Involvement of the Mannose Receptor in Infection of Macrophages by Influenza Virus

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Received 4 October 1999/Accepted 1 March 2000

Influenza viruses A/PR/8/34 (PR8; H1N1), A/Aichi/68 X-31 (HKx31; H3N2), and A/Beijing/89 X-109 (BJx109; H3N2) show marked differences in their ability to infect murine macrophages, including resident alveolar and peritoneal macrophages as well as the macrophage-derived cell line J774. The hierarchy in infectivity of the viruses (PR8 < HKx31 < BJx109) resembles that of their reactivity with mannose-binding lectins of the collectin family. Since the macrophage mannose receptor recognizes the same spectrum of monosaccharides as the collectins do, we investigated the possible involvement of this receptor in infection of macrophages by influenza virus. In competitive binding studies, the binding of 125I-labeled mannosylated bovine serum albumin to macrophages was inhibited by the purified hemagglutinin and neuraminidase (HANA) glycoproteins of influenza virus but not by HANA that had been treated with periodate to oxidize its oligosaccharide side chains. The inhibitory activity of HANA from the three strains of virus differed markedly and correlated with the infectivity of each virus for macrophages. Infection of macrophages, but not MDCK cells, by influenza virus was inhibited by yeast mannan. A variant line of J774 cells, J774E, which expresses elevated levels of the mannose receptor, was more readily infected than J774, and the sensitivity of J774E cells to infection was greatly reduced by culture in the presence of β-mannose, which down-modulated mannose receptor expression. Together, the data implicate the mannose receptor as a major endocytic receptor in the infectious entry of influenza virus, and perhaps other enveloped viruses, into murine macrophages.

Infection of host cells by influenza virus is mediated by binding of the viral hemagglutinin (HA) to sialylated cell surface molecules, followed by receptor-mediated endocytosis and acid-activated membrane fusion in endosomes (22). Many different sialylated glycoproteins and glycolipids on the cell membrane may function as primary receptors for influenza virus attachment, but not all binding leads to infection (for example, see references 8 and 49). With the exception of recent studies on influenza C virus, little is known about the identity of the functional receptor(s) that initiates the infectious process. Influenza C virus differs from influenza A and B viruses in recognizing the less common N-acetyl-9-O-acetylmuraminic acid, rather than N-acetylneuraminic acid, as its specific receptor determinant. Zimmer et al. (52) have identified a major mucin-type glycoprotein on the surface of Madin-Darby canine kidney type I cells, gp40, that binds influenza C virus and is subject to constitutive endocytosis and that may represent the functional receptor for influenza C virus in this cell type.

In this study we focus on the infectious entry of influenza A virus into macrophages (Mφ). Influenza virus infects Mφ, and viral proteins are expressed, but replication is abortive and little or no infectious virus is produced (38, 46). By acting as a “dead end” for the virus, Mφ play an important role in early host defense against influenza virus infection. Furthermore, infection of Mφ leads to the production of proinflammatory cytokines and alpha/beta interferon (IFN-α/β), which will further act to limit virus spread (31, 32).

We observed a marked difference among three strains of influenza A virus in their ability to infect murine Mφ. Interestingly, the relative infectivity of the viruses for Mφ paralleled their sensitivity to the collectins serum mannose binding lectin (MBL) and lung surfactant protein D (36). These are soluble collagenous Ca2+-dependent (C-type) lectins involved in innate host defense (15), which bind to oligosaccharide moieties on influenza virus glycoproteins and mediate viral aggregation, opsonization, and neutralization of virus infectivity (2, 14). The collectin sensitivity of influenza viruses is related to the level of glycosylation of the viral glycoproteins (36).

