Influenza A virus M1 blocks the classical complement pathway through interacting with C1qA

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The matrix (M1) protein of influenza A virus is a conserved multifunctional protein that plays essential roles in regulating the viral life cycle. This study demonstrated that M1 is able to interact with complement C1qA and plays an important inhibitory function in the classical complement pathway. The N-terminal domain of M1 protein was required for its binding to the globular region of C1qA. As a consequence, M1 blocked the interaction between C1qA and heat-aggregated IgG in vitro and inhibited haemolysis. It was shown that M1 protein prevented the complement-mediated neutralization of influenza virus in vitro. In addition, studies on mice indicated that the administration of M1 could promote a higher virus propagation rate in lung and shortened survival of mice infected with the virus. Taken together, these results suggest strongly that the M1 protein plays a critical role in protecting influenza virus from the host innate immune system.

INTRODUCTION

The genome of influenza A virus is composed of eight segments of single-stranded RNA, which exist as a viral ribonucleoprotein (vRNP) complex with nucleocapsid protein (NP) and RNA-dependent RNA polymerase (Neumann et al., 2004). vRNP is surrounded by matrix protein 1 (M1), which is the most abundant protein in influenza virus particles (Nayak et al., 2004). Influenza A virus M1 protein is relatively conserved and consists of two globular regions (aa 1–164 and 165–252). The structure of the N-terminal fragment of the M1 protein determined by X-ray crystallography analysis shows the existence of nine helices and eight loops (Arzt et al., 2001; Harris et al., 2001; Sha & Luo, 1997), whilst the structure of the C-terminal domain of M1 is not yet available. It is widely accepted that M1 plays a significant role in many aspects of the virus life cycle. M1 interacts with RNA and RNP complexes (Baudin et al., 2001; Bui et al., 1996; Elster et al., 1997; Huang et al., 2001; Wakefield & Brownlee, 1989; Watanabe et al., 1996; Ye et al., 1999) and viral envelope protein (Ali et al., 2000; Barman et al., 2001), and is involved in transcription inhibition (Baudin et al., 2001; Elster et al., 1997; Watanabe et al., 1996), RNP nuclear import/export and the budding process (Akarsu et al., 2003; Bourmakina & Garcia-Sastre, 2005; Nayak et al., 2004). M1 expressed on the infected cell surface is responsible for cross-reactive recognition by cytotoxic T lymphocytes (Braciale, 1977; Reis & Schulman, 1980). Several other studies have also suggested that some M1 mutations affect virus particle morphology (Burleigh et al., 2005) and are involved in virus–host protein interactions (Nagata et al., 2008).

Several host proteins have been found to associate with M1, including the globular domain of the histone octamer (Garcia-Robles et al., 2005), heat-shock protein 70 (Watanabe et al., 2006) cytoskeletal elements (Avalos et al., 1997), the cellular receptor of activated C kinase 1 (Reinhardt & Wolff, 2000) and caspase 8 (Zhirkov et al., 2002). These interactions imply a broad range of roles of biological significance, such as vRNP export and viral morphogenesis. Extracellular signal-regulated kinase (ERK), downstream of the Ras-activated factor Raf/MEK/ERK pathway, phosphorylates M1. When cells infected with influenza virus are treated with a MEK-specific inhibitor, NP and vRNP complexes accumulate in the nucleus (Pleschkha et al., 2001). A recent report indicated that cyclophilin A interacts with M1 and impairs early virus replication (Liu et al., 2009). The interactions between M1 and host proteins are critical for viral propagation, but it is
unknown whether M1 protein has any effects on host immune responses.

A number of studies have focused on the mechanism of influenza virus neutralization by serum. It had been found that human serum can neutralize strain A/WSN/33 virus efficiently through specific antibodies and the C1q–C4-mediated complement pathway (Beebe et al., 1983). In a study carried out with mouse serum, researchers found that C1q but not C3 was needed for virus neutralization and that a heat-resistant factor also contributed to virus neutralization (Mozdzanowska et al., 2006). A recent study indicated that natural IgM could restore virus neutralization activity to antibody-deficient serum and that the mechanism of virus neutralization by natural IgM was associated with virion aggregation, which was dependent on C1q–C4 of the complement pathway, but not on C5-mediated viral lysis (Jayasekera et al., 2007). Taken together, these data indicate the involvement of complement, or at least C1q, in influenza virus neutralization by serum.

