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SELECTION OF INFLUENZA VIRUS VARIANTS BASED ON SIALYLOLIGOSACCHARIDE RECEPTOR SPECIFICITY

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Abstract - Influenza viruses bind to host cells by attachment to sialic acid containing oligosaccharides on cell surface glycoproteins and glycolipids. We have examined the receptor specificity of influenza viruses using an approach employing highly purified mammalian sialyltransferases. In conjunction with bacterial sialidases these specific enzymes are used to modify erythrocytes or tissue culture cells to contain a single sialyloligosaccharide receptor determinant of defined sequence. By analysis of viral binding to or infection of the derivatized cells, the receptor specificity of the virus can be deduced. Influenza viruses may exhibit strict and varied receptor binding properties. Although the biological significance of receptor specificity in influenza viruses is not fully understood, host species of influenza may exert selective pressures resulting in the emergence of a receptor variant with properties optimal for growth in that host. The nature of such selective pressures and the selection of receptor binding variants *in vitro* is discussed.

INTRODUCTION

Influenza viruses infect a variety of species including man, pigs, horses, seals and birds (Ref. 1). The virus has a membrane envelope with two types of surface glycoproteins, the hemagglutinin and the neuraminidase, both of which interact with sialyloligosaccharides on host cells. Over thirty years ago it was found that pretreatment of erythrocytes or host cells with bacterial sialidase abolished viral adsorption and/or infection, demonstrating that sialic acid is an essential feature of the receptor determinant (Ref. 2,3). It is the hemagglutinin which mediates attachment to the cell, named for its activity in causing viral agglutination of erythrocytes. The neuraminidase is capable of hydrolyzing sialic acid from receptors, and at high levels in an infected cell aids in elution of the budding virus from the host membrane. Early observations suggested that influenza virus hemagglutinins could differ in their binding properties. Notably, graded sialidase treatment of erythrocytes abolished agglutination of certain viruses before that of others (Ref. 4) and soluble sialoglycoproteins acting as receptor analogs caused inhibition of hemagglutination only for some viruses (Ref. 5,6).

In recent years we have sought to examine the detailed binding specificity of influenza virus hemagglutinins to understand the basis of differential receptor binding properties observed previously, and to determine how such specificity may influence the biology of the virus. Because sialyloligosaccharides of cell surface glycoproteins and glycolipids exhibit considerable diversity, the interaction of the virus with cell surface molecules may be very complex. To reduce this complexity, enzymatic modification of cell surface oligosaccharides is used to prepare cells with a single sialyloligosaccharide receptor determinant. Analysis of the ability of influenza viruses to bind to these cells has revealed that influenza virus hemagglutinins recognize aspects of oligosaccharide structure beyond the terminal sialic acid. Indeed, two influenza isolates may exhibit very different receptor specificities. As discussed below, receptor specific viruses may be differentially subject to selective pressures at the level of virus adsorption to the host cell surface.

ENZYMATIC MODIFICATION OF CELL SURFACE SIALYLOLIGOSACCHARIDES

As depicted in Fig. 1, cells may in principle be modified to carry sialic acid in a single sequence by a two-step enzymatic procedure. This has been successfully applied to analysis of animal virus receptors on erythrocytes and tissue culture cells (Ref. 7,8). First, virus receptors are destroyed by removal of terminal sialic acid (SA) residues from

