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Data Article

Dataset of antigenic distance measures, hemagglutination inhibition, viral lung titers, and weight loss in mice and ferrets when exposed to HA-based vaccination or sub-lethal A(H1) influenza infection

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In this data article, different antigenic distances measures are presented with matching immunological and virological data and is related to the research paper entitled, "Influenza hemag-

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glutinin antigenic distance measures capture trends in HAI differences and infection outcomes but are not suitable predictive tools". The data was collected from H1 influenza hemagglutinin-based vaccine studies in the mouse and ferret animal models. Mice were prime-boost-boost vaccinated with either wild-type or computationally optimized broadly reactive antigen (COBRA) HA-based virus-like-particles and then challenged with one of two challenge viruses. Serum was collected before challenge and tested for hemagglutination inhibition (HAI) titers for a panel of different H1 viruses isolated from different host species (human and swine). Viral lung titers and weight loss were recorded after challenge. Ferrets were sublethally infected with H1 viruses and serum was collected two weeks later for generation of HAI titers against a panel of H1 viruses. Antigenic distances measures ($p_{sequence}$, p_{HA1}, $p_{all\text{-}epitope}$, $p_{epitope}$) were determined for the vaccine/pre-immune strain compared to the HAI antigen/challenge strain. The antigenic distances were paired to the HAI titers in both mice and ferrets and then with the viral lung titers or weight loss in mice. Linear regression analysis was performed with HAI titer and with the change in HAI titer to the homologous match. Analysis was performed on different subsets of the data depending on viral host origin. These data can be used for future computational antigenic distance studies.

Specifications Table

Subject	Immunology
Specific subject area	Elicited antibody responses to influenza HA-based vaccination and pre-immunity
Type of data	Table Graph
How data were acquired	In vitro HAI assay, in vitro viral plaque assay on day 3, mouse weights as a percentage of day 0, protein sequence comparison
Data format	Raw Cleaned Filtered
Parameters for data collection	Only H1 influenza was analysed. Antigenic distances were determined for virus combinations available in the HAI assay and mouse challenge as long as a $p_{epitope}$ was obtainable. Serum was collected two weeks after the second boost or sub-lethal challenge for the maximum antibody elicitation. Lung viral titers and weights were recorded during each of their peaks, days 3 and 6, respectively.
Description of data collection	Antigenic distances were collected by comparing two H1 protein sequences and determining the differing number of amino acids per measurement. HAI titers were determined by measuring the minimum titer of RDE-treated sera needed to inhibit agglutination of turkey red blood cells by an antigen. Viral lung titers were determined by counting visible plaques after applying dilutions of lung homogenate to a confluent cell monolayer. Weights were recorded six days after challenge and subtracted from the pre-infection weight.
Data source location	Institution: University of Georgia City/Town/Region: Athens Country: United States
Data accessibility	Data was deposited in Mendeley Data Repository name: Dataset of antigenic distance measures, hemagglutination inhibition, viral lung titers, and weight loss in mice and ferrets when exposed HA-based vaccination or sub-lethal A(H1) influenza infection Data identification number: 10.17632/rssdph9527.1 Direct URL to data: https://data.mendeley.com/datasets/rssdph9527/draft?a=d1085b41-b72a-45ac-9243–544c8cb0b9a8
Related research article	Skarlupka AL, Handel A, Ross TM. Influenza hemagglutinin antigenic distance measures capture trends in HAI differences and infection outcomes, but are not suitable predictive tools [published online ahead of print, 2020 Jul 15]. Vaccine. 2020;S0264–410X(20)30,828–8. doi:10.1016/j.vaccine.2020.06.042

Value of the Data

- This data can be used for comparing the results of different methods used to calculate correlates of protection produced by different research groups and computing location- or technique-specific variation that may exist.
- Scientists across different fields ranging from immunology, vaccinology, computational biology and virology will benefit from this data.
- This data can be used to further investigate the virological and host-response differences between influenza viruses isolated from human versus swine origin.
- This raw data generated in both the mouse and ferret model can be used to further define and characterize the animal models used for human influenza vaccination and infection.
- Future antigenic distance or influenza protection modeling studies can incorporate and analyze the raw data.