The mannose receptor (MR) is an integral membrane protein that is expressed on tissue Mφ and immature dendritic cells and mediates the uptake of glycoproteins terminating in mannose, fucose, or N-acetylgalactosamine (34, 39, 41). Since the saccharide specificity of the MR overlaps that of the collectins (15), influenza virus glycoproteins represent potential ligands for this receptor. High-affinity ligand recognition by the MR is effected through clustering of its multiple C-type lectin domains (44). Following endocytosis of the receptor-ligand complex in clathrin-coated pits, bound ligand is released in the acidic environment of the endosome and the MR recycles back to the cell surface to mediate subsequent rounds of internalization (40).

The MR has various functions. It is involved in clearance from the circulation of endogenous proteins bearing high-mannose chains, including lyosomal hydrolases (40) and tissue plasminogen activator (29). It contributes to the acquired immune response by mediating the uptake of mannosylated antigens by dendritic cells for processing and presentation to T lymphocytes (11, 35, 39), and a soluble form of the MR present in serum may be involved in antigen transport and presentation of glycoconjugates to specialized antigen-presenting cells (21). The MR also plays a key role in innate immunity by binding to surface glycans on a wide range of bacterial, fungal, and par...
asitic pathogens and mediating their uptake by phagocytosis (27, 42). The role of the MR in viral infection is, however, largely unexplored. In this study we investigate the possible involvement of the MR in infectious entry of influenza virus into Mø.

MATERIALS AND METHODS

Viruses and viral glycoproteins. The influenza A viruses used in this study were the Mt. Sinai strain of A/PR/8/34 (PR8) and viruses BJx109 (H1N1) (32) and PR8 (H3N2), which are high-titer reassortants of PR8 with A/Beijing/353/99 (H3N2) and A/Aichi/68 (H3N2), respectively. In HKS31, all genes except those encoding the hemagglutinin (HA) and neuraminidase (NA) are derived from the PR8 parent (4). BJx109 has not been fully genotyped but is known to carry the HA and NA genes of A/Beijing/353/99 (H3N2) and the M, PA, and PB2 genes of PR8 (Alan Hampson, World Health Organization Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia, personal communication). Viruses were grown in eggs and purified from allantoic fluid as described previously (1). Infectivity titers of allantoic fluids were determined by plating on Madin-Darby canine kidney (MDCK) cells in the presence of trypsin (2). Viral HA and NA glycoproteins (hereafter denoted HANA) were prepared by treatment of purified virus with n-octyl-b-D-glucoside, followed by centrifugation to remove the viral cores and dialysis to remove the detergent, as described previously (36).

Periodate treatment of HANA. BJx109 HANA (350 μg/ml in 50 μl of Tris-buffered saline) was incubated with an equal volume of 0.022 M NaIO4 at room temperature for 30 min followed by 1.5 volumes of glycero (0.44%), 10 min to inactivate the NaIO4. For mock-treated samples, the periodate and glycero were mixed before the addition of HANA.

Mø. Resident peritoneal and alveolar Mø from BALB/c mice were cultured in Dulbecco minimal essential medium DMEM/F-12 (Gibco BRL, Grand Island, N.Y.) which had been supplemented with additional folic acid (6 μg/ml), l-asparagine (56 μg/ml), l-arginine (116 μg/ml), NaHCO3, (2 mg/ml), gentamicin (30 μg/ml), 10 mM HEPES, 0.05 mM 2-mercaptoethanol, and 10% fetal calf serum (FCS) and is referred to here as DF-10 medium. Peritoneal cells were obtained by peritoneal cavity with 5 ml of cold RPMI 1640 medium (Gibco) supplemented with 30 μg of gentamcin per ml and 10 U of heparin per ml (RPMG). To obtain alveolar cells, lungs were lavaged in situ five times with 1 ml of RPMG by means of a blunt 23-gauge needle inserted into the trachea. Peritoneal and alveolar cells were treated with Tris-NHCl (0.14 M NH4Cl in 17 mM Tris [pH 7.2]) to lyse erythrocytes, washed twice, and resuspended in DF-10, and the large Mø-like cells were counted.