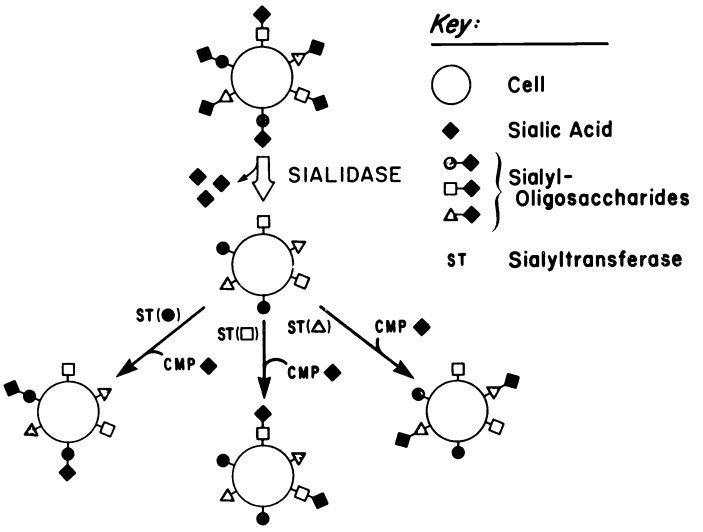

Fig. 1. Enzymatic modification of cell surfaces to contain sialyloligosaccharides of defined sequence.

cell surface oligosaccharides by treatment with Vibrio cholerae or Clostridium perfringens sialidase. The resulting asialo-cells are functionally unable to bind virus and/or are resistant to infection. Next the cells are washed free of sialidase and incubated with CMP-SA and a purified mammalian sialyltransferase (see Note a). These enzymes carry out the general reaction:

CMP-SA + HO-Acceptor → CMP + SA-O-Acceptor

and exhibit remarkable specificity for both donor and acceptor substrates. Thus, when reacted with sialidase-treated cells only oligosaccharides recognized as acceptor substrates are sialylated and the sequence elaborated on the cell is defined by the specificity of the sialyltransferase used (Ref. 9). Cells containing different sequences may be obtained by reaction of asialo cells with other sialyltransferases, each of which recognizes a different oligosaccharide acceptor substrate. Each cell preparation may then be tested for restoration of viral adsorption or infection.

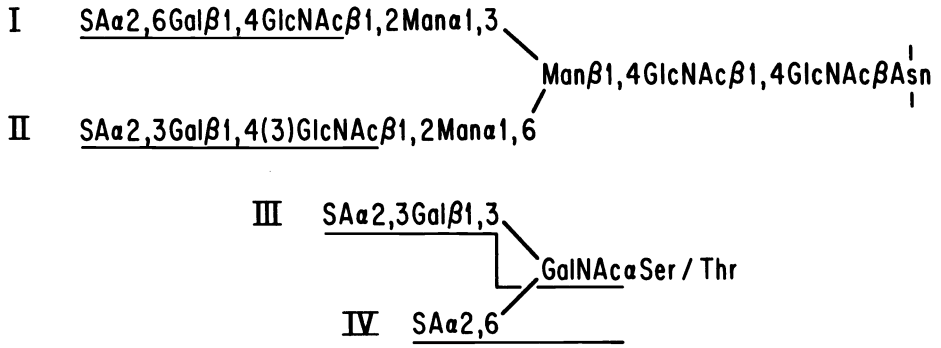

Fig. 2. Common sialylated sequences in glycoprotein oligosaccharides N-linked to asparagine and O-linked to threonine or serine. The underlined sequences indicate the products of four purified sialyltransferases mentioned in this report: I, the Galβ1,4GlcNAc α2,6 sialyltransferase (E.C. 2.4.99.1); II, The Galβ1,3(4)GlcNAc α2,3 sialyltransferase (E.C. 2.4.99.7); III, the Galβ1,3GalNAc α2,3 sialyltransferase (E.C. 2.4.99.4); and IV, the N-acetylgalactosaminide α2,6 sialyltransferase (E.C. 2.4.99.3).

Note a. Sialic acid is abbreviated SA. Unless otherwise indicated sialic acid refers to N-acetylneuraminic acid.

As many as twelve sialyltransferases may be required to elaborate all the oligosaccharide structures reported on glycoproteins and glycolipids to date. Of these, four sialyltransferases have been purified to homogeneity and their use as enzymatic tools in analysis of oligosaccharide structure and function has been described (Ref. 9,10). These four enzymes together elaborate the most common sialylated sequences found on glycoprotein oligosaccharides N-linked to asparagine and O-linked to threonine or serine as shown in Fig. 2. All four enzymes have been used in analysis of influenza virus receptor specificity.

