internalized (Fig. 2b). Approximately 65% of the infected monocytes showed endocytosed viral glycoproteins (without visually detectable viral glycoproteins remaining on the plasma membrane) after 1 h of incubation with PrV-specific IgG.

Since spontaneous endocytosis of viral glycoproteins gB, gE and the gE:gi complex from the plasma membrane has been described during early stages (≤ 6 h p.i.) of PrV infection (Tirabassi & Enquist, 1998), we determined whether the addition of PrV-specific antibodies is necessary for the observed endocytosis to occur. Therefore, plasma membrane glycoproteins of monocytes were biotinylated on ice at 13 h p.i. Afterwards, monocytes were incubated at 37 °C for 1 h in the presence or absence of PrV-specific antibodies. Cells were formaldehyde-fixed and permeabilized, and internalized biotinylated proteins were visualized. Fig. 3 shows that endocytosis only occurs in the presence of virus-specific antibodies. Furthermore, little or no biotinylated proteins are observed on the plasma membrane after incubation of the biotinylated cells with virus-specific IgG for 1 h. This suggests that most plasma membrane proteins become endocytosed as well. The most obvious explanation for this observation is that during the patching process of the abundantly presented viral glycoproteins, most other membrane proteins become caught and undergo co-endocytosis.

The use of antibodies (0.25 mg IgG/ml) derived from hyperimmune sera from pigs inoculated with different PrV parental strains (89V87, 75V19 and Ka) did not significantly alter the percentages of cells with endocytosed viral glycoproteins (64.8% ± 5.2%, 74.2% ± 11.7% and 62.1% ± 4.0%, respectively, 1 h post-antibody addition). Furthermore, the use of PrV-negative IgG (0.25 mg/ml) resulted in low background levels of endocytosed proteins (13.2% ± 0.1%) at 1 h post-antibody addition.

In all further experiments, antibodies derived from a PrV (89V87)-inoculated pig were used.

**Influence of infection status and concentration of antibodies on the antibody-induced endocytosis**

During earlier studies, it was found that the size of antibody-induced patches on PrV-infected SK cells gradually increases during antibody incubation (Favoreel et al., 1997). Viral glycoprotein capping only occurred once the patch size exceeded a minimal threshold size. Apart from antibody incubation time, the patch size was also affected by both the infection status of the cell (i.e. the amount of viral glycoproteins present on the plasma membrane) and the concentration of antibodies added. A minimal amount of viral glycoproteins on the plasma membrane (reached at 9 h p.i.) and a minimal concentration of antibodies (0.2 mg IgG/ml) were required to allow the formation of threshold-exceeding patches, resulting in a maximal conversion of patched to capped cells.

To evaluate whether a similar minimal threshold size of the viral glycoprotein patches has to be exceeded on monocytes
before endocytosis occurs, we examined the influence of infection status and the concentration of antibodies added.

Monocytes were incubated with porcine PrV polyclonal antibodies for 1 h at several time-points after PrV inoculation. From Fig. 4 and Fig. 1, it can be concluded that from the moment that the first viral glycoproteins appear on the plasma membrane of monocytes (7 h p.i.), antibody-induced endocytosis of the viral glycoproteins can take place. Inoculation periods between 7 and 15 h p.i. did not affect endocytosis significantly. Cells inoculated for 17 h showed a slightly reduced capacity to endocytise viral glycoproteins, probably due to a reduced capacity of the cell to perform energy-dependent processes as a consequence of the infection. All subsequent experiments were performed with cells at 13 h p.i.

Infected monocytes were incubated with different concentrations of PrV-specific antibodies for 1 h at 37 °C. Fig. 5 shows that as low a concentration of IgG as 0.04 mg/ml is sufficient to allow viral glycoprotein endocytosis.

In conclusion, it can be stated that both very low amounts of viral glycoproteins on the plasma membrane and very low concentrations of antibodies are sufficient to induce viral glycoprotein endocytosis. This indicates that antibody-induced endocytosis of viral glycoproteins is a highly efficient process and that the minimal threshold size of the patches,
Antibody-induced endocytosis of PrV glycoproteins

which must be exceeded for endocytosis to occur, is very small.

