Receptor-binding specificity of pandemic influenza A (H1N1) 2009 virus determined by carbohydrate microarray

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To the Editor:

Since it first emerged in North America in mid February 2009, the novel influenza A(H1N1) virus has spread to most other regions of the world, causing the WHO to declare an emergent pandemic1. The virus is a reassortant with six of its eight genes, including the haemagglutinin (HA) gene, originating from “North American triple reassortant” swine H1 viruses2. Although these swine viruses have caused sporadic human infection in recent years, onward transmission of infection was limited3. In relation to the increased transmissibility of the novel ‘swine’ virus and its establishment in the human population, the receptor specificity of the HA is a key determinant. The receptors are sialyl glycans which vary in distribution in tissues of different species and determine host range and tissue tropism, as well as the capacity of animal viruses to initiate a human pandemic4.

Soundararajan et al.5, have made a prediction of the receptor-binding specificity of a representative pandemic H1N1 2009 virus, A/California/4/2009 (Cal/09). The authors took account of the differences in amino acid residues in the receptor binding site of Cal/09 from those of previous human H1N1 HAs and constructed homology-based HA-glycan structural complexes with “human-type” oligosaccharide receptors, namely sialyl oligosaccharides terminating with N-acetylneuraminic acid α2-6-linked to galactose (Neu5Ac α2-6Gal), and “avian-type” terminating with Neu5Ac β2-3Gal. From these models Soundararajan et al. predicted that Cal/09 would be able to make optimal contacts with β2-6-sialylated glycans, a feature shared with other human H1N1 HAs and, in addition, make contacts with β2-3 sialylated glycans. However, features governing the receptor specificity of HA are complex and it is not always possible at present to draw definitive conclusions merely from sequence analysis and homology modelling studies.

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Note: Supplementary information is available.
We have compared directly, by carbohydrate microarray analysis, the receptor-binding characteristics of two isolates of the novel pandemic H1N1 virus, Cal/09 and A/Hamburg/5/2009 (Ham/09), with those of a seasonal human H1N1 virus, A/Memphis/14/96-M (Mem/96), as representative of a virus well adapted to humans\(^6\). As the HA of the novel H1N1 pandemic virus originated from a virus similar to “triple reassortant” swine H1N1 viruses, we compared one such example, A/Iowa/1/2006 (Iowa/06), isolated from a human infection\(^3\), and an ‘older’ close relative of classical swine H1N1 viruses, A/New Jersey/76 (NJ/76), the human isolate which initiated the concern of a pandemic threat in 1976\(^7\).

Our analysis system\(^8,9\) is based on the neoglycolipid (NGL) technology, which has been validated as an effective approach for presenting oligosaccharides for carbohydrate ligand assignments, not only for soluble carbohydrate-binding proteins but also for whole cells, bacteria\(^10\) and virus-like particles\(^11\) that express carbohydrate-binding proteins many of which have low affinities. The special advantage of the lipid-linked probes non-covalently presented on nitrocellulose matrix is their clustered state and an element of mobility that confer high avidity. Eighty six sequence-defined oligosaccharide probes were incorporated (Supplementary Table 1). These included eighty sialyl-terminating oligosaccharide probes with differing backbone types, chain lengths and branching patterns, also various sialylation, fucosylation and sulphation patterns, representative of N- and O-glycans and glycolipids. Six neutral probes served as negative controls.

We observed a clear distinction between the receptor-binding repertoire of the pandemic H1N1 viruses Cal/09 and Ham/09 and that of the seasonal H1N1 virus Mem/96 (Fig. 1). The Cal/09 and Ham/09 viruses bound not only to the majority of \(\alpha_2\)-linked sialyl sequences included irrespective of the backbone chain length and type, but they also bound to a considerable range of \(\alpha_2\)-linked sialyl sequences. In contrast, Mem/96 bound exclusively to \(\alpha_2\)-linked sialyl sequences, and the binding was almost always to those with tetrasaccharide or longer backbones irrespective of the backbone type (type 1, Gal \(\beta_1\)-GlcNAc or type 2, Gal \(\beta_1\)-4GlcNAc; Supplementary Table 1 and Supplementary Fig. S1). Even at a high virus concentration, no binding to the \(\alpha_2\)-linked sialyl sequences was observed (Supplementary Fig. S2). Features of the differential binding between the pandemic H1N1 viruses and the seasonal H1N1 virus Mem/96 are highlighted in Table 1 with selected probes that have closely related backbone sequences.