The mouse Mø lines J774 and J774E were cultured in α-minimal essential medium (Gibco) supplemented with 2 mM glutamine, 2 mM pyruvate, 30 μg of gentamicin per ml, 60 mM thiguanine, and 10% FCS (α-MEM-10). These cell lines were provided by Philip Stahl, Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Mo.

For infection studies, Møs were seeded in 250 ml into the wells of eight-well glass chamber slides (Lab-Tek; Nunc, Naperville, Ill.) and incubated overnight, and the supernatant was removed. A redissolved suspension of Mø or J774E in suspension was used in these preparations to exceed 90%. Infection of Mø by influenza virus. Mø monolayers in eight-well chamber slides were washed with serum-free medium and incubated for 1 h at 37°C with influenza virus (10 PFU unless otherwise stated) in 300 μl. Unadsorbed virus was then removed, and incubation of the cells in serum-free medium was continued for a further 7 to 9 h. The cell monolayers were then washed in phosphate-buffered saline (PBS), fixed in acetone, and stained with a 1:1,000 dilution of a monoclonal antibody (MAb A-3) specific for the nucleoprotein of type A influenza virus to visualize the nuclei for 2 h with 1% Triton-X-100. Immunofluorescence was observed using a confocal laser scanning microscope. Binding of 125I-HANA to murine Mø. Binding assays were conducted in Tris-buffered saline (0.05 M Tris-HCl, 0.15 M NaCl [pH 7.2]) supplemented with 20% fetal calf serum (fcs) (binding buffer).

Assay of virus adsorption. To assay the adsorption of BJx109 and PR8 viruses to Mø, peritoneal Mø in chamber slides were incubated with 3 × 106 PFU virus in 0.1 ml of serum-free medium for 1 h and washed, and the absorbed virus was chloroform treated at the monolayer for 2 h followed by fixation in 1% glutaraldehyde (Sigma no. N-7885; 20 mU in 0.1 ml of serum-free medium). All steps were carried out at 4°C to inhibit viral entry. The eluates were removed, 2.5 mM 2,3-decachloro-2-decyloxy-V-acetylneuraminic acid (DDN, Boehringer, Mannheim, Germany) was added to inhibit the residual viral HA activity, and the titer of infectious virus was determined by plaquing on MDCK cells as described previously (2). Preliminary experiments had shown that the presence of bacterial NA in virus samples had an adverse effect on plaquing efficiency if it were expressed through the inhibition of interaction of HA with cell membrane NA activity, and the titer of infectious virus was determined by plaquing on MDCK cells as described previously (2). Preliminary experiments had shown that the presence of bacterial NA in virus samples had an adverse effect on plaquing efficiency if it were expressed through the interaction of HA with cell membrane NA activity.

Radiolodination. Mannosylated bovine serum albumin (mBSA; 31 μg of mannose per mol of BSA) was purchased from E. Y. Laboratories Inc. (San Mateo, Calif.). Concanavalin A (ConA) was obtained from Boehringer Mannheim Corp., Indianapolis, Ind. ConA, mBSA and purified influenza HANA glycoproteins were labeled by 125I-1 mol modification (18) of the chloramine-T method described by Greenwood (13).

Mø binding assays. (i) 125I-labeled mBSA. Binding assays were conducted in Tris-buffered saline (0.05 M Tris-HCl, 0.15 M NaCl [pH 7.2]) supplemented with 20% fetal calf serum (fcs) (binding buffer).

Sialidase treatment of Mø. To assay the adsorption of BJx109 and PR8 viruses to Mø, peritoneal Mø in chamber slides were incubated with 3 × 106 PFU virus in 0.1 ml of serum-free DF-10 medium for 1 h at 37°C. Mock-treated cells were incubated similarly in serum-free medium alone. The cells were then washed three times and resuspended in binding buffer for binding studies (see above) or in serum-free medium for infection studies. For infection, 106 sialidase- or mock-treated Mø were incubated for 30 min at 4°C with 6 × 106 PFU of 125I-HANA (1 × 106 cpm/μg) was added to suspensions of 5 × 105 cells and no correction was made for nonspecific binding.