VARIATION IN RECEPTOR SPECIFICITY OF INFLUENZA VIRUS HEMAGGLUTININS

Binding to resialylated erythrocytes. To date nearly thirty influenza virus isolates have been examined for their agglutination of human erythrocyte preparations derivatized to carry the four sialyloligosaccharide sequences shown in Fig. 2 (Ref. 7,11,12). Over 90% of the sialic acid incorporated by the sialyltransferases is attached to glycoprotein oligosaccharides, most being transferred to glycophorin which contains one N-linked oligosaccharide and 15 O-linked oligosaccharides similar to those shown in Fig. 2 (Ref. 7). In the following discussion the sialyloligosaccharide sequences will be abbreviated by a disaccharide sequence and a Roman numeral which refers to the complete structure in Fig. 2. The largest differences in receptor binding properties are seen by comparison of the binding of viruses to erythrocytes containing the SAα2,6Gal(I) and SAα2,3Gal(III) sequences. Examples are shown in Fig. 3.

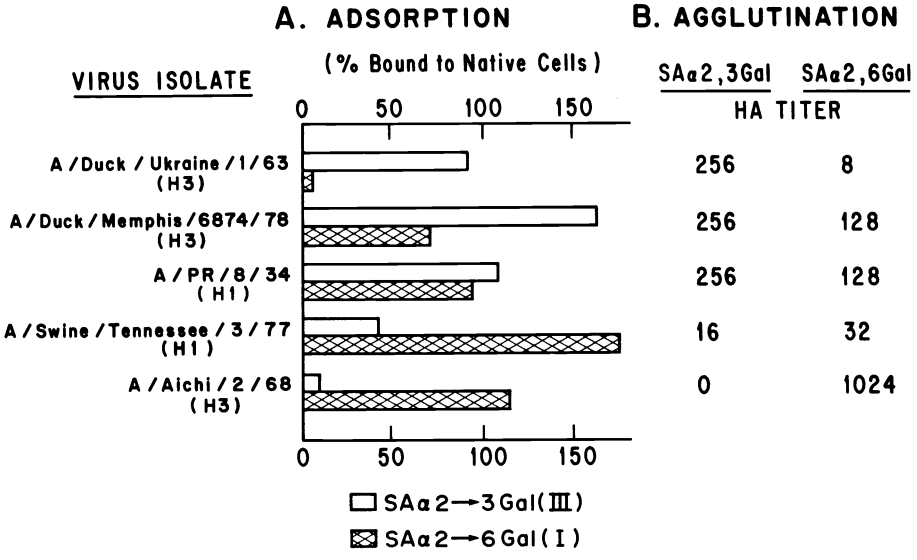

Fig. 3. Differential binding of influenza virus to erythrocytes containing the SAα2,3Gal(III) or SAα2,6Gal(I) linkages. Two methods of analyzing viral attachment are shown for each of several influenza virus isolates. In panel A direct adsorption to the cells is assessed by mixing the virus with a 5% suspension of red cells, removing unbound virus by repeated centrifugation and suspension in fresh buffer, and then monitoring the virus bound by quantitation of the viral neuraminidase. Results are expressed as a % of virus bound to native (unmodified) erythrocytes (Ref. 12). In panel B, serial two fold dilutions of concentrated virus are made in a microtiter plate, and each dilution is tested for its ability to agglutinate a suspension of the derivatized erythrocytes. The hemagglutination (HA) titer is expressed as the highest dilution which causes hemagglutination.