Co-endocytosis of MHC I with viral glycoproteins

PrV-infected monocytes, at different stages during incubation with PrV-specific antibodies (rim, patch and endocytosed viral glycoproteins) were formaldehyde-fixed, permeabilized, and MHC I was stained as described in Methods. As seen in Fig. 6, MHC I co-localizes well with patched and endocytosed viral glycoproteins.

Glycoproteins gB and gD are very important for antibody-induced endocytosis

Experiments with PrV (Ka) and isogenic gB, gC, gD, gE, gl, gE:gl, gG, gH, gK, gM and gN deletion mutants were performed to investigate which viral envelope glycoprotein(s) may play a role in antibody-induced redistribution of viral glycoproteins. None of the mutants used altered the expression of viral glycoproteins on the plasma membrane significantly, nor did they affect antibody-induced patching of the viral glycoproteins.

PrV (Ka)-infected monocytes showed slightly reduced endocytosis of viral glycoproteins after 1 h of antibody incubation, compared to cells inoculated with strain PrV (89V87). Table 1 shows the percentage of monocytes infected with different PrV strains with endocytosed viral glycoproteins after 1 h of incubation with antibodies. Infection with PrV (Ka) gB and gD deletion mutant strains resulted in a dramatic decrease of viral glycoprotein endocytosis ($P < 0.001$), while deletion of the gE:gl complex (but not gE or gl alone) resulted in a less pronounced, yet significant decrease in endocytosis ($P < 0.05$).

These data indicate that gB and gD are very important for viral glycoprotein endocytosis, while the gE:gl complex seems to enhance the efficiency of the process.

Viability of PrV-infected monocytes in the presence or absence of virus-specific antibodies

To evaluate whether the presence of virus-specific antibodies has an effect on the viability of the cells during infection, monocytes were inoculated with PrV (89V87) at an m.o.i. of 10. At 1-5 h p.i., 0.2 mg IgG/ml virus-specific
antibodies was added. Viability of the cells (mock-infected or PrV-infected in the continuous presence or absence of antibodies) was determined at different time-points after inoculation by incubating the cells for 3 min with 1 µg/ml propidium iodide, which specifically stains dead cells. Afterwards, fluorescence intensity of the cells was measured by flow cytometry. As seen in Fig. 7, viability did not alter significantly between 0 and 17 h p.i., neither in the absence nor in the presence of antibodies. Around 24 h p.i., viability started to decrease, independent of the presence of antibodies. In the absence of antibodies, the percentage of living cells dropped to approximately 19% at 75 h p.i., while 37% of the cells remained alive at the same time-point in the presence of antibodies. In both cases, all cells were infected, as determined by immunofluorescence staining with PrV-specific antibodies. Whether or not the presence of antibodies may protect cells from being destroyed might be an interesting issue for further research.

In conclusion, we can state that the presence of PrV-specific antibodies, and hence antibody-induced endocytosis of viral glycoproteins, has no short-term effect on cell viability. The slight drop in endocytosis capacity of cells inoculated for 17 h (Fig. 5) can indeed be explained by a decreased viability of the cells.

When infected cells were incubated in the presence of virus-specific antibodies, cells with endocytosed viral glycoproteins without visually detectable levels of viral glycoproteins on their plasma membrane were observed at all time-points between 7 and 17 h p.i. (data not shown). Since the experiments were performed in the continuous presence of antibodies, this
Table 1. Reduction of antibody-induced viral glycoprotein endocytosis in monocytes inoculated with different PrV deletion strains, compared to endocytosis observed with PrV (Ka) parental strain-infected cells

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Cells with endocytosed viral glycoproteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrV gB⁻</td>
<td>19.7±2.8**</td>
</tr>
<tr>
<td>PrV gC⁻</td>
<td>93.5±9.3</td>
</tr>
<tr>
<td>PrV gD⁻</td>
<td>21.4±2.0**</td>
</tr>
<tr>
<td>PrV gE⁻</td>
<td>101.5±19.5</td>
</tr>
<tr>
<td>PrV gL⁻</td>
<td>129.0±18.1</td>
</tr>
<tr>
<td>PrV gE:gi⁻</td>
<td>54.6±8.8*</td>
</tr>
<tr>
<td>PrV gG⁻</td>
<td>102.7±7.7</td>
</tr>
<tr>
<td>PrV gH⁻</td>
<td>100.0±17.3</td>
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<tr>
<td>PrV gK⁻</td>
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<td>PrV gL⁻</td>
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</tr>
<tr>
<td>PrV gM⁻</td>
<td>95.4±7.4</td>
</tr>
<tr>
<td>PrV gN⁻</td>
<td>92.1±4.7</td>
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Antibody-induced endocytosis of PrV glycoproteins

