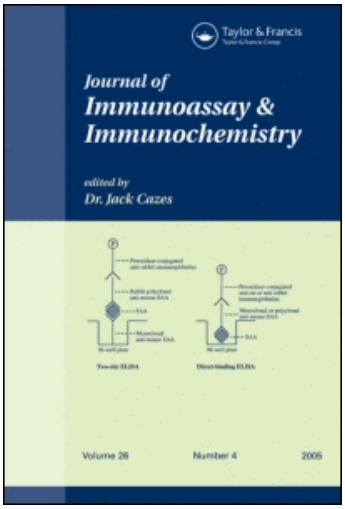

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Antigenicity of a Type A Influenza Virus Through Comparison of Hemagglutination Inhibition and Mass Spectrometry Immunoassays

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Abstract: The antigenicity of a type A (H1N1) influenza strain has been characterised through the application of mass spectrometry (MS) and hemagglutination-inhibition immunoassays performed in parallel. Two monoclonal antibodies were found to be highly and equally specific in HI assays against influenza strain A/New Caledonia/20/99 while the MS immunoassay demonstrated that both antibodies recognise the same epitopic peptide localised to residues 225-232 of the hemagglutinin HA1 subunit whose C-terminal residues reside in close proximity to the receptor binding site. Both immunoassays showed no binding of a monoclonal antibody that recognizes the hemagglutinin antigen of type B strains.

Keywords: Antigenicity, Hemagglutination inhibition, Immunoassay, Influenza, Mass spectrometry

INTRODUCTION

Annual outbreaks of influenza are most often associated with strains that are closely related to those that have appeared in circulation in previous years.[1] Antigenic variations among strains due to antigenic drift occur gradually as a result of the accumulation of replication errors in the genes

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that encode the two major surface antigens of the virus: hemagglutinin (HA) and neuraminidase (NA).[2] When reassortment of the segmented influenza RNA genome occurs, following the simultaneous infection of a host with two strains, the genes encoding HA or NA reassort and the virus undergoes an antigenic shift.[3] The antigenicity of the reassorted strain differs significantly from those that it originated from and possesses the potential to cause a pandemic outbreak.[3] Monitoring this antigenic variation is of major importance and forms the basis of current influenza surveillance strategies.

Three influenza pandemics last century resulted in tens of millions of deaths worldwide.[4] The emergence of a highly pathogenic avian influenza (H5N1) [5] and swine (H1N1) strains and their ability to infect a human host have renewed concerns about the global impact of the virus on human health.[5]

Widespread international travel allows a highly virulent strain of the virus to be rapidly transmitted throughout the human population.[6] Thus future pandemics will likely develop more rapidly providing a relatively short window in which to survey and assess the nature of the virus and its antigenicity. The need to develop new and improved surveillance methods in this regard has been recognised.[7]

The basis of any surveillance strategy is the unambiguous identification of the circulating influenza strains. Among the methods recommended by the World Health Organisation (WHO) are reverse transcription polymerase chain reaction (RT-PCR), enzyme immunoassays (EIA) for antigen detection, and various serological approaches including hemagglutination-inhibition (HI) and microneutralization (MN) assays. Each method has its own advantages and disadvantages[8,9] with respect to the time required for analysis, the need for specialised instrumentation and/or research facilities, and the level and reliability of information derived from the results.

The hemagglutination-inhibition assay is well established for the classification of hemagglutinating viruses.[10] The attachment of the influenza virus to Red Blood Cells (RBCs) results in their agglutination at a certain virus titre that impedes cell sedimentation. Hemagglutination is inhibited if an antibody is bound near to the receptor binding sites of the hemagglutinin antigen that in turn prevents the virus from attaching itself to the RBCs. The inhibition of hemagglutination by antisera or monoclonal antibodies (mAbs) is utilized in the HI assay to determine the serotype, and even groups within a given serotype, of the influenza virus.[10,11] While this serological assay forms the basis of present day screening of the virus, it is not without its limitations. For example, non specific hemagglutination inhibitors in animal sera can lead to false positive results unless removed or destroyed[11,12] and further problems can result from the varying abilities of virus strains to hemagglutinate RBCs of different animal species.[12] In addition to

these practical considerations, the HI assay provides no molecular detail or information about the structural changes that underlie antigenic drift or shift.

