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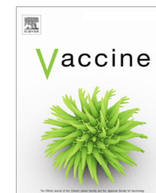

Proteomic analysis of influenza haemagglutinin-specific antibodies following vaccination reveals convergent immunoglobulin variable region signatures

Penelope J. Adamson [a,*], Mahmood A. Al Kindi [b,1], Jing J. Wang [b], Alex D. Colella [c], Timothy K. Chataway [c], Nikolai Petrovsky [d], Tom P. Gordon [b], David L. Gordon [a]

[a] Department of Microbiology and Infectious Diseases, Flinders University and SA Pathology, Flinders Medical Centre, Bedford Park, SA 5042, Australia
[b] Department of Immunology, Flinders University and SA Pathology, Flinders Medical Centre, Bedford Park, SA 5042, Australia
[c] Flinders Proteomic Facility, Flinders University, Flinders Medical Centre, Bedford Park, SA 5042, Australia
[d] Department of Endocrinology, Flinders University and Vaxine Pty Ltd., Flinders Medical Centre, Bedford Park, SA 5042, Australia

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ABSTRACT

Analysis of the anti-haemagglutinin serum antibody proteome from six H1N1pdm09 influenza A vaccinated subjects demonstrated restricted IgG1 heavy chain species encoded by IGHV5-51 and IGHV3-7 gene families in 2 subjects and either IGHV5-51 or IGHV3-7 in 4 individuals. All subjects exhibited a dominant IGKV3-20 light chain, however 5 subjects also exhibited IGKV3-11 and IGKV4-1 families. Sequences were closely aligned with the matched germline sequence, with few shared mutations. This study illustrates the feasibility of using a proteomic approach to determine the expressed V region signatures of serum antibodies induced by vaccination.

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1. Introduction

The propensity for influenza A viruses to evade the immune system remains a significant public health problem with annual seasonal outbreaks continuing despite widespread vaccination programs. While the viral genomic flexibility of influenza A (antigenic drift) allows immune evasion seasonally, occasionally there is a more significant recombination event between influenza strains (antigenic shift), potentially from different species, resulting in a pandemic, such as occurred in 2009 [1]. The 2009 swine origin H1N1 pandemic highlighted significant limitations in public health responses and vaccine production but also deficiencies in our understanding of detailed specific immune responses to infection and vaccination.

Currently vaccination is the principal means of preventing influenza A infection, however it requires accurate epidemiological prediction of circulating strains to be effective. Production of a broadly neutralising antibody response to influenza vaccination would negate the need to continually monitor and adjust vaccine formulations, saving both money and the need for annual vaccination. However, achieving this would require a better understanding of the immune response to influenza vaccination.

Recently, our understanding of the specific antibody response to infection has improved with the development of new techniques to examine antibody sequences at a more detailed level. We have moved from serological assays which provide limited information regarding presence of antibody, to recent advances including single cell sorting and high throughput DNA sequencing methods which allow systems wide interrogation of human B-cell repertoires [2–5]. These approaches provide important information at the level of the genome and are advantageous as they can provide complete antibody heavy (H)- and light (L)-chain pairing, including details of

Abbreviations: IgV, immunoglobulin variable; rHA, recombinant haemagglutinin; H, heavy; L, light; D, diversity; J, joining; K, kappa; IMGT, ImMunoGeneTics; CDR, complementarity determining region; BCR, B-cell receptor; PBMC, peripheral blood mononuclear cell.

* Corresponding author.
E-mail addresses: penelope.adamson@sa.gov.au (P.J. Adamson), dralkindi@squ.edu.om (M.A. Al Kindi), jing.wang@flinders.edu.au (J.J. Wang), Alexander.Colella@sa.gov.au (A.D. Colella), tim.chataway@flinders.edu.au (T.K. Chataway), nikolai.petrovsky@flinders.edu.au (N. Petrovsky), t.gordon@flinders.edu.au (T.P. Gordon), d.gordon@flinders.edu.au (D.L. Gordon).
[1] Present address: Department of Microbiology and Immunology, Sultan Qaboos University Hospital, Muscat 123, Oman.

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all variable (V), diversity (D) and joining (J) regions. However even as these methods are becoming more financially and technically viable, they may not necessarily reflect the actual antibodies being produced nor their relative quantities. A system which actually characterises and sequences the circulating antibodies responding to disease would be highly informative.

An alternative to genomic investigation is analysis of the serum proteome. Within our facility a novel proteomic methodology has been developed involving purification of antigen-specific immunoglobulin (Ig) followed by high resolution mass spectrometric sequencing to identify H- and L-chain V region signatures. This method has been used with success for the autoantibodies involved in systemic lupus erythematosus and Sjögren's syndrome [6]. Here we apply this proteomic approach to characterise the clonality, including expression of shared clonotypic antibodies, and repertoire properties of the response to recombinant influenza H1N1 haemagglutinin vaccination in six individuals at the level of the secreted (serum) proteome.

