Kent Academic Repository

Full text document (pdf)

Citation for published version

Molesti, Eleonora and Ferrara, Francesca and Lapini, Giulia and Montomoli, Emanuele and Temperton, Nigel J. (2014) Discordant Correlation between Serological Assays Observed When Measuring Heterosubtypic Responses against Avian Influenza H5 and H7 Viruses in Unexposed Individuals. In: International Meeting on Emerging Diseases and Surveillance (IMED 2014), 31 Oct - 03

DOI

Poster 22.028

Link to record in KAR

https://kar.kent.ac.uk/43720/

Document Version

Presentation

Copyright & reuse

Content in the Kent Academic Repository is made available for research purposes. Unless otherwise stated all content is protected by copyright and in the absence of an open licence (eg Creative Commons), permissions for further reuse of content should be sought from the publisher, author or other copyright holder.

Versions of research

The version in the Kent Academic Repository may differ from the final published version. Users are advised to check **http://kar.kent.ac.uk** for the status of the paper. **Users should always cite the published version of record.**

Enquiries

For any further enquiries regarding the licence status of this document, please contact: **researchsupport@kent.ac.uk**

If you believe this document infringes copyright then please contact the KAR admin team with the take-down information provided at **http://kar.kent.ac.uk/contact.html**

KAR Kent Academic Repository

University of Kent

University of Kent

Discordant Correlation between Serological Assays Observed When Measuring Heterosubtypic Responses against Avian Influenza H5 and H7 Viruses in Unexposed Individuals

Eleonora Molesti[1], Francesca Ferrara[1], Giulia Lapini[2], Emanuele Montomoli[2] and Nigel Temperton[1]

[1]Viral Pseudotype Unit (Medway), School of Pharmacy, University of Kent, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK
[2]Department of Molecular and Developmental Medicine, University of Siena, Via Aldo Moro 3, 53100 Siena, Italy