Although overall, the strongest binding of the H1N1 pandemic viruses was to \(\alpha_2\)-linked sialyl sequences, binding of Cal/09 to some of the \(\alpha_2\)-linked sialyl sequences was comparable to that of the corresponding \(\alpha_2\)-linked sialyl probes, notably probes 18 and 20, based on disaccharide backbones, and 40, based on a branched hexasaccharide-backbone. Also in this relatively high binding category were sialyl Lewis\(^x\)-related probes 22 and 29, with di- and tetra-saccharide backbones, and the sulphated sialyl-Lewis\(^x\) probe 35 with sulphate on N-acetylglucosamine (Supplementary Table 1). Thus our results using viruses are in accord with the prediction\(^5\) of dual specificity based on modelling of the HA protein of the Cal/09 virus. They contrast with those reported by Maines \textit{et al.}\(^12\) who examined the binding of the soluble recombinant HA of Cal/09 to a limited set of biotinylated sialyl (poly)N-acetyllactosamine probes presented on immobilized streptavidin. The authors detected binding to an \(\alpha_2\)-linked sialyl sequence with tetrasaccharide backbone, but little or no binding to \(\alpha_2\)-linked sialyl probes with di-, tetra- or hexasaccharide backbones or to an \(\alpha_2\)-linked probe with a disaccharide backbone. As the affinities of individual HA molecules for their oligosaccharide receptors are low, multivalent interaction with receptors is necessary for high affinity cooperative binding of virus. Thus differences in clustering of the probes\(^10\), on the one hand, and of the HAs, presented on virus rather than as antibody complexes\(^12\), on the other, are likely to account in part for the apparent higher avidity of binding in the present experiments.
Some differences between the two pandemic H1N1 isolates were, however, apparent in our analyses (Fig. 1, Table 1, Supplementary Table 1 and Fig. S1): Cal/09 bound more strongly overall to α2-3-linked sialyl sequences and it bound to several short sequences (disaccharide backbones), for example probes 8, 10, 12 and 20, that gave little or no binding with Ham/09. Moreover, Cal/09 bound to α2-8-linked polysialyl sequences as found on brain ganglioside GD3 (probes 70/71) and at the outer arms of N-glycans of the neural cell adhesion molecule N-CAM (probes 75-84). These differences were less apparent when lower concentrations of virus were used, whereupon binding was predominantly to α2-6-linked sialyl probes and only weak binding to the α2-3-linked probes 27-29 could be discerned (Supplementary Fig. S3). There are three amino acid differences between the HAs of Ham/09 and Cal/09, S83P, A197T and V321I, which may account, at least in part, for these differences in binding. Whereas the residues in Cal/09 are less common, the sequence of Ham/09 is representative of the consensus sequence for the majority of recently circulating H1N1 pandemic viruses.

The patterns of receptor binding of the pandemic H1N1viruses, Cal/09 and Ham/09, were similar overall to those observed for the “triple reassortant” H1N1 virus Iowa/06 and the ‘classical swine’ H1N1 virus NJ/76 (Fig. 1, Table 1, Supplementary Table 1 and Fig.S1), except that binding to α2-3-linked 4-O-acetylated sialyl probes 15/16 was observed only with the latter two viruses. This form of sialic acid has been identified in a number of animal species and in trace amounts in humans. The binding pattern of X31, a reassortant virus containing the HA and neuraminidase (NA) of A/Aichi/2/68 (H3N2) from the 1968 pandemic, also showed similarities to those of the two pandemic H1N1 isolates, with preferential binding to α2-6-linked and lower binding to the α2-3-linked sialyl sequences (Fig. 1, Table 1, Supplementary Table 1 and Fig. S1). The pattern of binding to the α2-3 sequences was more reminiscent of Ham/09, whereas the α2-8 binding resembled Cal/09, but was more apparent even at low concentrations of virus (Supplementary Figure S5).