Sialidase treatment of Mø. To assay the adsorption of BJx109 and PR8 viruses to Mø, peritoneal Mø in chamber slides were incubated with 3 × 106 PFU virus in 0.1 ml of serum-free DF-10 medium for 1 h at 37°C. Mock-treated cells were incubated similarly in serum-free medium alone. The cells were then washed three times and resuspended in binding buffer for binding studies (see above) or in serum-free medium for infection studies. For infection, 106 sialidase- or mock-treated Mø were incubated for 30 min at 4°C with 6 × 106 PFU of BJx109 virus in 0.5 ml, after which the cells were pelleted by centrifugation, washed, and incubated in serum-free medium. The cells were then resuspended in binding buffer containing 1% Triton-X-100 and 1% Nonidet P40. The Mø were then incubated with 125I-HANA (1 × 106 cpm/μg) for 30 min at 37°C, washed, and resuspended in binding buffer (see below) for binding experiments or in DF-10 for microscopy. Microscopic examination of cytoscintigraphic samples stained with Diff Quick (Lab Aids, Nanbehen, Victoria, Australia) showed the proportion of Mø in these preparations to exceed 90%.

Infection of Mø by influenza virus. Mø monolayers in eight-well chamber slides were washed with serum-free medium and incubated for 1 h at 37°C with influenza virus (10 PFU unless otherwise stated) in 300 μl. Unadsorbed virus was then removed, and incubation of the cells in serum-free medium was continued for a further 7 to 9 h. The cell monolayers were then washed in phosphate-buffered saline (PBS), fixed in acetone, and stained with a 1:1,000 dilution of a monoclonal antibody (MAb A-3) specific for the nucleoprotein of type A influenza virus to visualize the nuclei for 2 h with 1% Triton-X-100 and for 20 min at 37°C in 200 μl of medium containing the saccharide at 1.5 times its final concentration, and the percentage of fluorescing cells was determined. MAb A-3 was provided by Nancy Cox, Influenza Branch, Centers for Disease Control and Prevention, Atlanta, Ga.

To test the effect of mann on viral infection, cells were preincubated for 20 min at 37°C in 200 μl of medium containing the saccharide at 1.5 times its final concentration, and the percentage of fluorescing cells was determined. MAb A-3 was provided by Nancy Cox, Influenza Branch, Centers for Disease Control and Prevention, Atlanta, Ga.

RESULTS

Infection of murine Mø by different strains of influenza virus. We observed a marked difference among three strains of influenza A virus, BJx109, HKx31 and PR8, in their ability to infect murine Mø, as assessed by immunofluorescence microscopy at 8 to 10 h postinfection. This difference in infectivity was observed with resident peritoneal and alveolar Mø from BALB/c mice and with the murine Mø cell line J774 (Table 1), as well as with peritoneal Mø from C57BL/10 and CBA mice (data not shown). BJx109 infected each of the Mø populations.
most efficiently, HKx31 gave intermediate levels of infection, and PR8 infected only a small percentage of cells. For PR8 virus, immunofluorescent staining at 24 and 48 h postinfection revealed no further increase in infection and minimal cytopathic effect was observed. In contrast, Mφ infected with BJx109 and HKx31 viruses showed extensive cytopathic effect by 24 h postinfection. Assay of Mφ culture supernatants for infectious virus by plaquing on MDCK cells in the presence of trypsin revealed no increase in virus titer at 24 h postinfection compared to 2 h, with the latter titer representing virus inoculum that had spontaneously eluted from the cells (data not shown). These observations are consistent with the reports of others that influenza virus infection of Mφ is abortive (38, 46).