Two types of assays are shown, direct adsorption (panel A) in which virus is bound to erythrocytes and the washed cells assessed for associated viral neuraminidase, and hemagglutination, in which the ability of the virus to cause agglutination of the cells is examined. Good correspondence between the two binding assays is observed. In general, three main binding types are found. Some viruses preferentially bind cells containing the SAα2,6Gal(I) sequence, some preferentially bind cells containing the SAα2,3Gal(III) sequence, and some bind cells containing either sequence. Viruses that bind to cells containing the SAα2,3Gal(III) sequence also bind the SAα2,3Gal(II) sequence. However, the converse is not true. Several viruses have been found to bind cells containing the

SAα2,3Gal(II) sequence and the SAα2,6Gal(I) sequence, but in contrast do not bind to cells containing the SAα2,3Gal(III) sequence. Such subtleties indicate that it is difficult to strictly classify influenza virus receptor specificity into a few receptor binding types. Virtually all influenza viruses tested bind weakly if at all to cells containing the SAα2,6GalNAc(IV) linkage (Ref. 12).

As shown by the work of Schauer and colleagues, the sialic acids are a family of at least 23 natural compounds which can differ by their N- and O-acyl substituents (Ref. 13). The type of sialic acids found in glycoconjugates varies greatly from species to species. In most of the experiments described here, the sialic acid used is N-acetylneuraminic acid (Neu5Ac), the predominant sialic acid in humans. Recently we have prepared the sialyltransferase donor substrates CMP-N-glycollylneuraminic acid (CMP-Neu5Gc) and CMP-9-O-acetyl-N-acetylneuraminic acid (CMP-9-O-Ac-Neu5Ac) in addition to CMP-N-acetylneuraminic acid (CMP-Neu5Ac). With these analogs it has been possible to prepare erythrocytes containing the SAα2,6Gal(I) and SAα2,3Gal(III) linkages with each of the corresponding sialic acids. From initial experiments examining the adsorption of influenza viruses to these cells, several conclusions can be drawn. Some viruses bind equally well to cells containing Neu5Ac or Neu5Gc while others bind only the former. None of the viruses which bind the SAα2,6Gal(I) sequence with Neu5Ac will bind the same sequence with 9-OAc-Neu5Ac. Thus, the 9-O-acetyl substitution appears to interfere with binding. These results indicate that influenza virus hemagglutinins can distinguish between naturally occurring sialic acid analogs in addition to the sequence to which the sialic acid is attached.

Interaction of influenza viruses with neoglycoproteins. Analysis of the binding of influenza viruses to receptor modified erythrocytes has proven a sensitive and reliable method of distinguishing differences in receptor binding properties. Yet the assay is still quite complex. With the ultimate goal of a simplified binding assay which enables quantitative analysis of relative binding affinities for small molecular weight oligosaccharides, we have begun to examine the interaction of influenza viruses with neoglycoproteins. Starting with the procedure of Lee *et al.* (Ref. 14) for coupling the methyl imidate of N-acetylglucosamine to bovine serum albumin (BSA), galactose and sialic acid (Neu5Ac) have been added enzymatically (see Ref. 9) to yield the sequences SAα2,6Galβ1,4-GlcNAc and SAα2,3Galβ1,4GlcNAc-BSA at 20-30 mol/mol protein. As shown in Table 1 these

TABLE 1. Hemagglutination inhibition of a SAα2,6Gal specific influenza virus by sialylated neoglycoproteins.

A virus (M1/5) exhibiting specificity for binding the SAα2,6Gal linkage was tested for inhibition of hemagglutination by sialylated neoglycoproteins and free sialyloligosaccharides. A fixed amount of virus was mixed with serial dilutions of each derivative in a microtiter plate, and then challenged with a suspension of native erythrocytes. Results are expressed as the minimum concentration (based on sialic acid content) required to inhibit hemagglutination. Sialylated neoglycoproteins were prepared by coupling to bovine serum albumin (BSA) by the method of Lee *et al.* (14) followed by enzymatic addition of galactose with the N-acetylglucosaminide β1,4 galactosyltransferase and N-acetylneuraminic acid with the Galβ1,4GlcNAc α2,6 sialyltransferase or the Galβ1,3(4)GlcNAc α2,3 sialyltransferase. In this way derivatives containing the SAα2,6Galβ1,4 GlcNAc and SAα2,3Galβ1,4GlcNAc sequences were obtained with 33 mol and 22 mol oligosaccharide per mol bovine serum albumin, respectively. The corresponding free trisaccharides were similarly prepared by sialylation of β-methyl-N-acetyllactosamine.