Fig. 7. Viability of monocytes during antibody incubation. Monocytes were mock-infected (△) or inoculated with PrV. Curves show the viability of the cells at different time-points post-inoculation, assessed by flow cytometric analysis after propidium iodide staining of dead cells, in the continuous presence (starting 1.5 h p.i.) (○) or absence ([ ]) of virus-specific antibodies.

result indicates that newly synthesized viral glycoproteins are endocytosed rapidly, or, alternatively, that viral glycoprotein synthesis is blocked during antibody incubation.

Discussion

Addition of PrV-specific antibodies to PrV-infected porcine blood monocytes in vitro induces aggregation and internalization of the plasma membrane-anchored viral glycoproteins. Earlier studies (Favoreel et al., 1997) already showed an antibody-induced redistribution (aggregation and capping) of viral glycoproteins on the plasma membrane of PrV-infected SK cells in vitro. This redistribution, however, finally led to shedding of the glycoproteins into the surrounding medium, while the current observations show an internalization of the viral glycoproteins. In both cases, the redistribution ultimately leads to the occurrence of PrV-infected cells without visually detectable levels of viral glycoproteins on their plasma membrane.

Spontaneous endocytosis of viral glycoproteins gB, gE and the gE:gl complex (but not gl alone or gC) during early stages of PrV-infection (up to 6 h p.i.) has already been described (Tirabassi & Enquist, 1998). Viral glycoproteins, expressed on the plasma membrane at later stages of infection did not undergo spontaneous endocytosis. Here we report that the addition of virus-specific porcine polyclonal antibodies ( IgG) to PrV-infected monocytes, the natural carrier of the virus in the blood (Nauwynck & Pensaert, 1994), induces internalization of all major viral glycoproteins, even at late stages of virus infection (17 h p.i.). Addition of antibodies was necessary for endocytosis to occur, since biotinylated viral glycoproteins did not undergo spontaneous endocytosis at these infection stages.

Antibody-induced endocytosis is a fast and efficient process. Endocytosis started at 10 min post-antibody addition, and was completed in 65% of the infected cells at 1 h post-antibody addition. Only very small amounts of viral glycoproteins on the plasma membrane (reached at 7 h p.i.) and very low concentrations of antibodies (0.04 mg IgG/ml, derived from hyperimmune animals, and <0.25 mg IgG/ml, derived from pigs, 3 weeks after infection) were needed to induce endocytosis.

Experiments using PrV strains carrying deletions in the genes encoding the 11 different viral glycoproteins showed that gB and gD play a very important role (80% reduction in endocytosis, P<0.001) and the gE:gl complex has a smaller role (45% reduction in endocytosis, P<0.05).

The gE:gl complex plays a more important role during antibody-induced redistribution of viral glycoproteins on PrV-infected SK cells (60% reduction in glycoprotein capping, P<0.01). This could at least partially be explained by the Fc receptor activity of this complex (Favoreel et al., 1997). It was hypothesized that antibody bipolar bridging was needed for the formation of large patches. Since the size of the patches has to exceed a minimal threshold size before capping occurs (Favoreel et al., 1997), antibody bipolar bridging could stimulate viral glycoprotein capping.

Two possible explanations can be given why deleting the gE:gl complex does not result in an equally dramatic decrease in viral glycoprotein endocytosis in monocytes as the observed reduction in capping in SK cells. Firstly, the monocyte, in contrast to the SK cell, expresses a variety of natural Fc receptors (Alberts et al., 1994), which may be able to (partially) take over the function of the gE:gl complex. Secondly, the current results show that the minimal size of the patches

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required for internalization of the viral glycoproteins to occur is much smaller than the patch size required for viral glycoprotein capping to occur in SK cells. Therefore, the role of antibody bipolar bridging in the whole process could be much less important in monocytes than in SK cells.