These structural changes can be addressed by employing the reverse transcription polymerase chain reaction (RT-PCR) with subsequent sequencing of the products.[13,14] The basis of any RT-PCR approach is the targeted amplification of defined sequences within the segmented influenza RNA genome. Subtyping of the virus can be achieved by using sets of primers for conserved sequences of the different hemagglutinin and neuraminidase antigens across particular viral subtypes.[15–17] PCR sequencing of the genes that encode the hemagglutinin and neuraminidase antigens can identify mutations that may predict antigenic change, though this is not explicitly measured. RT-PCR experiments alone do not provide molecular detail about the antigenicity of the virus.

To address the shortcomings of the above approaches, a mass spectrometry (MS) immunoassay was developed.[18] It has been successfully applied to characterise the influenza virus at the protein level in which both the primary structure and antigenicity of viral antigens can be established in a single step.[19] This immunoassay is based on a comparison of MALDI mass spectra obtained following proteolytic digestion of the whole virus[19] or an electrophoretically-separated antigen[20,21] either before or after treatment with monoclonal antibodies. It was developed following the realisation that specific immune complexes could be preserved on conventional MALDI targets without immobilization of either antigen or antibody.[18] The mass maps recorded provide information on the primary structures of the viral antigens and, through database searching, their similarity to known viral antigen sequences. Further, a comparison of the MALDI mass spectra recorded before and after antibody treatment enables the antigenicity of a strain to be assessed and specific epitopes to be localised. While successfully demonstrated for whole virus,[19] the initial separation of antigens by gel electrophoresis prior to application of the immunoassay improves the sequence coverage of antigens in the mass maps and thus the likelihood that the binding of epitopic peptides can be followed.[20,21]

This article compares the effectiveness and validity of the MS assay for the screening of viral isolates by presenting a side-by-side comparison of the results from the application of the MS and HI immunoassays for three monoclonal antibodies targeted to a influenza A (H1N1) strain A/New Caledonia/20/99 together with one monoclonal antibody to a type B strain as a separate control. Immunodominant epitopes within the hemagglutinin antigen of this strain are identified and the results of both complementary assays are shown to be largely in accord.

EXPERIMENTAL

Virus Strains

All influenza strains used in this study were obtained from Advanced ImmunoChemicals Inc. (Long Beach, California, USA) after growth in the allantoic fluid of 10–11 day old embryonated eggs. All influenza strains used A/New Caledonia/20/99 IVR116 (H1N1), A/Beijing/262/95 (H1N1), B/Tokyo/53/99 and B/Victoria/504/2000 were chemically inactivated with 0.05% beta propiolactone and preserved in 0.1% sodium azide (NaN_3) and 0.005% thimerosal.

Monoclonal Antibodies

Bioreactor preparations (miniPERM) of monoclonal antibodies designated mAb127, mAb128 and mAb154 were kindly provided by Elizabeth Pietrzykowski (CSL Limited, Melbourne, Australia). The mAbs were used without further purification with the levels of antibody present estimated based on optical density measurements at 280 nm, and the separation and detection of their heavy and light chains by polyacrylamide gel electrophoresis. The PAGE experiments indicate the purity of the antibodies exceeds 95%. mAb127 and mAb128 are specific for influenza A virus of subtype H1N1 and were obtained using strain A/New Caledonia/20/99 as the immunogen. mAb154 was prepared using strain B/Jiangsu/10/2003 of the Yamagata/16/88 lineage as immunogen. Prior to their use for mass spectrometry experiments, the mAb storage buffer was exchanged with 50 mM NaCl, 25 mM NH_4HCO_3, 5 mM Tris-HCl pH 8.0 by centrifugation through a PES membrane microcentrifuge filter with a MWCO of 30 kDa. Sodium azide was added as a preservative to a final concentration of 0.1%. The concentration of the mAbs was determined using the Bradford method.[22]

Anti influenza A clone IVC102 (mAb IVC102) was obtained from Advanced ImmunoChemicals Inc. (Long Beach, California, USA). Aliquots of the mAb were completely dried in a vacuum concentrator and resuspended in binding buffer (50 mM NaCl, 25 mM NH_4HCO_3, 5 mM Tris-HCl pH 8.0) immediately prior to the use for the MS immunoassay.