To understand the function of M1 in more detail, we took the approach of yeast two-hybrid screening to identify host proteins that interact with M1. In the present study, the complement component C1qA was identified as an M1-binding protein. Further analysis showed that M1 interacted with the globular region of C1qA. The globular region of C1qA is the binding domain for the antigen–antibody complex. Our data suggest that the M1 protein can efficiently suppress the classical pathway and protect virus from being neutralized. We found that mice administrated with M1(1–170) had enhanced influenza virus propagation in lung and shortened survival rate when compared with mice treated with M1(171–252). Taken together, our results indicate that influenza virus M1 protein has an important role in protecting the virus from the host innate immune system via its interaction with complement C1qA.

**METHODS**

**Cell lines, viruses and antibodies.** Madin–Darby canine kidney (MDCK) cells and human embryo kidney 293T cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS; PAA). Influenza virus strain A/WSN/33 (H1N1) was generated by reverse genetics (Neumann et al., 1999) and propagated in MDCK cells. Mouse anti-M1 monoclonal antibody was prepared as described previously (Koestler et al., 1984). Rabbit anti-human C1qA and rabbit anti-NP polyclonal antibodies were generated by immunizing rabbits with glutathione S-transferase (GST)–C1qA(24–70) fusion and His-tagged NP protein, respectively. The antibodies were affinity purified with antigen-conjugated agarose. Monoclonal anti-Myc (9E10) and anti-His antibodies were purchased from Santa Cruz Biotechnology. Mouse anti-FLAG (M2) antibody was purchased from Sigma.

**Construction of plasmids.** Full-length and truncated forms of M1 were subcloned into pET30a (Novagen). Full-length C1qA and truncated forms were subcloned into pGEX-6P-1 (Pharmacia). Full-length M1, C1qA and truncated C1qA were subcloned into pENTR vector (Invitrogen) and transferred into pDEST-Myc or pDEST-FLAG expression vector (Invitrogen) using an LR Clonase enzyme mix kit (Invitrogen) following the manufacturer’s instructions.

**Yeast two-hybrid system.** A Matchmaker Two-hybrid System 3 (Clontech) was used to screen host proteins that interacted with M1. In brief, the bait construct pGBKTK7-M1 and a human kidney cDNA library in pACT2 (prey) were co-transformed into yeast strain AH109. Transformants were selected for growth on medium lacking His, Leu and Trp (His/-/Leu/-/Trp-). The colonies were then transferred to Ade+/His+/Leu+/Trp+ plates containing X-Gal. Blue colonies were selected and cultured in Ade+/His+/Leu+/Trp+ broth and lysed for plasmid extraction. The plasmids were amplified and the target insertions were verified by sequencing. To confirm interaction between M1 and host proteins, the two yeast expression plasmids were co-transformed into yeast strain SFY-526 and β-galactosidase assays were performed according to manufacturer’s instruction.

**Protein purification, and GST and His pull-down assays.** GST fusion and His-tagged proteins were purified with Sepharose 4B–glutathione (Pharmacia) and Ni-NTA (Qiagen), respectively. For the GST pull-down assay, 5 μg GST or GST–C1qg fusion protein bound to Sepharose 4B–glutathione was incubated with purified recombinant M1 or FLAG–M1 293T cell lysate at 4°C for 4 h. The beads were washed five times with PBS with 0.1% Triton X-100, and bound protein was eluted by boiling in SDS loading buffer and subjected to Western blot analysis. For the His pull-down assay, 5 μg Ni-NTA-bound His-tagged full-length and truncated M1 proteins were incubated with 1 μg recombinant GST–C1qA in His pull-down buffer (200 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4, 1 mM MgCl2, 0.1% Triton X-100, 20 mM imidazole, 1 mg BSA ml−1) at 4°C for 2 h. After incubation, the beads were centrifuged and washed with pull-down buffer. Bound proteins were eluted with 50 μl pull-down buffer supplemented with 200 mM imidazole and analysed by Western blot analysis with rabbit polyclonal anti-C1qA antibody.

**Co-immunoprecipitation.** 293T cells were transfected with FLAG-tagged and Myc-tagged plasmid. At 36 h post-transfection, cells were washed with cold PBS and lysed in lysis buffer [1% Triton X-100, 150 mM NaCl, 20 mM HEPES (pH 7.5), 10% glycerol, 1 mM EDTA] with protease inhibitor cocktail (Roche). The lysates were immunoprecipitated with anti-FLAG beads at 4°C for 4 h. The beads were washed five times with lysis buffer. Bound proteins were eluted by boiling with SDS loading buffer for 10 min and subjected to Western blot analysis with anti-Myc monoclonal antibody.

