A Comparison of Commercially Available H5N1 Hemagglutinin Antibodies: Polyclonal Anti-H5 Antibodies Can Detect All Variants of H5 Hemagglutinin While Monoclonal Antibodies Show Specificity

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Abstract

Rapid detection of the H5N1 virus is critical to the prevention of bird-to-human and human-to-human spread of this disease. While the U.S. Food and Drug Administration has approved a real-time PCR assay for the identification of influenza A/H5 virus, a more rapid assay might make use of antibodies specific to the H5 strain. Recently, five polyclonal and five monoclonal antibodies capable of detecting the H5 hemagglutinin have been developed. Here, we report the use of ELISA-based assays to assess the specificity and sensitivity of these antibodies. All of the antibodies could detect 10ng of peptide corresponding to the region of the H5 protein used to generate the respective antibodies. Furthermore, all monoclonal antibodies and all but one polyclonal antibody showed no reactivity to the corresponding regions of human-specific influenza hemagglutinin (H1, H3 and influenza B), demonstrating the specificity of these antibodies for H5. Sequence analyses of the hemagglutinin from H5N1strains from infected patients in Egypt and Indonesia in 2007 indicate that the viral genome has drifted slightly in the three years since its reappearance in 2004. While all of the monoclonal antibodies were able to recognize the 2007 Indonesian variant of H5, they showed much less reactivity to the H5 sequence from the Egyptian patients. All five of the polyclonal antibodies were able to recognize both the Indonesian and Egyptian variants. These results suggest that while all of these antibodies could be used to detect H5N1 influenza, the polyclonal antibodies may be more useful for the initial detection of a broad range of H5 strains exhibiting sequence variation and the monoclonal antibodies could determine which H5 variations were present.

Introduction

Influenza A virus is a major public health threat, killing more than 30,000 people per year in the USA (1). Novel influenza virus strains caused by genetic drift and viral recombination emerge periodically to which humans have little or no immunity, resulting in devastating pandemics. Influenza A can exist in a variety of animals; however it is in birds that all subtypes can be found (2). These subtypes are classified based on the combination of the virus coat glycoproteins hemagglutinin (HA) and neuraminidase (NA) subtypes. During 1997, an H5N1 avian influenza virus was determined to be the cause of death in 6 of 18 infected patients in Hong Kong (3). The more recent virulent strain of H5N1 is now seen in Africa and Europe, as well as in southeast Asia. There is some evidence of human to human spread of this virus, but it is thought that the transmission efficiency was fairly low (4). HA interacts with cell surface proteins containing oligosaccharides with terminal sialyl residues. Virus isolated from a human infected with the H5N1 strain in 1997 could bind to oligosaccharides from human as well as avian sources, indicating its species-jumping ability (5). Sequence analysis of viruses obtained from Indonesian and Egyptian patients infected with H5N1 indicates the influenza virus genome has drifted somewhat from what was first reported. We describe here the specificity of several polyclonal and monoclonal antibodies for H5 and their ability to recognize the Indonesian and Egyptian variants of H5.

Results – H5 Hemagglutinin ELISA Experiments with Peptide Targets

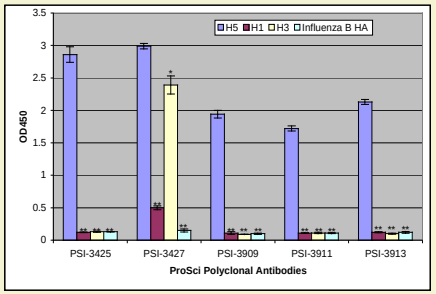

Figure 1. Cross-reactivity of Polyclonal anti-H5 Antibodies. Standard ELISA was used to test the specificity ProSci polyclonal antibodies using 10ng peptides corresponding the immunogenic peptide and the equivalent sequences from H1, H3, and influenza B hemagglutinin. The data represent the mean ± SEM of four different samples for each peptide. *, $p < 0.05$; **, $p < 0.01$ compared to that of the immunogenic peptide (Student's t test).