the UNIVERSITY of GREENWICH

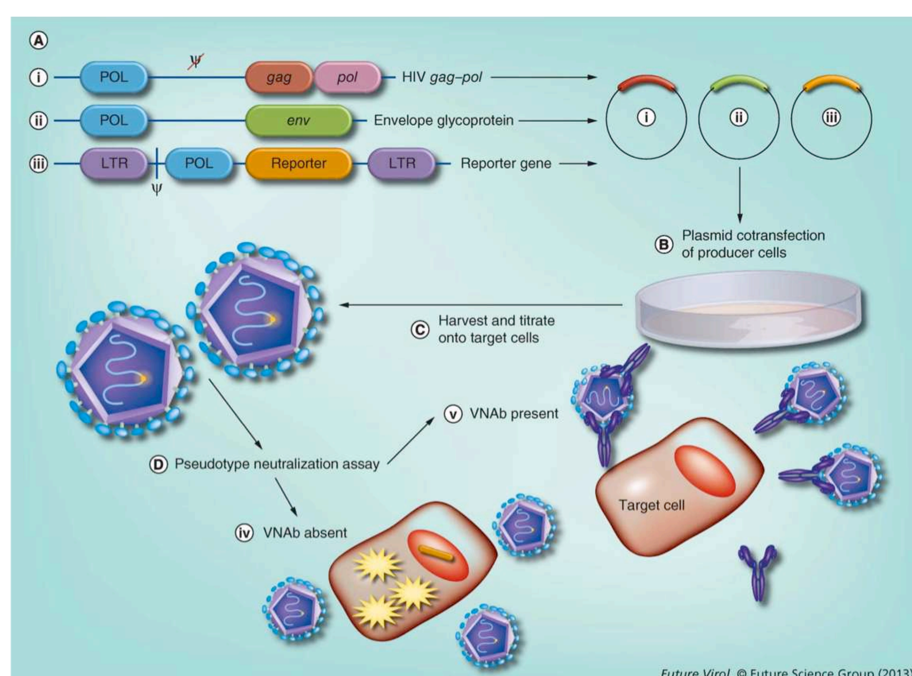
ABSTRACT

The human population is constantly exposed to multiple influenza A subtypes due to zoonotic spillover and rapid viral evolution driven by intrinsic error-prone replication and immunological pressure. In this context, antibody responses directed against the HA protein are of importance since they have been shown to correlate with protective immunity. Serological techniques, detecting these responses, play a critical role for influenza surveillance, vaccine development, and assessment. As the recent human pandemics and avian influenza outbreaks have demonstrated, there is an urgent need to be better prepared to assess the contribution of the antibody response to protection against newly emerged viruses and to evaluate the extent of pre-existing heterosubtypic immunity in populations. In this study, 68 serum samples collected from the Italian population between 1992 and 2007 were found to be positive for antibodies against H5N1 as determined by single radial hemolysis (SRH), but most were negative when evaluated using haemagglutination inhibition (HI) and microneutralisation (MN) assays. As a result of these discordant serological findings, the increased sensitivity of lentiviral pseudotypes was exploited in pseudotype-based neutralisation (pp-NT) assays and the results obtained provide further insight into the complex nature of humoral immunity against influenza A viruses.

MATERIALS AND METHODS

Serum Samples: A panel of human sera was provided by the Department of Molecular and Developmental Medicine, University of Siena. It consisted of 68 sera collected from Italian subjects (age between 6m and 92yr) from 1992 to 2007 that were previously found to be positive against HPAI H5N1 A/Vietnam/1194/2004 as determined by the SRH assay. The sero-positivity of these sera was also confirmed by removing nonspecific antibodies by their adsorption with a 1:1 volume mixture of A/New Caledonia/20/1999 H1N1 and A/California/7/2004 H3N2 viruses. The positive controls used in pp-NT assays were reference sheep sera against A/England/427/1988 (H3N2) and NIBRG-14 (H5N1) provided by the National Institute for Biological Standards and Control (NIBSC, United Kingdom), avian sera against A/African starling/England/983/1979 (H7N1) provided by the Animal Health and Veterinary Laboratories Agency (AHVLA), and monoclonal antihuman influenza A (H1N1, H2N2) antibody (mAb C179) (Takara, Clonotech).

Pseudotype Construction and Firefly Luciferase pp-NT Assays: Lentiviral pseudotypes with HA glycoproteins derived from HPAI virus A/Vietnam/1194/2004 (H5N1), A/Indonesia/5/2005 (H5N1), and HPAI virus A/chicken/Italy/13474/1999 (H7N1) were produced by cotransfection of 293T/17 cells with the respective HA plasmid (pI.18 backbone), the HIV gag-pol plasmid p8.91, and the reporter plasmid pCSFLW (expressing Firefly luciferase) using the Fugene-6 transfection reagent. Neuraminidase activity was provided by exogenous bacterial NA addition for the release of pseudotypes from producer cells. Serum samples (2.5 μL) were twofold serially diluted in culture medium and mixed with pseudotype virus (10e6 (RLU) luciferase input) at a 1:1 v/v ratio. After incubation at 37 °C for 1hr, 1e4 293T/17 cells were added to each well of a white 96-well flat bottomed tissue culture plate. RLU were evaluated 48hr later by luminometry using the Bright-Glo assay system (Promega, UK). End-point neutralizing antibody titres were calculated using GraphPad Prism 6. IC50 pseudotype neutralization titres are expressed as the reciprocal of the serum dilution that results in a 50% inhibition of pseudotype virus entry.