Hemagglutination-Inhibition Assay

HI assays were performed as described by the WHO with minor modifications.[12] Briefly, two-fold serial dilutions of stock monoclonal antibody solutions where prepared in V-shaped microtitre plates (25 µL per dilution point). Each dilution point was mixed with the same volume

of a standardized solution of a viral test strain (4 HA units in 25 μL) and incubated for 30 min at room temperature. 50 μL of a 1% fowl erythrocyte solution in PBS was added and inhibition of hemagglutination was visually assessed after incubation for 60 min at room temperature.

Western Blot

Western blot analysis of viral proteins was performed as described by Towbin[23] with minor modifications. The viral antigens separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were blotted onto a PVDF membrane (Pall Corporation, Pensacola, Florida, USA). The membrane was blocked with a 5% solution of skim milk powder in PBS buffer containing 0.1% Tween20. It was subsequently incubated with a 1 $\mu g \cdot mL^{-1}$ solution of the primary antibody in the same buffer. The presence of bound antibody was visualised using a HRP-conjugated, anti-mouse antibody (Bio RAD, Hercules, California, USA) employing enhanced chemoluminescence (ECL) for exposure of an X-ray film.

Hemagglutinin Separation and Digestion

Viral proteins were separated by SDS-PAGE as previously described.[20] 20 μg of virus was added to each lane of a SDS-PAGE gel. Hemagglutinin represents some 35% of total virus.[24] The hemagglutinin-containing bands were excised and subjected to in gel tryptic digestion. The tryptic peptides were recovered in binding buffer (50 mM NaCl, 25 mM NH_4HCO_3, 5 mM Tris-HCl pH 8.0) for utilization in the MS immunoassays.

Mass Spectrometry Immunoassays

MS immunoassays were conducted as described previously with minor modifications.[20] In brief, 14 μg of the tryptic peptides, generated from the combined HA bands of two gel lanes, were mixed with two-fold the amount of mAb (by weight) in solution. Binding buffer (50 mM NaCl, 25 mM NH_4HCO_3, 5 mM Tris-HCl pH 8.0) was added to achieve a total volume of 25 μL. The mixture was incubated at 4°C. Samples for MS analysis were taken immediately after addition of the antibody ($t = 0$) and after 24 h. An aliquot of the untreated antigen preparation, and a second aliquot treated with a monoclonal antibody specific to type B influenza were run in parallel under the same conditions as non binding controls. MALDI-MS analysis was performed on a QSTAR-XL hybrid Q-TOF mass spectrometer (Applied Biosystems) in the positive ion TOF-MS mode. 1 μL of sample was diluted with 3 μL matrix solution

($10\,mg{\cdot}mL^{-1}$ α-cyano-4-hydroxycinnaminic acid, 50% acetonitrile in water). 1 μL of the analyte and matrix solution was spotted onto a MALDI target and dried by evaporation. Each sample was measured in triplicate from different spots on the MALDI target using an average of 200 laser shots per spectra.

MALDI mass spectra for the control (no mAb) and antibody treated samples were compared for evidence of mAb-peptide binding through a comparison of the relative areas for the ion signals across both spectra relative to the sum of the areas for all nucleoprotein derived peptides present. An absolute reduction in relative area of at least 15%[20,21] identifies an antigenic peptide.

RESULTS

Antigenicity of Influenza A Strain A/New Caledonia/20/99 by Hemagglutination-Inhibition

Figure 1 shows the application of the hemagglutination-inhibition assay for monoclonal antibodies designated mAb127, mAb128 and mAb IVC102