**ELISA assays.** ELISA was performed using Maxisorb plates (Nunc). Plates were coated with proteins diluted in coating buffer [100 mM Na2CO3/NaHCO3 (pH 9.6)] at 4°C overnight followed by blocking with 10% PBS in PBS for 2 h at 37°C. All subsequent steps were performed in PBS containing 0.05% Tween 20 unless otherwise indicated, and each step was followed by three washes with PBS/0.05% Tween 20. The enzymic activity of horseradish peroxidase (HRP) was measured by the addition of 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma) and H2O2. Absorbance was measured using a microplate reader (Tecan Sunrise).

To assess the effect of M1 on C1q and IgG binding, plates were coated with 5 μg purified GST–C1qA(23–245) ml−1. Heat-aggregated IgG (100 μl, 2 μg ml−1; Zhongshan Goldenbridge Biotechnology) was added in the presence of M1 at various concentrations. IgG bound to C1qA was detected with HRP-conjugated goat anti-human IgG antibody. A405 (blank) was the absorbance obtained when no IgG was added, whilst A405 (100%) was the absorbance obtained when IgG was added in the absence of M1. The percentage inhibition was calculated using the formula 1−[A405 (measurement)−A405 (blank)]/[A405 (100%)−A405 (blank)].
To detect anti-M1 antibody in serum, plates were coated with 5 μg M1(1–170) ml⁻¹. The indicated amount of serum was added and bound antibody was detected with HRP-conjugated goat anti-mouse antibody.

**Virus neutralization assays.** Human serum was obtained from a healthy volunteer and stored at -80 °C. Influenza virus A/WSN/33 (50 μl, 10⁶ p.f.u. ml⁻¹) was mixed with 10 μl human serum or heat-inactivated serum in the presence of 20 μM M1 or BSA at 37 °C for 30 min. The samples were analysed immediately by plaque assay.

**Plaque assay.** MDCK cell monolayers in 35 mm dishes were washed with PBS and serial dilutions of virus were adsorbed to the cells for 2 h. Unadsorbed virus was removed by washing with serum-free DMEM, and the cell monolayers were then overlaid with DMEM supplemented with 3 % low-melting-point agarose and 2 μg TPCK-treated trypsin (Sigma) ml⁻¹. After 3 days of incubation, visible plaques were counted and virus titres were calculated. All data were expressed as the mean of triplicate samples.

**Haemolytic assay.** To determine the haemolytic activity of the classical component activity, sheep red blood cells (SRBCs) were sensitized using rabbit anti-SRBC polyclonal antibody. Antibody-sensitized SRBCs (10 μl) were then incubated with 10 μl human serum in the presence of the indicated concentration of M1(1–170) or BSA at a final volume of 1 ml PBS at 37 °C for 30 min and then centrifuged at 2000 g for 1 min. The A₅₅₀ of the supernatant was measured against a reagent blank in which SRBCs were incubated with PBS. The percentage haemolysis was calculated by normalizing against the absorbance of a mixture of red blood cells and distilled water, which showed complete haemolysis.

**Mice and virus infection.** Four-week-old specific-pathogen-free BALB/c mice were used in these studies. Experiments were performed in accordance with institutional guidelines. M1 administration and mice infection were performed according to a modification of a standard procedure (Jayasekera et al., 2007). Briefly, mice were anaesthetized with ether and then administration with 200 μl M1(1–170) or M1(171–252) (1 μg ml⁻¹) via the tail vein. The mice were then immediately infected intranasally with 25 μl influenza virus (2 x 10⁵ p.f.u. ml⁻¹). At 3 days post-infection, three mice from both groups were sacrificed and virus titres in the lungs were determined by plaque assay. Mice were examined daily for survival.