The reassortant HKx31 (H3N2) virus is known to derive all of its genes for internal components from A/PR/8/34 (H1N1) virus (4); hence, the difference in ability of these two viruses to infect murine Mφ most probably reflects a difference in their surface glycoproteins. The low infectivity of PR8 for Mφ was not typical of other H1N1 subtype viruses, however, since the quantity of infectious virus that could be eluted from Mφ monolayers with V. cholerae NA following adsorption of virus for 1 h at 4°C was shown to be very similar for PR8 and BJx109 viruses (1.8 × 10^4 to 7.5 × 10^4 and 3.1 × 10^4 to 17.1 × 10^4 PFU, respectively, in three experiments).

A particular feature of the HA molecule of PR8 (Mt. Sinai) is the absence of carbohydrate from the globular head of the molecule and its overall lack of high-mannose-type glycans (9, 25). In contrast, BJx109 and HKx31 viruses carry 4 and 2 potential glycosylation sites on the head of HA, respectively (37, 45). In a previous study we have shown that differences in glycosylation of the HA molecules of influenza viruses markedly affect their interaction with collectins, the collagenous mannann-binding C-type lectins that are present in serum and pulmonary fluids (36). We observed here that the hierarchy in the ability of the three viruses to infect Mφ (BJx109 > HKx31 > PR8) paralleled their sensitivity to collectins. Since the Mφ MR recognizes the same spectrum of monosaccharides as the collectins do (15) and functions in both endocytosis and phagocytosis (41), we investigated a possible role for the MR in infection of Mφ by influenza virus.

**Interaction of influenza virus glycoproteins with the Mφ MR.** To determine whether influenza viruses interact with the MR, we examined the ability of purified HANA viral glycoproteins to inhibit the binding of a known ligand of this receptor, 125I-labeled mBSA, to peritoneal Mφ. We established in a separate experiment that 125I-mBSA and HANA do not themselves interact, by demonstrating the failure of 125I-mBSA to bind to HANA-coated microtiter wells under conditions where the binding of specific antibody to such wells and the binding of 125I-mBSA to wells coated with the collectin MBL were readily demonstrated (data not shown). Any inhibition of binding of 125I-mBSA to Mφ by HANA should therefore indicate direct interaction of HANA with the MR.

Specific binding of 125I-mBSA to macrophages was inhibited by EDTA and by D-mannose, as expected (Fig. 1). Binding was also strongly inhibited by the HANA glycoproteins of BJx109 virus, and this inhibitory capacity was lost if the HANA was treated with periodate to oxidize viral carbohydrate.

When HANA preparations of the three viruses were compared, marked differences in their apparent avidity for the MR were evident: inhibition of 125I-mBSA binding was strongest with BJx109, intermediate with HKx31, and weakest with PR8 HANA (Fig. 2A). The inhibitory activities of the three HANA preparations correlated directly with their content of high-mannose or hybrid-type glycans, as indicated by binding of 125I-labeled ConA to purified viruses of the three strains (Fig. 2B) (3, 28), and paralleled the efficiency with which the respective viruses infect Mφ (Table 1). Taken together with the effect of periodate treatment mentioned above, these data imply a direct interaction of influenza virus HANA glycoproteins, through their carbohydrate, with the lectin domain(s) of the MR and are consistent with an involvement of the MR in infection of Mφ by influenza virus.

**Effect of mannan on infection of Mφ by influenza virus.** Since binding of mannosylated ligands to the MR is inhibited by yeast mannans, it was of interest to investigate the effect of mannan on infection of macrophages by influenza virus. Mφ monolayers in chamber slides were incubated for 1 h with 10^6 PFU of influenza virus in the presence or absence of mannan (5 mg/ml). Following removal of unbound virus, the cells were washed and incubated for a further 8 h, again in the presence or absence of mannan, and infection was assessed by immunofluorescence.