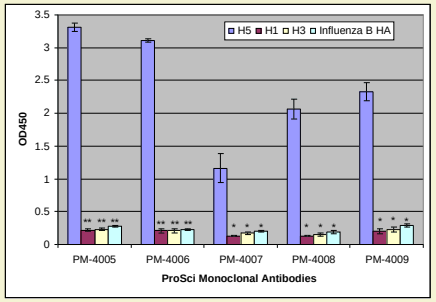

Figure 2. Specificity of Monoclonal anti-H5 Antibodies. Standard ELISA was used to test the specificity ProSci monoclonal antibodies using 50ng peptides corresponding the immunogenic peptide and the equivalent sequences from H1, H3, and influenza B hemagglutinin. The data represent the mean ± SEM of four different samples for each peptide. *, $p < 0.05$; **, $p < 0.01$ compared to that of the immunogenic peptide (Student's t test).

A. `2004:  R N S P Q R E R R R K K R G`
`Indo.: R N S P Q R E S R R K K R G`
`Egypt: R N S P Q G E R R R K K R G`

B. `2004:  C Y P G D F N D Y E E L K H L`
`Indo.: C Y P G S F N D Y E E L K H L`
`Egypt: C Y P G N F N D Y E E L K H L`

C. `2004:  A P E Y A Y K I V K K G D`
`Egypt: A P E N A Y K I V K K G D`

Figure 3. Sequence Analysis of Indonesian and Egyptian Strains. Genetic drift has led to hemagglutinin sequence differences in H5N1 viruses isolated from Indonesian and Egyptian patients in early 2007 compared to the original sequence isolated from poultry in China in 2004. A) Hemagglutinin sequence from aa334 – 347 that is recognized by the PSI-3425, PM-4005 and PM-4006 antibodies. B) Hemagglutinin sequence from aa106 – 120 that is recognized by the PSI-3427 antibody. C) Hemagglutinin sequence from aa265 – 277 that is recognized by the PSI-3911, PM-4007, PM-4008 and PM-4009 antibodies.

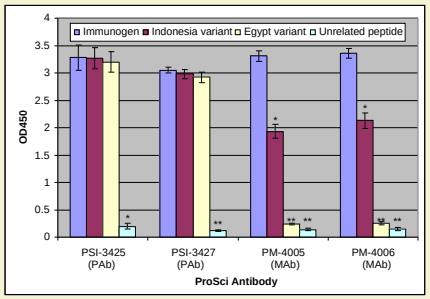

Figure 4. Detection of the Indonesian and Egyptian Variants of H5. Standard ELISA was used to test the ability of ProSci antibodies to recognize variant hemagglutinin sequences using 50ng peptides corresponding the immunogenic peptide and the equivalent peptide sequences from H5N1 isolated from Indonesian and Egyptian patients respectively. The data represent the mean ± SEM of four different samples for each peptide. *, $p < 0.05$; **, $p < 0.01$ compared to that of the immunogenic peptide (Student's t test).

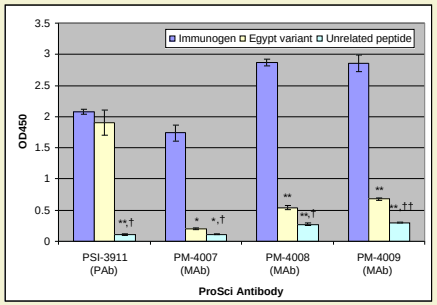

Figure 5. Detection of the Egyptian Variant of H5. Standard ELISA was used to test the ability of ProSci antibodies to recognize variant hemagglutinin sequences using 50ng peptides corresponding the immunogenic peptide and the equivalent peptide sequences from H5N1 isolated from Egyptian patients. The data represent the mean ± SEM of four different samples for each peptide. *, $p < 0.05$; **, $p < 0.01$ compared to that of the immunogenic peptide; †, $p < 0.05$; ††, $p < 0.01$ compared to that of the peptide corresponding to the Egyptian variant (Student's t test).

Summary

- Of the five polyclonal anti-hemagglutinin antibodies developed, four are highly specific for H5, with PSI-3427 showing strong cross-reactivity to H3 and a lesser amount towards H1 hemagglutinin.
- All five monoclonal anti-hemagglutinin antibodies are highly specific for H5 hemagglutinin.
- Genetic drift has led to some sequence variation in the hemagglutinin isolated from Indonesian and Egyptian patients infected with H5N1 in early 2007.
  - The polyclonal antibodies tested can recognize both variants in addition to the original sequence.
  - Two of the monoclonal antibodies (PM-4005 and PM-4006) can recognize the Indonesian but not Egyptian variant hemagglutinin; the other three (PM-4007, PM-4008 and PM-4009) can recognize the Egyptian variant, but at a much reduced level.

References

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