**RESULTS**

**Influenza A virus M1 protein interacts with C1qA**

To explore the function of the M1 protein, we attempted to search for cellular proteins that interacted with it. We screened a human kidney cDNA library by using a yeast two-hybrid system. The full-length M1 coding sequence was cloned into the pGBK7 vector to generate pGBK7-M1 as bait. The bait construct was used to co-transfect yeast strain AH109 with the cDNA library in pACT2, followed by screening on Leu⁻ Trp⁻ His⁻ plates. This screening yielded a cDNA clone encoding the complement fragment C1qA, suggesting that C1qA peptide interacts with M1. To confirm the interaction between M1 and C1qA, GST pull-down assays were carried out. GST and GST–C1qA fusion proteins bound to Sepharose 4B–glutathione were incubated with cell lysates prepared from pDEST-FLAG-M1-transfected 293T cells. Complexes were washed and resolved by SDS-PAGE. M1 proteins were detected in the GST–C1qA pull-down materials by Western blotting with anti-FLAG antibody, indicating that GST–C1qA fusion protein could interact with FLAG–M1 from the cell lysate (Fig. 1a). In a similar experiment, GST–C1qA fusion protein was found to bind to purified recombinant His–M1 fusion protein but not to GST alone (Fig. 1b). To examine the interaction of M1 and C1qA in cells, FLAG-tagged M1 and Myc-tagged C1qA were transiently expressed in 293T cells and cell lysates were immunoprecipitated with anti-FLAG antibody and immunoblotted with anti-Myc antibody. The result showed that FLAG–M1 indeed interacted with Myc–C1qA (Fig. 1c). Furthermore, FLAG–C1qA expressed in cells bound to M1 but not to NP, indicating that C1qA interacts specifically with M1 (Fig. 1d).

**Fig. 1.** Influenza virus matrix protein M1 interacts with C1qA. (a, b) GST pull-down assay. Cell lysates from pDEST-FLAG-M1 transfected 293T cells or purified His–M1 proteins were incubated with GST and GST–C1qA, respectively. M1 protein associated with C1qA was analysed by Western blotting with anti-FLAG antibody (a) and anti-His antibody (b). (c, d) Co-immunoprecipitation analysis. (c) 293T cells were transfected with pDEST-Myc–C1qA and pDEST-FLAG-M1 or pDEST-FLAG-M1 alone. Cell lysates were immunoprecipitated with anti-FLAG antibody and immunoblotted with anti-Myc antibody. (d) 293T cells were transfected with pDEST-FLAG-C1qA or pcDNA3.1 and then infected with A/WSN/33 virus (m.o.i. of 1) for 12 h. Cell lysates were immunoprecipitated with anti-Myc antibody and immunoblotted with anti-M1 or anti-NP antibody.
To characterize further the interaction between M1 and C1qA, we attempted to map the region of M1 required for interaction with the complement fragment. A series of truncated mutants of M1 was constructed (Liu et al., 2009) (Fig. 2a) and applied to a His pull-down assay. As shown in Fig. 2(b), the N-terminal aa 1–170 of M1 were sufficient to bind C1qA. A GST pull-down assay also showed that GST–C1q bound to M1(1–170) but not M1(171–252) (Fig. 2c), indicating that M1(1–170) was required for its binding to C1qA. A previous study showed that the C1qA globular head region plays an important role in the classical complement pathway (Kaul & Loos, 1995). In the present study, we demonstrated that M1(1–170) interacted with the C1qA globular head region (aa 110–245) but not with C1qA(1–109) by a GST pull-down assay (Fig. 3a). Co-immunoprecipitation analysis also indicated that full-length M1 interacted with the C1qA globular region (Fig. 3b). As the C1qA globular region plays an important role in the classical complement pathway, our results implied that M1 may subvert the host classical complement system during virus infection.

**M1 blocks C1qA-mediated classical complement activity**

C1qA is the first subcomponent of the C1 complex in the classical complement pathway and plays a key role in recognizing immunoglobulin through its globular head region (Kishore & Reid, 2000). Therefore, we hypothesized that the binding of M1 to the globular head region of C1qA could impair complement activity. To assess the effect of M1 on the interaction between C1qA and IgG, we first used an ELISA to examine the changes in binding of IgG to C1qA coated on plates with an increasing M1 protein concentration. As shown in Fig. 4, M1 was found to block the binding of IgG to C1qA in a dose-dependent manner. This suggested that M1 may be a potent inhibitor of the classical complement pathway. We then used haemolytic assays to measure the classical pathway complement activation and the effect of M1 protein on this. The data showed that M1 blocked the complement-dependent haemolysis of SRBCs in a dose-dependent manner, indicating that M1 could interfere efficiently with the classical complement pathway (Fig. 5).

**M1 counteracts neutralization of virus by human serum**

It has been shown that influenza virus can be neutralized *in vitro* by the classical complement pathway (Beebe et al., 1983; Jayasekera et al., 2007; Mozdzanowska et al., 2006). As our results showed that M1 interacted with C1qA and blocked classical pathway activation, we asked whether M1 was able to protect influenza virus from being neutralized by serum. To address this question, influenza virus A/WSN/33 was incubated with normal or heat-inactivated human serum in the presence or absence of M1 protein for 30 min. After treatment, the virus was titrated by plaque assay. As shown in Fig. 6, when human serum was pre-incubated with M1 protein, virus neutralization was partly counteracted as the virus titre was fourfold higher than that in the control. However, there was no significant difference.