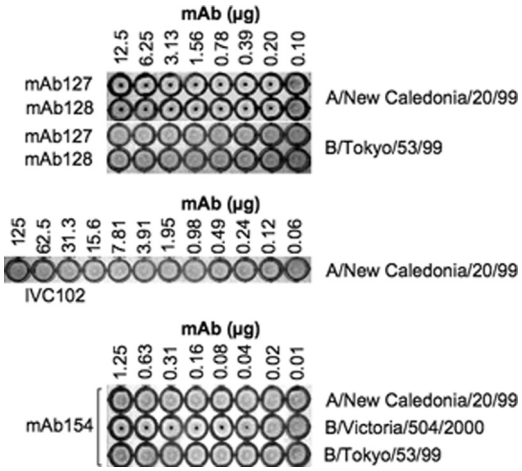

Figure 1. Results of hemagglutination-inhibition assays. Serially diluted (1/2) monoclonal antibody solutions (of mAb127, mAb128, mAb154 and mAb IVC102) were tested in HI assays against a standardised concentration of influenza virus (across strains A/New Caledonia/20/99, B/Tokyo/53/99 and B/Victoria/504/2000) in the presence of red blood cells. Where there is sufficient antibody present to bind to the hemagglutinin of the virus particles and prevent their agglutination to the red blood cells, the blood cells "button" at the bottom of the plate.

against the influenza A (H1N1) strain A/New Caledonia/20/99 and B-type strain B/Tokyo/53/99. In these assays, serially diluted (1/2) antibody solution is treated with a standardised quantity of virus in the presence of erythrocytes. Where sufficient antibody is present to bind to the hemagglutinin of the virus particles and prevent their agglutination to the red blood cells, the blood cells "button" at the bottom of the plate. Antibodies mAb127 and mAb128 inhibit hemagglutination against the type A virus down to concentrations of 2 $ng \cdot \mu L^{-1}$ (0.2 μg mAb). They fail to do so at all concentrations up to 125 $ng \cdot \mu L^{-1}$ (12.5 μg mAb) against the type B virus. In contrast, the monoclonal antibody mAb154 to type B viruses of the Yamagata/16/88 lineage inhibits hemagglutination of the B/Victoria/504/2000 strain down to 0.4 $ng \cdot \mu L^{-1}$ (0.04 μg mAb), but not the B/Tokyo/53/99 strain of a different lineage due to differences in the nature of the targeted epitope. This antibody similarly does not inhibit hemagglutination in the presence of the type A strain.

Antigenic Characterisation of Influenza Type A Strain A/New Caledonia/20/99 by Mass Spectrometry

To examine the region of the type A hemagglutinin targeted by the monoclonal antibodies, mAb128 was initially chosen for the implementation of the MS immunoassay against the New Caledonia strain. As has been described previously for this strain,[20] the hemagglutinin antigen migrates on a SDS-PAGE gel together with nucleoprotein (NP) and appears at approximately 55 kDa (Figure 2). The identity of these and the other antigens were confirmed from their mass maps. Although unresolved, hemagglutinin has been found to be slightly enriched toward the top of the visible HA/NP band (see Figure 3) and this region was therefore chosen as the major source of the HA antigen. Digestion of the HA-enriched proportion of the band with trypsin, and the recovery of cleaved peptides from this gel region, was followed by incubation of aliquots of this antigen preparation with either mAb128 or an influenza B-specific monoclonal antibody (mAb154) as a non-binding control. The untreated and antibody-treated solutions were incubated for 24 hours and then analysed by mass spectrometry.

The MALDI mass spectra contain ion signals associated with the tryptic peptides derived from either the HA or NP antigen (see Figure 3). The identity of each of the peptides was established based upon an alignment of their masses with those generated theoretically through the *in silico* digestion of the hemagglutinin and nucleoprotein antigen of the same and related strains using the Mascot mass fingerprint search algorithm of the MSDB database[25] with the identities of the most abundant peptide ions confirmed by tandem mass spectrometry (Figure 4).

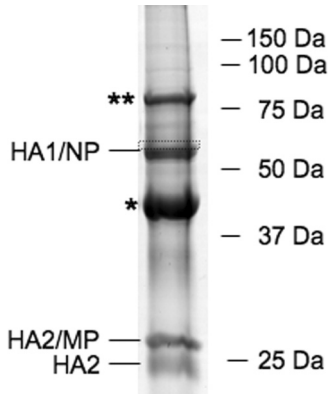

Figure 2. SDS-PAGE separation of the antigens of type A influenza (strain A/New Caledonia/20/99). The location of the antigens is denoted: P for unseparated polymerase subunits, NP nucleoprotein, MP matrix protein, and HA1 and HA2 for hemagglutinin subunits 1 or 2, respectively. Ovalbumin (*) and ovotransferrin (**) were both detected as a result of the virus being grown in the allantoic fluid of chicken eggs. The boxed area at the top of the combined HA1 and NP band was excised for tryptic digestion and the mass map of this region is shown in Figure 3.