Sialylated Derivative	Amount Required for Inhibition of Hemagglutination
	mM
Neoglycoproteins	
SAα2,6Galβ1,4GlcNAc-BSA	0.07
SAα2,3Galβ1,4GlcNAc-BSA	>0.7*
Sialyloligosaccharides	
SAα2,6Galβ1,4GlcNAcβCH$_3$	>5*
SAα2,3Galβ1,4GlcNAcβCH$_3$	>5*

* No inhibition at highest concentration tested.

were tested for their ability to inhibit hemagglutination by a laboratory strain of influenza virus, M1/5, which exhibits a receptor specificity with preferential binding of the SAα2,6Gal(I) sequence. Of the two neoglycoproteins tested only the one containing the SAα2,6Gal linkage inhibited hemagglutination, in keeping with the known specificity of the M1/5 virus hemagglutinin. Also tested as inhibitors of hemagglutination were two trisaccharides identical in sequence to those attached to the multivalent neoglycoproteins (Table 1). Neither of the free trisaccharides was an inhibitor of hemagglutination at the highest concentration tested. This result suggests that a multivalent ligand such as the neoglycoprotein is required to produce a receptor analog sufficiently avid to inhibit hemagglutination. The neoglycoprotein containing the SAα2,6Galβ1,4GlcNAc sequence has recently been coupled to cyanogen bromide activated agarose and the resulting conjugate tested for adsorption of the M1/5 virus. Specific binding of the M1/5 virus to neoglycoprotein-agarose relative to bovine serum albumin-agarose used as a control suggests the basis for an in vitro binding assay using neoglycoproteins.

RECEPTOR SPECIFICITY BASED ON SPECIES OF ORIGIN

One of the most interesting epidemiological observations for influenza viruses concerns the origin of the human Hong Kong virus which emerged in the human population in 1968 producing world wide pandemic. The hemagglutinin, also a major antigen of influenza virus, was antigenically distinct from previous influenza viruses, but was virtually identical serologically to hemagglutinins of avian and equine viruses isolated five years earlier in 1963 (Ref. 15,16). It is generally believed that the Hong Kong virus arose from genetic reassortment between the previously circulating human virus or 'Asian flu' and an animal virus which contributed the hemagglutinin gene (Ref. 16,17). Indeed, the prototype Hong Kong influenza hemagglutinin exhibits 96% sequence homology with that of a potential avian virus progenitor, A/duck/Ukraine/1/63 (Ref. 18,19). Influenza isolates with hemagglutinins bearing this strong antigenic and structural similarity have been classified as the H3 serotype regardless of the species of origin.

Despite the remarkable structural similarity, a survey of influenza isolates with the H3 hemagglutinin revealed very different receptor binding properties that correlated with species of origin (Ref. 12,20). These are summarized in Table 2. When compared for binding erythrocytes containing the SAα2,6Gal(I) sequence and the SAα2,3Gal(III) sequence, seven human isolates exhibited strong preferential binding to the former, while four avian and equine isolates preferentially bound the latter. Furthermore, upon screening of glycoprotein inhibitors of hemagglutination, a glycoprotein present in horse serum, α_2 macroglobin, was a potent inhibitor only of the human isolates (Ref. 20). These observations suggest the possibility that each influenza virus host species may exert selective pressures favoring propagation of a virus with characteristic receptor binding properties.