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**Fig. 2.** M1(1–170) binds C1qA. (a) Diagram of the series of deletion mutants of M1 and their interaction with C1qA, determined by a His pull-down assay and/or by immunoprecipitation (IP). ND, Not determined. (b) His pull-down assay. His-tagged recombinant M1 and its deletion mutants were incubated with GST–C1qA. Bound proteins were eluted and analysed by Western blotting with anti-C1qA antibody. CBB, Coomassie brilliant blue staining. (c) His–M1(1–170) or M1(171–252) were incubated with GST or GST–C1qA. Proteins associated with C1qA were analysed by Western blotting analysis and detected with anti-His or anti-M1 antibody.
in the virus titre with or without M1 when the serum was heat-inactivated. Taken together, these results indicated that M1 can counteract the factors in serum that neutralize influenza virus and are sensitive to heat inactivation. It is likely that the complement components in the serum are the factors involved in neutralizing the virus and that this neutralization can be inhibited in the presence of M1 proteins.

**Mice become more susceptible to virus infection after administration with M1**

Finally, we asked whether M1 administration facilitated influenza virus propagation *in vivo*. To address this question, BALB/c mice were administrated with 200 μg M1(1–170) or M1(171–252) and immediately infected intranasally with influenza virus (5 × 10^6 p.f.u.). Virus titres in the lungs of these mice were measured at 72 h post-infection. As seen in Fig. 7(a), the virus titre in M1(1–170)-administered mice was approximately threefold higher than that in M1(171–252)-treated mice. Consistent with these results, the survival period of mice administrated M1(1–170) was on average reduced by 1 day compared with mice administrated with M1(171–252) (Fig. 7b). As only M1(1–170), which binds to C1qA, is known to make mice more vulnerable to viral infection, this suggested that M1 may facilitate virus replication by counteracting virus neutralization by the C1qA-mediated complement pathway.

**DISCUSSION**

Viruses have developed numerous strategies to counteract the classical complement system. Many virus proteins have been found to regulate complement activation and their functions to cover almost every step of the classical complement activation pathway (Favoreel *et al.*, 2003). Herpes simplex virus expresses Fc receptors on the surface of infected cells, which inhibits efficient activation of antibody-dependent complementarily components (Frank & Friedman, 1989). The VCP protein of vaccinia virus and SPICE protein of variola virus have been shown to inactivate C3b and C4b as co-factor I activity. Herpesvirus saimiri CCPH protein effectively inhibits C3 convertase activity and reduces cell-surface deposition of C3b. Cowpox virus IMP protein limits macrophage infiltration by downregulating complement proteins C3a, C4a and C5a (Howard *et al.*, 1998; Kotwal *et al.*, 1998). Glycoprotein C of herpes simplex virus mediates inhibition of C5 binding to C3b (Friedman *et al.*, 1984; Hung *et al.*, 1992, 1994) and the HV5-15 protein of herpesvirus saimiri inhibits complement activity after C3b deposition, indicating terminal complement inhibition (Rother *et al.*, 1994). However, so far, little is known about whether influenza
virus has developed a strategy to suppress the classical complement pathway.

Previous studies have focused mainly on the mechanism of virus neutralization by complement. Human serum was found to neutralize influenza virus efficiently by an antibody-dependent mechanism, and complement components (C1, C3 and C4) were also indispensable for neutralization, although membrane attack pathways were not needed for the process (Beebe et al., 1983). A recent study demonstrated that, in mice, natural antibody rather than specific antibody mediated neutralization of influenza virus. It had been confirmed that C1, C3 and C4 are required in the process (Jayasekera et al., 2007). It should be noted that all of these previous studies are consistent with C1 being indispensable in the neutralization of influenza virus by complement. The present study also confirmed that the virion could be neutralized by complement (Fig. 6). Taken together, these results indicated that the influenza virion can be neutralized efficiently by complement and that neutralization is dependent on complement C1. Influenza virus may develop some strategies to block this complement C1-dependent neutralization. A recent study reported that human astrovirus coat protein inhibits complement activation via C1 (Bonaparte et al., 2008), which provides a good example of virus targeting complement by binding with C1.