No appreciable differences in the ions detected, nor their relative intensities, are apparent between the mixture without antibody or that treated with influenza B-specific antibody mAb154 (Figure 3). In contrast, treatment of the peptide mixture with monoclonal antibody mAb128 to type A influenza, results in a significant decrease of the relative intensity of the HA-derived peptide ion at m/z 961.5 (Figure 3). The identity of this peptide, and others, was confirmed by tandem mass spectrometry. It comprised residues 225-232 (numbered according to the precursor protein HA0) with sequence RFTPEIAK (Figure 4).

A summary of the average relative area data obtained from three spectra each recorded for the no-antibody, mAb154 treated and mAb128 treated samples are shown in Figure 5. The areas for all peptides ions from both HA and NP in each spectrum were plotted relative to the sum of the areas for all NP-derived peptide ions since the antibody targets the HA antigen. The binding of peptide HA[225-232] at m/z 961.52 to mAb 128 is evident by an absolute decrease in the relative area of its ion signal of 19% and 36% compared with that for the no antibody and mAb154 treated samples respectively. This is the only peptide observed to bind to the antibody as established by an absolute decrease in the relative area of the ion of greater than 15%.[20,21] Figure 5 illustrates that there is no such change in the relative area (within experimental

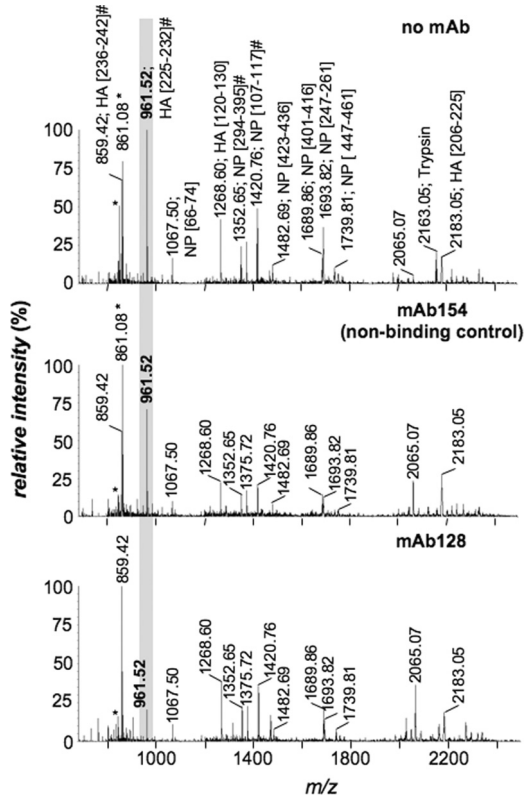

Figure 3. MALDI mass spectra of the tryptic peptides derived from the top of the HA1/NP band (see Figure 2) incubated with either mAb128 (anti influenza A H1N1), mAb154 (anti influenza B) or with binding buffer alone (no mAb). Note that the signal at m/z 861.1 (asterisk) is associated with matrix cluster ions that appear with variable intensity due to their sparodic production during the ionisation process. All peptide ions denoted # have been sequenced by tandem mass spectrometry. Sequence coverage for the HA and NP antigens is 14% and 25%, respectively.

error) of the other peptide ion signals. Noteworthy is that the peptide ion at m/z 2183.07 that corresponds to residues 206-224 directly preceding the epitopic peptide was also found not to bind to mAb128 since the magnitude of the change in its relative area cannot be attributed to a specific binding of the mAb to this peptide. The same is true for the peptide HA[236-242] at m/z 859.43, which flanks the binding peptide on the C-terminal side. Its relative area increases after treatment with antibody mAb128, a likely consequence of more of the peptide being ionised as it no longer competes for ionisation with peptide HA[225-232].

