Phenotypic Variations in Low Pathogenic H1N1 Avian Influenza Viruses Resulting from Genotypic Differences Among Isolates

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Materials and Methods

Type A influenza isolates possessing the hemagglutinin (HA) - neuraminidase (NA) combination of H1N1 have been of great interest to both public health and veterinary scientists and officials since the occurrence of the 1918 Spanish Flu pandemic and the emergence of avian influenza (H5N1) "influenza virus". H1N1 type A influenza viruses have been recovered from wild and domestic birds, swine and humans but the isolates studied are not uniformly distributed for all age and sex strata. However, the host range in this host

Introduction

Waterfowl-origin H1N1 type A influenza viruses in our repository appeared to demonstrate differences in infectivity and hemagglutinin titers when propagated in embryonating-chicken-eggs. Upon the completion of genomic analysis it was determined that all genomic segments were waterfowl-origin, though determined aa sequence homology was as low as 70.5 percent between some RNA segments, thus eliminating the possibility that genetic reassortment with mammalian influenza viruses was responsible for the observed phenotypic variations. It was concluded that the phenotypic differences observed in this study were due to the nucleotide and amino acid substitutions in wild- and domestic-bird-origin AIV isolates, and not due to genetic recombination among H1N1 viruses from different species of birds. Only the aa sequence identities were not associated with previously reported amino acid substitutions affecting replication of human-origin influenza viruses in embryonating chicken eggs.

Results

Initial Virus Isolation

These experiments were designed to test whether the NS genomic segment of isolates within the study was non structural allele A or B. (5) It was determined based on the phylogenetic tree that all the isolates in this study were allele A. All four isolates exhibited the lowest HA titer among the four isolates throughout the six limiting dilution, the GMT at 48 hours post inoculation was 9.85.

Summary

This study confirmed genotypic differences existed in isolates serologically characterized as H1N1 AIV. Amino acid homology varied from 70-100% among the genonomic segments but, it was determined that all genonomic segments were likely waterfowl-origin. Thus the possibility that genetic reassortment with type A influenza viruses from poultry, humans and other mammalian was not responsible for the phenotypic variations, resulting in the rejection of hypothesis two. Therefore we conclude that the phenotypic differences observed in this study were a result of accumulated nucleotide point mutations and amino acid substitutions observed among the isolates, thus supporting hypothesis two.

Backstrations confirmed the EID50 of the 0.1L challenge inoculums for 87-430, 93-06, 93-13, and 93-52 were 1x10^5, 17 EID50/0.1mL, 1x10^3, 76 EID50/0.1mL, 1x10^5, 67 EID50/0.1mL, and 1x10^3, 30 EID50/0.1mL, respectively.

Sequence Analysis

Nucleotide homology among isolates ranged from 93.3% with the matrix gene (M1) to 71.1% with the non-structural gene (NS3) (Table 4). The matrix gene was embryonic the most conserved in this study, had nucleotide (nt) and amino acid (aa) identities at 93.3% and 100%, respectively. The largest identity differences among the isolates were demonstrated in the non structural genonomic segments with only 71.1% nt identity, and the NS1 protein with 70.5% aa identity, with all other proteins having identities within the range of 79.5-100%. (Table 4 and 5).

Using information published by Suarez, et al. a phylogenetic tree was created to determine whether the NS genomic segment of isolates within the study was non structural allele A or B. (5) It was determined based on the phylogenetic tree that all the isolates in this study were allele A.

Table 3: HA Titers (HA GMTs) of Each Egg Passage (at the highest dilution)

<table>
<thead>
<tr>
<th>Isolate Name</th>
<th>Passage #</th>
<th>87-430</th>
<th>93-95</th>
<th>93-118</th>
<th>02-350</th>
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</thead>
<tbody>
<tr>
<td>A/green-winged</td>
<td>87-430</td>
<td>8.5</td>
<td>8.33</td>
<td>8.48</td>
<td>6.0</td>
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<tr>
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<td>93-95</td>
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<td>8.33</td>
<td>9.50</td>
<td>7.50</td>
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<tr>
<td></td>
<td>93-118</td>
<td>8.50</td>
<td>8.33</td>
<td>9.50</td>
<td>7.50</td>
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<tr>
<td></td>
<td>02-350</td>
<td>8.50</td>
<td>8.33</td>
<td>9.50</td>
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Table 4: Changes in Amino Acid Sequences of the Four H1N1 AIV Isolates

<table>
<thead>
<tr>
<th>Gene/Protein</th>
<th>NS1</th>
<th>NS2</th>
<th>NS3</th>
<th>NS4</th>
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<tbody>
<tr>
<td>A/green-winged</td>
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