The presence of mannan throughout the experiment led to marked inhibition of infection of Mφ by each of the three viruses (Fig. 3A). Under the same conditions, mannan had no effect on the ability of these viruses to infect MDCK cells, which lack an MR (data not shown). When mannan was included only for the first hour of the experiment (i.e., during the

### Table 1. Differences in the ability of influenza virus strains to infect murine Mφ

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<thead>
<tr>
<th>Virus</th>
<th>% of infected cells</th>
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<tbody>
<tr>
<td></td>
<td>Peritoneal Mφ</td>
</tr>
<tr>
<td>BJx109</td>
<td>59 ± 11</td>
</tr>
<tr>
<td>HKx31</td>
<td>35 ± 7</td>
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<tr>
<td>PR8</td>
<td>3 ± 1</td>
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* Mφ monolayers in chamber slides were infected for 1 h with 10^6 PFU of influenza virus. At 8 to 10 h postinfection, cells were stained by immunofluorescence for expression of influenza virus NP.

* Data represent the mean percent infection ± 1 standard error of the mean from three experiments. MDCK cells infected under the same conditions showed >95% infection by all three viruses.
virus adsorption and early-entry phase), it was less effective at inhibiting Mφ infection (Fig. 3B), suggesting that virus adsorption to sialylated receptors was not blocked by this treatment and that the process of infection could resume once mannan was removed. Consistent with this finding, mannan had no effect on the binding of 125I-labeled HANA glycoproteins of BJx109 to peritoneal Mφ (data not shown). Mannan added after 1 h had little inhibitory effect on infection of Mφ by BJx109, indicating that it does not block postentry stages of influenza virus replication or gene expression in Mφ. Together, these results point to the effect of mannan on infection being mediated at the stage of virus entry, possibly through an effect on the MR.

Effect of different levels of MR expression on infection of Mφ by influenza virus. To further assess the role of the MR in influenza virus infection, we compared the sensitivities of two murine Mφ lines, J774 and J774E, to infection by influenza virus. J774E is a variant line of J774 that was selected on the basis of its increased expression of the MR (10). Binding studies with 125I-mBSA confirmed the differing MR expression of the two cell lines (Fig. 4A), with binding to each cell line being saturable and being three- to fourfold higher for J774E than for J774. Scatchard analysis of the data yielded similar dissociation constants for binding of ligand to the two cell lines (Kd = 10 and 11.5 nM for J774 and J774E, respectively), indicating that the different levels of binding reflect a difference in receptor number rather than receptor affinity.

In infection studies, each of the three strains of influenza virus showed higher infectivity for J774E than for J774 cells (Fig. 4B). Although we cannot rule out the possibility that J774 and J774E differ in ways other than MR expression, the higher infectivity of influenza viruses for J774E is consistent with involvement of MR in the infectious process.

The effect of downregulation of MR expression on J774E cells was also examined. Culture of J774E cells in 25 mM α-mannose for 2 h resulted in a marked (five- to sixfold) reduction in MR expression as assessed by binding of 125I-mBSA to the cells after they had been washed free of mannose (Fig. 5A). The sensitivity of J774E cells to infection by BJx109 virus was likewise markedly reduced (Fig. 5B); in this experiment, 25 mM α-mannose was present throughout the infection and subsequent culture period. Under the same experimental conditions, α-mannose had no effect on infection of MDCK cells by BJx109 virus (data not shown), arguing against a nonspecific effect of this sugar on cell susceptibility to influenza virus or on influenza virus replication per se. Taken together, the results of this and the previous experiment indicate a close association between levels of MR expression and sensitivity to influenza virus infection of the J774 and J774E Mφ cell lines.