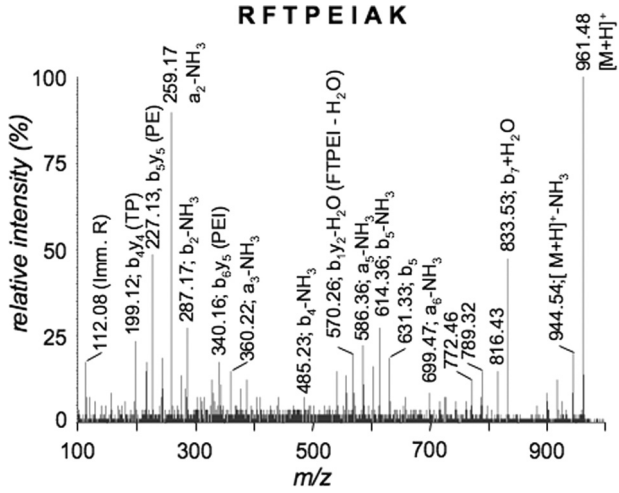

Figure 4. Tandem mass spectrum of the peptide ion at m/z 961.5 from which its amino acid sequence (RFTPEIAK) can be derived.

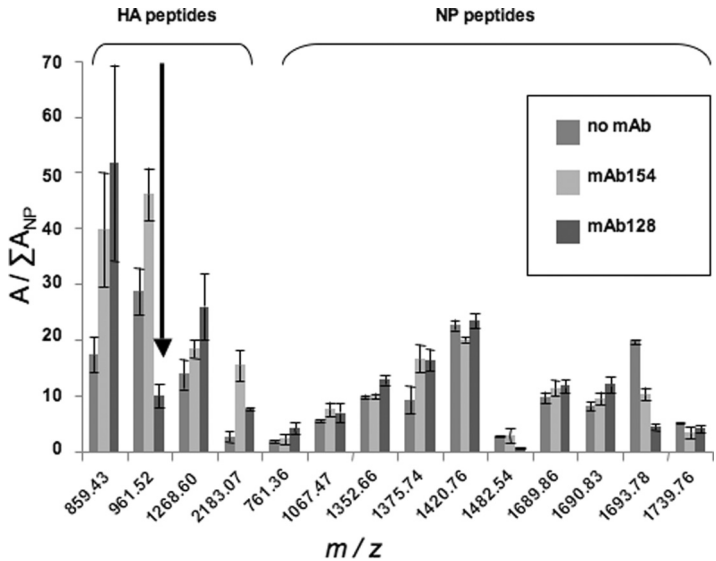

Figure 5. Plot of relative ion areas for all ions labelled in Figure 3 (averaged across three datasets). The area for each ion signal was measured relative to the sum of the areas for all nucleoprotein-derived peptide ion signals within the same spectrum. The binding of peptides to antibody was assessed based on an absolute decrease in relative area of >15% after treatment with antibody as reported previously (Morrissey & Downard, 2006; Morrissey et al., 2007).

HI Assay with Anti Influenza a Monoclonal Antibody IVC102

The larger peptide at m/z 2183.0 has previously been found to constitute an epitope recognised by another monoclonal antibody (mAb IVC102) employing the MS immunoassay. The IVC102 antibody binds to the peptide associated with residues 206-224 of the hemagglutinin antigen as well as the peptide HA 226-232 which flanks this region on the C-terminal side as previously reported (20). A HI assay was performed with it against the New Caledonia strain. Surprisingly, mAb IVC102 failed to inhibit hemagglutination of red blood cells in the presence of the A/New Caledonia/20/99 strain even where the undiluted antibody solution was used (Figure 1). This is in contrast to the results for the two independently derived monoclonal antibodies mAb127 and mAb128 that were reactive against H1-type hemagglutinin and could effect inhibition with 0.2 μg of antibody.