TABLE 2. Contrasting receptor binding properties of H3 hemagglutinins of human, avian and equine influenza virus isolates

Source of Virus	Preferred Receptor Determinant	Hemagglutination Inhibition by α_2 Macroglobulin
Human Isolates (1963-1977)	SAα2,6Gal(I)	+++
Avian and Equine (1963-1978)	SAα2,3Gal(III)	±

SELECTION OF RECEPTOR SPECIFIC VARIANTS OF INFLUENZA VIRUS

It is well documented that two animal species may elaborate quite different sialyloligosaccharides on their glycoproteins. Examples for soluble glycoproteins are mucins (Ref. 13,21) and the serum glycoprotein α_1-acid glycoprotein (Ref. 22,23) and for a membrane glycoprotein, erythrocyte glycophorin (Ref. 24,25). We have been interested in how such variation could lead to differential selection of receptor variants of influenza virus in different host species. Two mechanisms of selection have been tested; selection by inhibition of infection with soluble glycoproteins acting as receptor analogs; and selection by differential adsorption to cell surface receptors.

Glycoprotein inhibitors of infection. The extreme sensitivity of human H3 isolates to inhibition of hemagglutination by horse serum or equine α_2 macroglobulin (Table II) suggested that such inhibitors might provide selective pressure by preventing the inhibitor sensitive phenotype from binding to cells. Precedence for this idea was obtained by

Choppin and Tamm (Ref. 6) who observed selection of an inhibitor insensitive variant from an inhibitor sensitive human H2 isolate when grown in ovo in the presence of horse serum. Subsequently, the inhibitor sensitive (RI/5$^+$) and inhibitor insensitive (RI/5$^-$) viruses were found to exhibit strict specificity for binding the SAα2,6Gal(I) and SAα2,3GalIII) linkages, respectively (Ref. 26). Results in Fig. 4 assess the receptor specificity of a human H3 isolate (A/Memphis/102/72) grown in MDCK cells with no inhibitor, in the presence of horse serum (see also Ref. 20), or in the presence of purified α_2 macroglobulin which accounts for virtually all the hemagglutination inhhbition activity in horse serum.

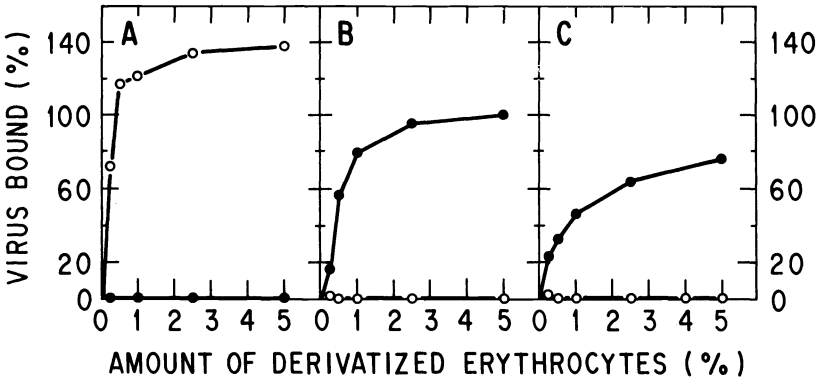

Fig. 4. Shift in receptor specificity of a human H3 isolate by growth in the presence of horse serum or equine α_2 macroglobulin. A/Memphis/102/72 was propagated in MDCK cells in the absence (panel A) or presence of either 10% horse serum (panel B) or 0.3 mg/ml equine α_2 macroglobulin (panel C). Receptor specificity of the progeny virus was assessed by adsorption to increasing amounts of receptor modified erythrocytes containing the SAα2,3Gal(III), ● ; or SAα2,6Gal(I), ○ ; linkages. Results are expressed as the amount of viral neuraminidase bound to cells as a % of the amount which bound to 5% native erythrocytes.