Sialic acid requirements for interaction of influenza virus with the MR and for viral infection. As described above, the blocking of 125I-mBSA binding to Mφ by HANA glycoproteins from the three strains of virus suggested a direct interaction of HANA with the MR mediated through the viral carbohydrate. Since the primary receptor for influenza virus is sialic acid and since the MR itself is a sialylated glycoprotein (19), it was also of interest to examine the sialic acid dependence or otherwise of the interaction of HANA glycoproteins with the MR. Peritoneal Mφ were treated with V. cholerae NA or mock treated and then tested for binding of 125I-mBSA in the presence or absence of BJx109 HANA. They were also tested for their...
ability to be infected by BJx109 virus. The effectiveness of the sialidase treatment was monitored by comparing the binding of 125I-labeled BJx109 HANA to treated and mock-treated Mφ; as shown in Fig. 6A, binding of 125I-labeled HANA was reduced by >90% following sialidase treatment.

Sialidase-treated Mφ retained the capacity for specific binding of 125I-mBSA (Fig. 6B), an observation consistent with the finding of Pontow et al. (33) that inhibition of sialylation of the glycans on newly synthesized MR did not affect the lectin activity of the receptor. Furthermore, BJx109 HANA blocked the binding of 125I-mBSA to sialidase-treated Mφ and control Mφ to a similar extent (Fig. 6B), indicating that interaction of HANA with the MR does not require sialic acid on the latter and can occur, as with other MR ligands, through direct binding of viral carbohydrate to the lectin domains of the MR. Infection of Mφ by BJx109 virus, however, was highly sialic acid dependent, in that 74% of mock-treated Mφ and only 10% of sialidase-treated Mφ became infected following incubation with BJx109 virus for 30 min at a multiplicity of infection of 3. The carbohydrate-mediated interaction of influenza virus glycoproteins with lectin domains of the MR is thus, on its own, not sufficient to mediate infection of Mφ by influenza virus in the absence of sialic acid.
binding assays with 125I-BJx109 HANA (A) and 125I-mBSA (B). Specific binding in medium alone (mock treated) for 60 min at 37°C, washed, and used in competitive binding experiments with 125I-mBSA, and the avidity of HANA for the MR correlated with the efficiency of infection by influenza virus also correlated with the level of expression of MΦ by influenza A virus. The study was prompted by our observation that the efficiency of infection of murine MΦ by three strains of influenza virus, BJx109, HKx31, and PR8, differed markedly and paralleled the sensitivity of the viruses to C-type lectins of the collectin family (36), whose carbohydrate specificity is similar to that of the MR. Evidence for a direct interaction of viral HANA glycoproteins with MR on the MΦ surface was obtained from competitive binding experiments with 125I-mBSA, and the avidity of HANA for the MR correlated with the efficiency of infection of MΦ by the three viruses in question. The efficiency of infection by influenza virus also correlated with the level of expression of MR on the MΦ. Furthermore, infection of MΦ was inhibited by yeast mannan, a known ligand of the MR, at a stage subsequent to virus adsorption. Given the known endocytic activity of the MR and the fact that uptake of influenza virus into an endosome following adsorption is an obligatory step in the infectious process, the present results suggest that uptake via the MR represents a major endocytic route for influenza virus into MΦ.

Since the MR is both sialylated and a lectin, interaction of influenza virus with this receptor might occur in two ways: by binding of the viral HA through its receptor binding site to sialoglycoproteins to the lectin domains of the MR. The competition experiments with 125I-mBSA indicated binding by the latter mechanism. Thus, (i) treatment of HANA glycoproteins with periodate destroyed their ability to inhibit the binding of 125I-mBSA to MΦ; (ii) the avidity of HANA preparations from the three viruses for the MR correlated directly with their high mannosyl and/or hybrid glycan content, as indicated by the ability of the viruses to bind ConA (3, 28); and (iii) HANA could block binding of 125I-mBSA to MΦ that had been extensively desialylated. We conclude that binding of the viral glycoproteins to the MR occurs predominantly through the viral carbohydrate and does not require interaction through sialic acid, although HA binding to sialic acid on the MR under normal circumstances is not excluded.