2. Materials and methods

2.1. Human samples

Archived sera, collected as part of a previous investigation in 2009 into the safety and immunogenicity of a novel pandemic recombinant haemagglutinin (rHA) vaccine (the FLU005 study) [7], was used in this study. Briefly, subjects received 45 μg rHA vaccine with or without Advax™ adjuvant on day 0 and at 3 weeks with sera collected on day 0 (baseline), week 3, week 6 and week 24. Baseline and week 3 sera were used for this study. Participant demographics are shown in Supplementary Table 1. This study was approved by the Southern Adelaide Clinical Research Ethics Committee.

2.2. Purification of anti-H1 antibodies

rHA was kindly provided by Protein Sciences Corporation. The protein is a full length recombinant haemagglutinin derived from the 2009 influenza H1 strain produced in a baculovirus expression system in cultured insect cells.

Serum anti-rHA immunoglobulins were purified from ELISA plates (Maxi-Sorp: Nunc) coated with 1 μg/ml rHA antigen in phosphate buffered saline (PBS), pH 7.4 in a method adapted from Al Kindi et al. [8]. Briefly, coated plates were blocked with 1% BSA (Sigma) in PBS, incubated with serum from each subject diluted 1:25 v/v in PBS at 37 °C for 90 min, washed six times with PBS, and anti-H1 antibodies were eluted three times with 0.1 M glycine and 0.5 M NaCl, pH 2.3. The antibody elution was neutralised with 1 M Tris-HCl, pH 8.0 and anti-H1 antibodies concentrated using a 10000 MWCO Amicon Ultra-15 centrifugal filter (Millipore). The antibody was further concentrated with a 10000 MWCO Amicon Ultra-0.5 centrifugal filter (Millipore), then reduced with 10 mM DTT (Astral Scientific) and alkylated with 20 mM iodoacetamide (Biorad) before an estimated 20–40 ng H- and 10–20 ng L-chains were separated using SDS-PAGE (Any kD Mini-PROTEAN® TGX Stain Free™ Protein Gel; Biorad).

2.3. Mass spectrometry (MS)

In-gel trypsin digestion was performed on H- and L-chain bands excised from gels using Trypsin Gold (Promega) as described previously [8]. Tryptic peptides were separated on a C18 reversed phase column with a 45 min acetonitrile gradient using an Ekspert nanoLC 400 HPLC (Eksigent, AB Sciex) coupled to a TripleTOF 5600+ mass spectrometer (AB Sciex) running in positive ion mode, with rolling collision energy and dynamic accumulation enabled. Due to the small volume of sera available only two technical replicates were analysed for all samples except sample 242 which only had one.

2.4. Protein sequence data analysis

Mass spectrometric sequence data was analysed using Peaks Studio v7.5 software (Bioinformatics Solution Inc.) using the combined ImMunoGeneTics (IMGT (F + ORF + in-frame P) version 3.1.2; accessed 28th July 2014; http://www.imgt.org), NCBI (NCBI Human Database; accessed 12th September 2016) and Uniprot 2015–08 databases. A database of IGH rearrangements for IGHV3-7 and IGHV5-51, including published CDR3 sequences [2,4,9–12], was created and also used for analysis of the sequence data. The parameters for database matching have been described previously and include a maximum of 2 missed cleavages, precursor m/z tolerance of $\leq$10 ppm; product ion error tolerance of 0.02 Da; precursor charge state of +2 to +4 [8]. The database matching allows a peptide sequence containing 2 amino acid substitutions to match to germline sequence. If a peptide contains more than 2 substitutions it will only be matched if a string of at least 5 consecutive amino acids within the peptide matches a germline sequence. It is possible to determine the number of non-mutated/germline peptides present by counting the number of spectra matched to the germline. This is true also for peptides containing substitutions. If amino acid substitutions occur within the same peptide they come from a single IGH chain, however it cannot be determined using this approach if mutations within different peptides along the length of the IGH family sequence are within the same IGH chain. Matched germline and mutated peptides cannot come from a single antibody and can be regarded as comprising intraclonal variants that have been somatically selected from the germline species during antibody maturation. This method can be used to estimate the abundance of each peptide by spectral counts, however the contribution of the peptides to each clonotype cannot be estimated.

The approach used to assign gene families involved identifying unique peptides to individual V-region families with an in-house programme (unpublished) using the IMGT database. The in-house software uses the BLAST algorithm to identify the presence of unique peptides for gene families. Peptides identified as unique were then confirmed manually using the PEAKS software. After confirmation of unique peptides, supporting peptides were identified and matched to the gene family sequence providing a more complete map of sequence information for each gene family.

3. Results

3.1. Serum anti-H1 antibodies express convergent IgV peptide signatures

Mass spectrometric sequence analysis was performed on in-gel tryptic digests of H- and L-chains of purified anti-rHA antibody from 6 subjects of the rHA vaccine trial. The haemagglutination inhibition (HI) assay results for the baseline sera from these subjects were low (Supplementary Table 1) and this was reflected in the rHA ELISA which showed low reactivity in the 2 samples (087 and 207) that were tested. Despite this low reactivity, affinity purification was attempted, however no immunoglobulin variable (IgV) region peptide signatures could be obtained for baseline sera (data not shown). Analysis of week 3 sera showed a common usage of either IGHV5-51 or IGHV3-7 H-chain species in 4 of 6 subjects, or both as seen in 2 subjects (Table 1, Supplementary Fig. 1), however none of the published HCDR3 sequences were represented in

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