The present study demonstrated that M1 may play a similar role in counteracting complement via C1. Firstly, M1 is highly abundant in influenza virus-infected cells and it is plausible that, at the late phase of virus infection, some host cells are so heavily destroyed that any matrix protein that is not assembled into virus particles is released and counteracts the complement system to protect new virus particles before they can attach to other cells. Secondly,
anti-M1 antibodies were detected in human serum and the
serum of virus-infected mice (see Supplementary Fig. S1, available in JGV Online), indicating that M1 was present in
body fluids of the host following influenza virus infection, probably due to the non-specific release of M1 as a result of
cell necrosis. These results indicate that M1 can bind to the
C1qA globular region to efficiently reduce the interaction
between C1qA and IgG and inhibit classical complement
activity-dependent haemolysis. These results suggest that
M1 is a potential complement pathway inhibitor by disrupting C1q-dependent classical complement activity.
C1q is a key component of the classical complement
pathway and acts as a recognition molecule that interacts
with antibody–antigen complexes to activate the
complement cascade. Some studies have shown that
specifically designed peptides that bind C1q can inhibit
the complement pathway (Roos et al., 2001). According to
the present results, the M1 protein may act as a virus-
designed complement inhibitor that is abundantly
expressed during virus infection and released during
infected cell necrosis to protect newly assembled virus
particles from being neutralized by complement.

The present study indicates that influenza A virus M1
protein blocks the complement pathway through its
interaction with C1qA. This finding may help to elucidate
the connection between the innate immune system and
influenza virus, and explain how influenza virus counteracts the innate immune system.

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REFERENCES

Akarsu, H., Burmeister, W. P., Petosa, C., Petit, I., Muller, C. W.,
protein-binding domain of the influenza A virus nuclear export
protein (NEP/NS2). *EMBO J* 22, 4646–4655.

virus assembly: effect of influenza virus glycoproteins on the

Arzt, S., Baudin, F., Barge, A., Timmins, P., Burmeister, W. P. &
on intact influenza virus M1 protein and from a new crystal form of its
N-terminal domain show that M1 is an elongated monomer. *Virology*
279, 439–446.

virus NP and M1 proteins with cellular cytoskeletal elements in

Transport of viral proteins to the apical membranes and interaction
of matrix protein with glycoproteins in the assembly of influenza

dissection of the membrane and RNP binding activities of influenza

of influenza virus by normal human sera: mechanisms involving

Bonaparte, R. S., Hair, P. S., Banthia, D., Marshall, D. M., Cunnion,
serum complement activation via C1, the first component of the

Bourmakina, S. V. & Garcia-Sastre, A. (2005). The morphology and
composition of influenza A virus particles are not affected by low

Braciale, T. J. (1977). Immunologic recognition of influenza virus-
infected cells. II. Expression of influenza A matrix protein on the
infected cell surface and its role in recognition by cross-reactive

low pH on nuclear transport of influenza virus ribonucleoproteins.

Influenza A viruses with mutations in the M1 helix six domain display

Influenza virus M1 protein binds to RNA through its nuclear

Favoreel, H. W., Van de Walle, G. R., Nauwynck, H. J. & Pensaert,

simplex virus type 1 Fc receptor: participation in bipolar bridging of

Friedman, H. M., Cohen, G. H., Eisenberg, R. J., Seidel, C. A. & Cines,
receptor for the C3b complement component on infected cells.

Garcia-Robles, I., Akarsu, H., Muller, C. W., Ruigrok, R. W. & Baudin,

Harris, A., Forouhar, F., Qiu, S., Sha, B. & Luo, M. (2001). The crystal
structure of the influenza matrix protein M1 at neutral pH: M1–M2
protein interfaces can rotate in the oligomeric structures of M1.
*Virology* 289, 34–44.

Howard, J., Justus, D. E., Totmenin, A. V., Schelchkunov, S. & Kotwal,
G. J. (1998). Molecular mimicry of the inflammation modulatory
proteins (IMPs) of poxviruses: evasion of the inflammatory response

Effect of influenza virus matrix protein and viral RNA on ribonucleoprotein

Hung, S. L., Srinivasan, S., Friedman, H. M., Eisenberg, R. J. &
Cohen, G. H. (1992). Structural basis of C3b binding by glycoprotein

Hung, S. L., Peng, C., Kostavasili, I., Friedman, H. M., Lambris, J. D.,
Eisenberg, R. J. & Cohen, G. H. (1994). The interaction of
glycoprotein C of herpes simplex virus types 1 and 2 with the

antibody and complement mediated neutralization of influenza virus

http://vir.sgmjournals.org