Production and neutralization of pseudotype viruses

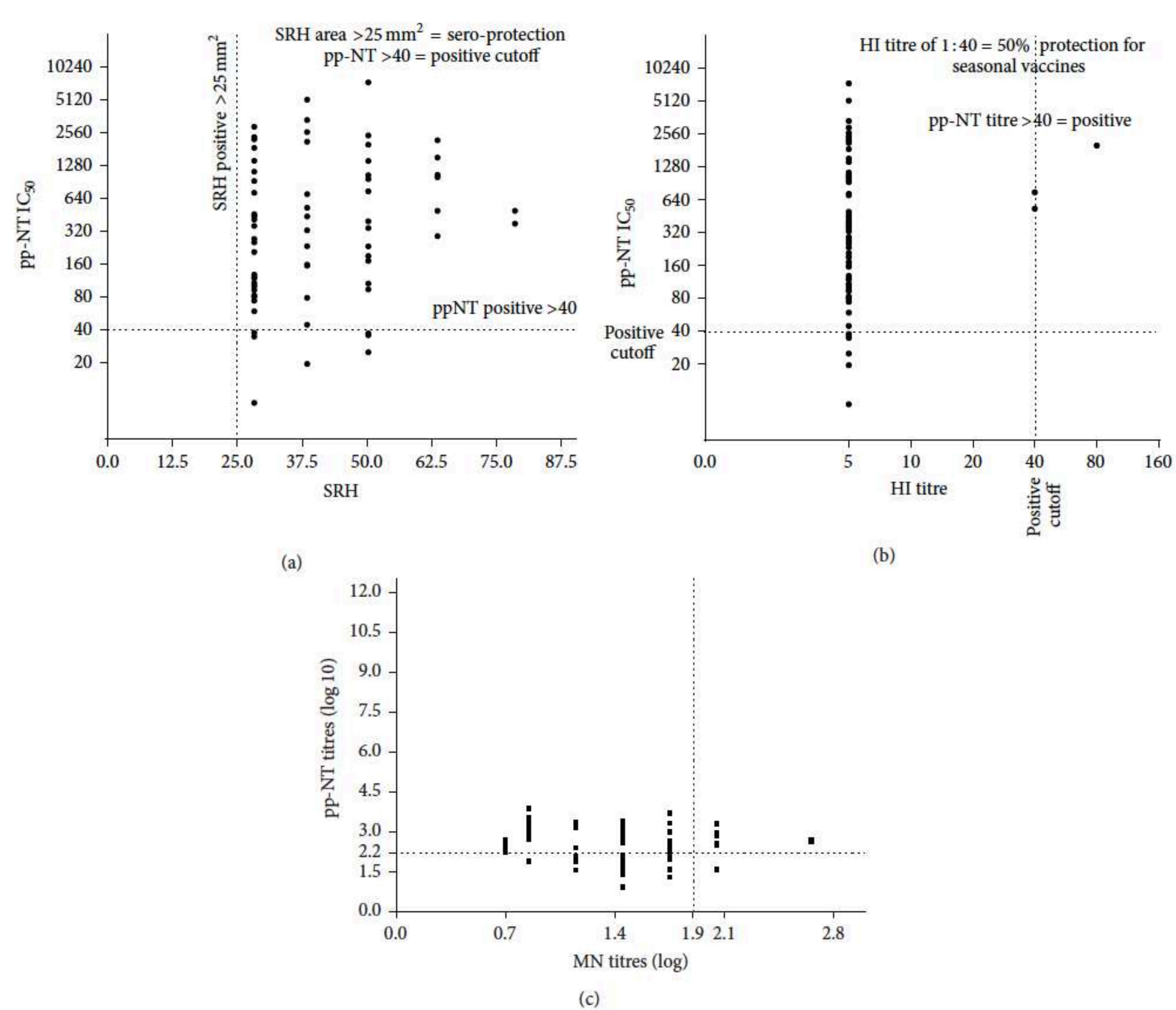
1. HIV gag-pol, envelope glycoprotein (HA) and reporter construct transfected into HEK293T/17 producer cells
2. 48 hours post-transfection, supernatant is harvested prior to titration onto susceptible target cells
3. Pseudotypes can be employed as surrogate viruses in virus neutralisation assays (pp-NT)

Single Radial Haemolysis (SRH) Assays: For the SRH assays, agarose immuno-plates were prepared with sheep and turkey erythrocytes 10% (v/v of assay buffer) sensitized by inactivated whole virus (antigens used: A/Vietnam/1194/2004 and A/Indonesia/5/2005) and with the addition of 5% guineapig complement. The amount of antigen was diluted in PBS to reach a final concentration of 2000 HU/mL. The size of the haemolysis zone around the well containing the serum is measured in mm and the diameter of haemolysis is then transformed in area (mm2) by using a Transidyne Calibrating Viewer.

Inhibition of Haemagglutination (HI) Assays: The haemagglutination inhibition (HI) assay was performed according to WHO recommendations using whole inactivated virus for the H5N1 strains: A/Vietnam/1194/2004 and A/Indonesia/5/2005.