The results show an important role for gB and gD, which are dispensable for viral glycoprotein capping in SK cells. Of possible interest in this context may be the presence of a YxxL-34x-YxxL amino acid sequence in the cytoplasmic tail of gB and a YxxF-7x-YxxL sequence in the tail of gD, with Y standing for tyrosine, x for any amino acid, L for leucine, I for isoleucine and F for phenylalanine. From the literature, it is known that receptors which can undergo endocytosis after binding their respective ligand contain several highly conserved amino acid sequences in their cytoplasmic tail. The most strict consensus sequence is YxxL-7x-YxxL (ARAM sequence, antigen recognition activation motif) (Reth, 1989). It has been assumed that binding of a ligand to these receptors induces aggregation (patching) of the receptors. By this change in conformation, a tyrosine kinase can bind to the tail of the receptor, phosphorylating the tyrosines in the ARAM sequence, which in turn causes receptors to be internalized. However, varicella-zoster virus gE undergoes spontaneous endocytosis, which is mediated by one YxxL sequence in its cytoplasmic tail (Olson & Grose, 1997). The possible role of the cytoplasmic tail, and, more specifically, the YxxL sequences in the tail of gB and gD might be worth investigating, since antibody-induced endocytosis of the viral glycoproteins can be blocked by the addition of genistein, a specific inhibitor of tyrosine kinases (unpublished observations). Therefore, experiments are planned to construct PrV mutant strains expressing carboxy-terminal truncated forms of gB and/or gD.

The present findings establish an interesting in vitro model to study the possibility that certain viral glycoproteins might be capable of transducing signals to the cell upon cross-linking.

Moreover, the findings may have significance for the pathogenicity of the virus. It is known that herpesviruses have developed multiple ways to evade the immune response (Banks & Rouse, 1992; Ploegh, 1998). This report shows that the continuous presence of virus-specific antibodies ensures the clearance of the plasma membrane of all visually detectable levels of viral glycoproteins up to 17 h p.i., without any effect on cell viability. Although the lack of visually detectable levels of viral glycoproteins does not exclude the possible presence of enough molecules for complement activation, it is interesting to further investigate whether the current model reflects a new immune-evasive strategy by PrV. Important to note in this context is our observation that MHC I undergoes co-endocytosis with viral glycoproteins after the addition of PrV-specific antibodies to PrV-infected monocytes. Since biotinylation experiments showed co-endocytosis of most plasma membrane proteins, MHC I co-endocytosis is most likely to be a passive phenomenon. However, a specific interaction between MHC I and one or more viral glycoproteins, similar to the murine cytomegalovirus gp34 association with MHC I (Ploegh, 1998), cannot be excluded, and may be worth investigating. Virus-induced (but not antibody-induced) endocytosis of MHC I is a phenomenon already described for human immunodeficiency virus (HIV). The HIV Nef protein modifies the endocytic machinery so that class I molecules are internalized (Collins et al., 1998). Notwithstanding the sensitivity of CTLs, this down-regulation appears sufficiently effective to block killing by class I-restricted anti-HIV CTLs in vitro (Collins et al., 1998). However, one has to consider the effect of MHC I clearance on natural killer (NK) cells. NK cells destroy a variety of virus-infected cells early during infection. They are normally prevented from killing their targets by inhibitory signals provided through interaction of receptors on the NK cells with self-MHC products (complexes of MHC with cell-derived peptides) (Kärre, 1997). If, as demonstrated here, MHC I could be completely removed from the cellular surface, cells may be more easily destroyed by NK cells. Therefore, the current in vitro results may indicate an important role for NK cells in controlling PrV infection. Earlier studies in vitro already suggested that NK cells may be crucial for lysis of PrV-infected cells (Kimman et al., 1996; Martin & Wardley, 1984), although this could not be demonstrated in vivo (Martin & Wardley, 1984).

In conclusion, it can be stated that antibody-induced endocytosis of viral glycoproteins and MHC I might reflect an interesting new type of immune evasion, used by PrV.

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References


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