In the absence of inhibitor the progeny virus exhibits the receptor specificity of the parent virus, binding with strict specificity to cells containing the SAα2,6Gal(I) sequence. In contrast, when grown in the presence of horse serum or equine α_2 macroglobulin, the progeny virus exhibits a specificity characteristic of the avian and equine isolates, binding preferentially to cells containing the SAα2,3Gal(III) sequence. In each case, the SAα2,3Gal(III) specific variants are, as expected, insensitive to inhibition of hemagglutination or infection by horse serum (see also Ref. 20,27). These results provide a rationale for selection and maintenance of a SAα2,3Gal(III) specific, inhibitor insensitive phenotype as observed in avian and equine isolates. But what is the molecular basis of the selection being observed? Amino acid sequences of the hemagglutinins of several plaque purified viruses obtained from experiments such as those shown in Fig. 4 have been deduced from the nucleotide sequence of the RNA genes by J.J. Skehel and R.S. Daniels at the NIMR, Mill Hill, London. The sequence of the SAα2,3Gal(III) specific,inhibitor insensitive variants differed from the sequence of the parent gene by a single nucleotide resulting in a change of amino acid 226 from leucine to glutamine (Ref. 27). This amino acid is located in the center of the proposed receptor binding pocket of the three dimentional crystal structure of the hemagglutinin reported by Wilson et al. (Ref. 28). How the difference in a single amino acid can mediate dramatic changes in receptor binding properties is still not clear.

It is also of interest to understand the molecular features of a glycoprotein inhibitor that enables it to combine with the hemaggutinin with sufficient avidity to inhibit infection. Molecular weight, high valency, and the spatial arrangement of the sialyloligosaccharides have been proposed to be important in the interaction of glycoprotein inhibitors with influenza viruses (Ref. 29,30). In view of the forgoing discussion, oligosaccharide structure must also be important. While equine α_2 macroglobulin is a potent inhibitor of infection of human H3 isolates, human α_2 macroglobulin is a poor inhibitor. Yet both glycoproteins have similar molecular weight and sialic acid content. Levinson et al. (Ref. 31) have suggested that 4-O-acetyl-N-acetylneuraminic (4-O-Ac-Neu5Ac) acids present in equine α_2 macroglobulin play an important role in its interaction with a human influenza isolate bearing the H2 hemagglutin. We have obtained similar evidence for human H3 isolates. However, the relative roles of valency, spatial grouping of the oligosaccharides, oligosaccharide sequence and O-acyl sialic acid substituents in the potency of a glycoprotein inhibitor of infection remain to be established.

Differential adsorption to cell surface receptors. Selection of the SAα2,6Gal(I) specific inhibitor sensitive properties observed for human H3 isolates cannot be readily explained by selective suppression of the contrasting SAα2,3Gal(III) specific phenotype with glycoprotein inhibitors of infection. Indeed, no glycoprotein yet tested is an effective inhibitor of hemagglutination of viruses with the inhibitor insensitive H3 hemagglutinin, including glycophorin which presumably is the predominant receptor on erythrocytes. An alternate means of selection for the SAα2,6Gal(I) specific inhibitor sensitive phenotype could be differential adsorption to cell surface receptors.

To test the potential for sialyloligosaccharides on host cells to select receptor specific variants of influenza virus, we have examined influenza virus infections of tissue culture cells enzymatically modified as outlined in Fig. 1 to carry sialyloligosaccharide receptor determinants of defined sequence. Previous studies with Sendai virus (Ref. 8) and polyoma virus (Ref. 32) have shown that tissue culture cells rendered resistant to infection by treatment with sialidase became susceptible to infection when their receptor determinant SAα2,3Gal(III) was restored by sialylation, but remained resistant to infection when the sequence SAα2,6Gal(I) was attached. Similar specificity is observed with influenza viruses when examined for infection of MDCK cells derivatized to contain the SAα2,6Gal(I), SAα2,3Gal(II) or SAα2,3Gal(III) linkages. That is, viruses which preferentially bound to erythrocytes containing either the SAα2,6Gal or SAα2,3Gal linkages exhibited similar selectivity in infection of resialylated MDCK cells containing the same sequences. However, the resialylated MDCK cells are not always infected at the same level as native cells. Nonetheless, mixed infections of resialylated cells with two viruses of contrasting receptor specificity revealed examples of selection based on viral adsorption to cell surface receptors. For example, a SAα2,3Gal specific virus (A/duck/Ukraine/1/63) was preferentially selected from a mixture with a SAα2,6Gal specific virus (A/RI/5^{+}/57) on resialylated cells containing either the SAα2,3Gal(II) or SAα2,3Gal(III) linkages, while no selection was observed when the same viruses were grown on native cells.