Characterisation of Influenza A Strain A/New Caledonia/20/99 by Western Blot Analysis

To seek independent evidence that mAb IVC102 recognises the hemagglutinin antigen of the New Caledonia strain, beyond that detected by the MS immunoassay,[20] a Western blot was performed with this antibody (Figure 6). This confirmed that mAb IVC102 specifically recognises the hemagglutinin antigen of the type A strain and not the type B strain on the same blot. However, the majority of mAb IVC102 was found to bind a protein with an apparent mass of approximately 25 kDa. Only

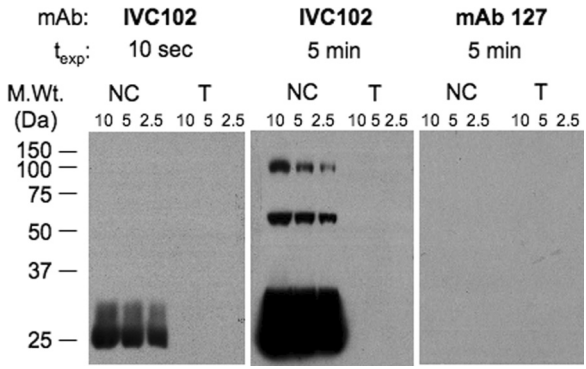

Figure 6. Western blots of viral antigens of type A New Caledonia/20/99 (NC) and type B Tokyo/53/99 (T) influenza from increasing levels of virus (from 2.5, 5 and 10 μg) targeted with monoclonal antibodies mAb IVC102 or mAb127 as primary antibodies. t_{exp} denotes the time of exposure of the X-ray film.

at longer exposure times (5 min) did a band appear at the expected location for the HA1 subunit at approximately 55 kDa together with a third band at approximately 90 kDa corresponding to the uncleaved hemagglutinin subunits.

MALDI mass maps (data not shown) of the electrophoretically separated viral proteins showed that the protein at 25 kDa contains the HA2 subunit of the hemagglutinin antigen as well as the matrix protein (MP). Thus it is concluded that the HA2 subunit contains the immunodominant epitope targeted by mAb IVC102. This is consistent with, and explains the results of, the HI assay since the binding of mAb IVC102 to the HA2 subunit would not necessarily inhibit virus-mediated hemagglutination of red blood cells. The binding of this antibody to a peptide segment of the HA1 subunit in the MS assay[20] is, on the other hand, a result of an intramolecular cross reactivity of the antibody. As only the HA1 subunit was recovered from the gel for the MS-based immunoassay, the binding of the antibody to the HA2 subunit was not assessed.

Interestingly, no binding was detectable when mAb127 (or mAb128) were used in a Western blot of the influenza A/New Caledonia/20/99 virus (Figure 6). This suggests that the epitope recognised by the latter two hemagglutination inhibiting mAbs is conformational whereas the epitope recognised by mAb IVC102 is linear and therefore detected by Western blot analysis.

DISCUSSION

The antigenicity of hemagglutinin can be assessed in the context of a reported structure for the protein of another type A (H1N1) influenza strain (A/Puerto Rico/8/34) with which it shares 87.5% sequence identity. The antigen is characterised by five distinct antigenic sites, denoted, Sa, Sb, Ca_1, Ca_2 and Cb. All are located at the globular head of the HA1 subunit of the protein in close proximity to the receptor binding site.[26]

The monoclonal antibodies mAb127 and mAb128 proved to be highly, and equally, effective at inhibiting the agglutination of red blood cells by the type A virus. The epitope recognised by these mAbs must therefore be located at an antigenic site atop the HA1 subunit. This was investigated using the MS immunoassay where the treatment of the digested HA antigen with either mAb resulted in a substantial and selective depletion of the peptide ion signals for HA residues 225-232 with sequence RFTPEIAK. No such depletion of the peptide ion signal was observed after incubation with influenza B-specific monoclonal antibody (mAb154) indicating that residues 225-232 are specifically recognised.

The MS immunoassay results indicate that antibodies mAb127 and mAb128 recognise the same epitope. This is consistent with their identical

performance in the HI assays. Moreover, both bind a different epitope than that recognised by mAb IVC102.[20] This underlines the selectivity of the MS immunoassay and demonstrates its ability to discriminate between different epitopes within the same antigen.