As observed by others (26, 43) and confirmed in this study, infection of MΦ by influenza virus is sialic acid dependent, as it is for other cell types. Interaction of the virus through its carbohydrate with the MR is thus clearly not sufficient to mediate infectious entry of the virus, even though, by analogy to other MR ligands, uptake of the virus into endosomes under these circumstances might be expected. Receptor binding by the HA, however, is now recognized to be required not only for binding and subsequent endocytosis of the virus by the host cell but also for efficient fusion of host and viral membranes in the endosome to bring about the entry of the viral nucleocapsid into the cytoplasm (23, 30). The latter requirement is thought to reflect the need for close apposition of viral and endosomal membranes and correct orientation of the HA at the time of the acid-induced conformational change in HA and exposure of the fusion peptide. Under normal circumstances, this apposition is mediated by binding of the HA to sialic acid on the endosomal membrane. Since the MR dissociates from its ligand at the pH of the endosome, it would be unable to substitute for sialic acid in providing this link in desialylated cells: virus particles that during endocytosis were bound only to the lectin domains of the MR would be released from the host membrane in the endosome and membrane fusion would not occur. The situation can be contrasted with the ability of influenza virus to infect desialylated MΦ in the presence of subneutralizing levels of antiviral antibody (26, 43). In that case, antibody bound to Fc receptors on the MΦ can act as a surrogate receptor for the virus, the antigen-antibody association being stable in the acidic environment of the endosome.

The dual dependence on sialic acid and the MR for infection of MΦ by influenza virus suggests the following model. Following, or coincident with, primary binding of virus to sialylated glycoprotein or glycolipid receptors on the cell surface, virus particles bind through their oligosaccharide moieties to the lectin domains of the MR. The avidity of the latter interaction, and hence the efficiency of endocytosis, will be determined by the nature and density of glycosylation of the HA and NA glycoproteins of the virus in question (16). Since the MR is itself sialylated, the sialic acid-binding requirement for infection may be met by the MR also, although whether sialic acid on the MR is accessible, of an appropriate type, and present in the correct linkage or conformation to be recognized by influenza virus is not known at present. Alternatively, neighboring sialylated receptors that are bound by the virus may be taken into the endosome along with the virus and the MR.

The finding that the HANA glycoproteins of PR8 (Mt. Sinai) virus interact poorly with the MR is consistent with the known paucity of high-mannose or hybrid-type glycans on its surface glycoproteins (Fig. 2B) (25) and the overall lack of glycosylation sites on the head of its HA molecule (9), and in the proposed model finding accounts for the low level of infection of MΦ by PR8 that we have observed here. Interestingly, we have found the Cambridge strain of PR8 virus to infect MΦ with three- to fivefold higher efficiency than the Mt. Sinai strain does. PR8 (Cambridge) carries a potential glycosylation site on the head of HA (at residue 131 in the H3 numbering) which is lacking in PR8 (Mt. Sinai) (9, 50). A difference in electrophoretic mobility of the HA molecules of the two viruses indicated that this site is glycosylated in the Cambridge strain, and PR8 (Cambridge) was shown to be more sensitive than PR8 (Mt. Sinai) to hemagglutination inhibition by the collectin MBL in mouse serum (G. Selvaraj, J. L. Miller, and E. M. Anders, unpublished data). These findings further implicate glycosylation as a factor in the infectivity of influenza virus for MΦ.

A difference in the substrain of PR8 virus used may in part explain the fact that other researchers studying the interaction of influenza virus with MΦ have not found the infectivity of
PR8 virus to be particularly low (31). Another important difference may lie in the Mφ populations used. Alternative or differential may lie in the MRPR8 virus to be particularly low (31). Another important difference between influenza virus strains in their interaction with dendritic cells that might relate to their interaction with the MR are under way in this laboratory.

ACKNOWLEDGMENTS

This work was supported by grant 970283 from the National Health and Medical Research Council of Australia. We thank Sharon Feigl for technical assistance.

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