Microneutralization (MN) Assays: Microneutralization (MN) assays were performed using wildtype H5N1 A/Indonesia/5/2005 (homologous strain of subclade 2.1 provided by CDC, Atlanta, GA) and wildtype H5N1 A/Vietnam/1194/2004 (homologous strain of subclade 1 provided by the CDC).

RESULTS



(a) Comparison of SRH assay versus pp-NT assay for antibody responses against the A/Vietnam/1194/2004 antigen. SRH titres expressed as diameter of haemolysis (in mm²) are plotted on the X-axis. SRH titres > 25mm2 are considered to be sero-protective and this cutoff is represented on the plot by a vertical dotted line. IC50 pseudotype neutralization titres expressed as the reciprocal of the serum dilution that results in a 50% inhibition of pseudotype virus entry are plotted on the Y-axis; (b) comparison of HI assay versus pp-NT assay for antibody responses against the A/Vietnam/1194/2004 antigen. HI titres expressed as the reciprocal of the highest dilution causing complete inhibition of haemagglutination are plotted on the X-axis. IC50 pseudotype neutralization titres expressed as the reciprocal of the serum dilution that results in a 50% inhibition of pseudotype virus entry are plotted on the Y-axis; (c) comparison of MN assay versus pseudotype based neutralization assay for antibody responses against the A/Vietnam/1194/2004 antigen. The vertical dashed lines indicate the value of MN log 10 titre = 1.9 (corresponding to a titre of 1:80) and the proposed threshold of protective antibodies; horizontal dashed line indicates the corresponding value of pp-NT log 10 titre = 2.55 (corresponding to a titre of 1:357 as calculated by Alberini *et al*, 2009).

CONCLUSIONS

1. It is postulated that this highly sensitive pseudotype neutralization assay is measuring broadly specific anti-stalk antibodies that have been elicited via exposure to seasonal H1 and H3 viruses and/or antibodies that recognize highly conserved sequences located underneath the RBS of individual subtypes.
2. The HI data shows that the antibody responses measured in these individuals are unlikely to be directed against the globular head.
4. No wild-type virus culture is necessary as the serological assay is entirely synthetic.
5. The influenza pseudotype assay is useful as an adjunct to the EMA/FDA approved serology assays, HI and SRH. It is a functional assay and quantifies neutralizing antibody responses against both the HA head (HA1) and stalk (HA2).
6. Pseudotype neutralization assays may prove easier to standardize as do not rely on erythrocytes which contribute to the high inter-laboratory variability of HI.
7. Compared to the routinely run HI, this assay is both “serum-sparing” and “antigen-sparing” and “bio-safe” (containment level 1 or 2).
8. Assay is straightforward to scale-up for large sample sizes making it ideal for sero-epidemiological studies.

REFERENCES

Overview of Serological Techniques for Influenza Vaccine Evaluation: Past, Present and Future.
Trombetta CM, Perini D, Mather S, Temperton N, Montomoli E. Vaccines 2014, 2, 707-734.

Discordant correlation between serological assays observed when measuring heterosubtypic responses against avian influenza H5 and H7 viruses in unexposed individuals.
Molesti E, Ferrara F, Lapini G, Montomoli E, Temperton N. Biomed Res Int. 2014;2014:231365. doi:10.1155/2014/231365. Epub 2014 Jun 11.

Pseudoparticle neutralization is a reliable assay to measure immunity and cross-reactivity to H5N1 influenza viruses.
Alberini I, Del Tordello E, Fasolo A, Temperton NJ, Galli G, Gentile C, Montomoli E, Hilbert AK, Banzhoff A, Del Giudice G, Donnelly JJ, Rappuoli R, Capecchi B. Vaccine. 2009 Oct 9;27(43):5998-6003. doi: 10.1016/j.vaccine.2009.07.079. Epub 2009 Aug 7.

A sensitive retroviral pseudotype assay for influenza H5N1-neutralizing antibodies.
Temperton NJ, Hoschler K, Major D, Nicolson C, Manvell R, Hien VM, Ha do Q, de Jong M, Zambon M, Takeuchi Y, Weiss RA. Influenza Other Respir Viruses. 2007 May;1(3):105-12. doi: 10.1111/j.1750-2659.2007.00016.x.

Email: n.temperton@kent.ac.uk Web: www.viralpseudotypeunit.info Twitter: @ViralPseudotype