These results demonstrate the potential of cell surface receptors to mediate selection of receptor specific viruses. Little is presently known about the sialyloligosaccharide structures present in cell surface glycoproteins and how these structures might differ from tissue to tissue and from species to species. Perhaps the best studies to date are the erythrocyte glycophorins which exhibit considerable species variation (Ref. 24,25). Sialylated glycolipids may also serve as cell surface receptors for influenza viruses (Ref. 33,34). Clearly, further investigation is required to assess the possible role of cell surface receptors in the selection of receptor specific variants of influenza virus *in vivo*.

CONCLUDING REMARKS

It is evident that influenza viruses exhibit considerable diversity in recognition of specific sialyloligosaccharide structures as cell surface receptor determinants. It is also apparent from laboratory models that such specificity can provide the basis for selection of receptor specific variants. Does such selection occur naturally? Indirect evidence comes from the correlation of receptor specificity and species of origin observed for influenza isolates bearing the H3 hemagglutinin (Ref. 12,20; Table II). There are also several instances in which changes in receptor binding properties have been documented to occur upon adaptation of human influenza viruses to growth *in vivo* (chicken embryos). Burnet and Bull (Ref. 35) and Stone (Ref. 36) found that human H1 viruses as isolated preferentially agglutinated human erythrocytes to a higher titer than chicken erythrocytes, but after growth *in ovo* agglutinated erythrocytes of both species equally well. More recently, growth of human influenza B viruses *in ovo* has been found to select for an antigenic variant different from that which predominates in the human host (Ref. 37). The original virus and egg adapted variant was also found to differ in their agglutination of erythrocytes of different species. In each of these cases the cause of selection or maintenance of variants with different receptor binding properties has not been identified. It will be of interest to identify the factors responsible for selection of receptor variants in natural hosts of influenza in order to better understand the role of receptor specificity in the biology of the virus.

Many biological roles have been ascribed to sialyloligosaccharides of glycoproteins and glycolipids (Ref. 38,39). In addition to influenza viruses and other animal viruses, a variety of agents including animal lectins (Ref. 38,40) tumor specific antibodies (Ref. 41,42) bacterial toxins (Ref. 39) mycoplasma (Ref. 39) and interferon (Ref. 43) use sialyloligosaccharides as cell surface receptors. Sialyloligosaccharides have also been suggested to play important roles in mediating a variety of biological processes such as clearance of serum glycoproteins and erythrocytes from blood (Ref. 39), cellular differentiation in slime mold (Ref. 44) migratory activity of macrophages (Ref. 45) and cell-cell adhesion of chicken hepatocytes (Ref. 46,47). Purified glycosyltransferases have been proposed in general to be valuable enzymatic tools in analysis of oligosaccharide

structure and function (Ref. 9). The use of sialyltransferases as applied here to the analysis of influenza virus receptor specificity may similarly yield valuable information concerning other biological roles of the sialic acids. Indeed, to date they have been employed in probing the role of the oligosaccharides of mammalian liver lectin in its binding activity (Ref. 48), the role of sialic acids in the M and N blood groups (Ref. 49), and in the nature of the cellular receptors of Mycoplasma pneumoniae (Ref. 50). The prospect of using combined chemical and enzymatic synthesis of sialyloligosaccharides of biological interest offers additional approaches to the analysis of oligosaccharide structure and function for the future.

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