Sequence alignment of HA derived from influenza strain A/New Caledonia/20/99 with the solved protein structure of HA derived from

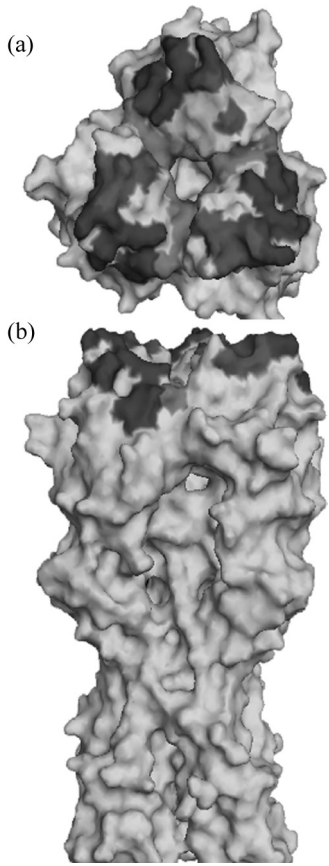

Figure 7. Structure of the hemagglutinin trimer derived from that for the H1N1 strain A/Puerto Rico/8/34 (PDB: 1RU7) (Gamblin et al., 2004). Atomic co-ordinates from the PDB data file were input into the Pymol viewer (version 0.99). A representation of the protein surface is shown (a) in top view, and (b) in side view. Residues corresponding to amino acids 225-232 in A/New Caledonia/20/99 are depicted in red. Peptide HA[206-225] (recognised by mAb IVC102) is shown in orange for one HA monomer. Antigenic sites Sa and Sb are highlighted in blue or purple, respectively, and the major elements of the receptor binding site are coloured in cyan.

A/Puerto Rico/8/34[27] reveals that the peptide comprising residues 225-232 is part of the globular head of the protein. Its C-terminal end is in close proximity to the antigenic site Sa[26] in the HA1 chain of a neighbouring HA monomer as well as the receptor binding site of its own HA1 chain (Figure 7). Most residues of the peptide HA[225-232], however, appear to be located at the base of a channel within the HA trimer (Figure 7). Thus the peptide likely comprises only a part of the epitope, and given that neither flanking peptides HA[206-224] nor HA[236-242] bind the antibodies, the results suggest that it is part of a larger conformational epitope. The location of its C-terminus, in close proximity to a known antigenic sites as well as the receptor binding site, supports this idea and it is also in agreement with the result that both mAb127 and mAb128 were tested negative in a Western blot against the A/New Caledonia/20/99 virus strain (Figure 6).

The mAb IVC102 recognises an epitope containing the tryptic cleavage product compromising residues 206-224 (numbered relative to the precursor protein HA0) of hemagglutinin derived from the H1N1 strains A/New Caledonia/20/99 and A/Beijing/262/95.[20] This sequence region overlaps with antigenic site Sb and contains parts of the "190-helix" which is involved in receptor binding[25,26] (Figure 7). However, the HI assay results of this study fail to validate this since mAb IVC102 failed to inhibit hemagglutination of red blood cells by the A/New Caledonia/20/99 strain. Moreover, an identical result was obtained using the A/Beijing/262/95 strain (data not shown). The application of a Western blot, on the other hand, proves the specificity of this monoclonal antibody for hemagglutinin derived from type A viruses but indicates that the immunodominant epitope resides on the HA2 chain of the antigen that forms the stem region of the protein. Thus the receptor binding site of the HA1 chain[28] is largely accessible to the virus and this explains why mAb IVC102 fails to inhibit hemagglutination. The previous MS immunoassay results[20] have thus been able to identify a less dominant epitope of the HA1 chain and demonstrate this antibody's intramolecular cross reactivity.

CONCLUSIONS

The antigenicity of the influenza virus can be established and epitopes defined using a mass spectrometry immunoassay and that the results are largely in accord with those observed in HI assays. In contrast to the HI assay, the MS immunoassay offers the ability to gain insights into the antigenicity of the viral proteins from a structural perspective. It can identify the nature of the recognised antigenic determinant and is capable of detecting either linear or discontinuous epitopes where proteolysis

proceeds incubation of the antigens or whole virus with antibody. The ability to establish viral antigenicity and the primary structure of the viral antigens in a single analysis by mass spectrometry complements the methods presently used in the surveillance of circulating influenza strains in cases where a deeper molecular insight into antigenicity is required.

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