These results indicate that no major change in receptor binding specificity of the HA was required for the emergent pandemic virus to acquire human-like characteristics and become established in the human population. Other factors are likely to have contributed to the sustained human-to-human transmission of the pandemic H1N1 viruses in contrast to the sporadic infections by swine viruses. For example, acquisition of a novel NA by genetic reassortment may have provided better complementarity between the functional characteristics of the HA and NA of the emergent virus.

The broader specificity for α2-3-in addition to α2-6-linked receptors is also pertinent to the greater virulence of the pandemic virus than seasonal influenza viruses observed in animal models, and its capacity to cause severe and fatal disease in humans, despite the generally mild nature of most infections. Binding to α2-3-linked receptors is thought to be associated with the ability of influenza viruses to infect the lower respiratory tract where there is a greater proportion of α2-3-relative to α2-6-sialyl glycans, although long chain α2-3-linked sialyl (poly-N-acetyllactosamine) sequences are present in ciliated bronchial epithelial cells in humans where they are the receptors for another human pathogen, *Mycoplasma pneumoniae*. The differences in receptor binding between the 2009 pandemic and the seasonal H1N1 viruses may therefore account, at least in part, for the higher virus replication and greater pathology reported in the lungs of ferrets, mice and non-human primates infected with pandemic viruses, compared to contemporary seasonal viruses.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.
Acknowledgments

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REFERENCES


Figure 1. Carbohydrate microarray analyses of the six viruses investigated
Numerical scores for the binding signals are shown as means of duplicate spots at 5 fmol/spot (with error bars). The microarrays consisted of eighty sialylated and six neutral lipid-linked oligosaccharide probes, printed on nitrocellulose-coated glass slides. These are listed in Supplementary Table 1 and arranged according to sialic acid linkage, oligosaccharide backbone chain length and sequence. The various types of terminal sialic acid linkage are indicated by the coloured panels as defined at the bottom of the figure.
Table 1
Features of binding to selected sialyl sequences

<table>
<thead>
<tr>
<th>Probe</th>
<th>Sequence</th>
<th>Fluorescence signal intensities</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Cal/09</td>
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<tr>
<td>12</td>
<td>NeuAco-3Galβ-4Glc-AO&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5191</td>
</tr>
<tr>
<td>16</td>
<td>Neu4,6Aco-3Galβ-4Glc-AO</td>
<td>-</td>
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<tr>
<td>52</td>
<td>NeuAco-6Galβ-4Glc-AO</td>
<td>12226</td>
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</tr>
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<td>NeuAco-6Galβ-4GlcNAc-AO</td>
<td>25526</td>
</tr>
<tr>
<td>23</td>
<td>NeuAco-3Galβ-3GlcNAcβ-3Galβ-4Glc-DH&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>40</td>
<td>NeuAco-3Galβ-3GlcNAcβ&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>NeuAco-6Galβ-4GlcNAcβ&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>6997</td>
</tr>
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<td>18824</td>
</tr>
</tbody>
</table>

<sup>a</sup>Probe, probe number and position in microarray;
<sup>b</sup>The selected α2-3-linked and α2-6-linked sialyl sequences are marked in red and blue, respectively; the type 1 (Galβ-3GlcNac) backbones are in green;
<sup>c</sup>AO, NGLs prepared from reducing oligosaccharides by oxime ligation with the amidooxy (AO) functionalized amino lipid, 1,2-dihexadecyl-rn-glycero-3-phosphoethanolamine (DHPE)<sup>19</sup>;
<sup>d</sup>- signal less than 1;
<sup>e</sup>DH, NGLs prepared from reducing oligosaccharides by reductive amination with DHPE<sup>20</sup>.