1. Data description

1.1. Raw data: the original datafiles

- H1 Sequence Comparisons_20190519.xlsx
  - This file contains all the antigenic distance measures ($p_{sequence}$, p_{HA1}, $p_{all\text{-}epitope}$, and $p_{epitope}$)
- accession.xlsx
  - This file contains the viral strains with their corresponding HA protein accession number
- Challenge.csv
  - This file contains the average lung viral titers in average PFU/lung at day 3 post-challenge and weight retained (Day 6 wt / Day 0 wt *100) for mice post-challenge
- mouse_hai.csv
  - This file contains all the HAI titers for mice vaccinated with VLP + adjuvant after prime-boost-boost. The reciprocal of the last serum dilution to agglutinate 0.8% turkey erythrocytes is reported
- pepitope_hai_ferret
  - This file contains all the HAI titers for ferrets sub-lethally infected with live H1N1 influenza virus. The reciprocal of the last serum dilution to agglutinate 0.8% turkey erythrocytes is reported

Processing Code: The code used to clean the raw data

- Ferret.Rmd
  - This file contains the code combining antigenic distance measures from "H1 Sequence Comparisons_20190519.xlsx" to the HAI titers in "pepitope_hai_ferret"
- Mouse.Rmd
  - This file contains the code combining antigenic distance measures from "H1 Sequence Comparisons_20190519.xlsx" to the HAI titers in "mouse_hai", and the challenge data in "Challenge.csv"
- README.md
  - This file contains an explanation of the files in the Mendeley Repository

Processed Data: The cleaned datafiles from processed code

- ferret.rds
  - This file contains the cleaned datafile for the ferret dataset
- Mouse.rds
  - This file contains the cleaned datafile for the mouse dataset

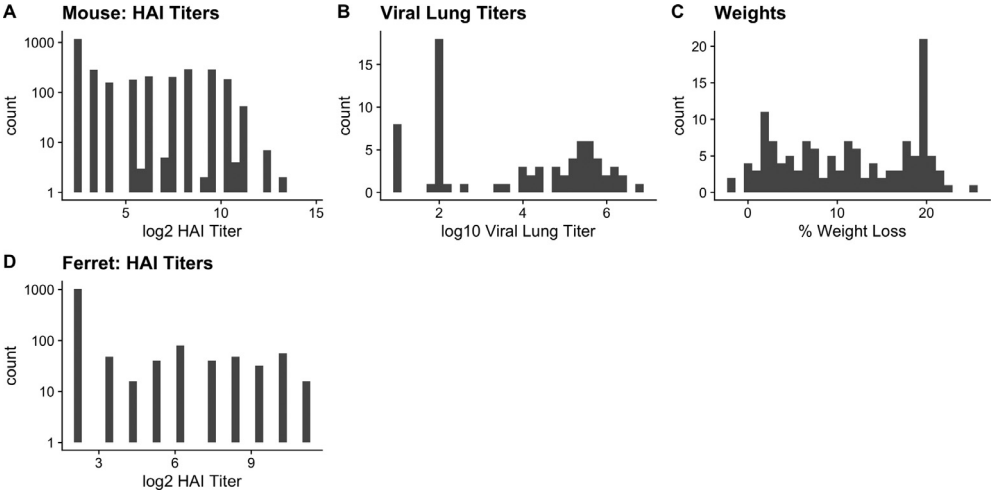

Fig. 1. Frequency distributions of the outcomes: mouse HAI titers (A), day 3 viral lung titers (B), day 6 percent weight loss (C), and ferret HAI titers (D).

Exploratory Code

- Exploratory.Rmd
  - This file contains the code that describes the distributions of different variables

Figures

- antigenic_distance_measures.tiff
  - This file is a histogram of the distribution of the antigenic measures
  - Fig. 2
- outcomes.tiff
  - This file is a histogram of the distribution of the HAI titers, and viral lung titers, and weight
  - Fig. 1

2. Experimental design, materials, and methods

2.1. Mouse vaccinations and infections

The mouse serological and virological data was generated previously [1]. Female BALB/c mice were maintained and housed according to USDA guidelines for laboratory animals. All procedures were approved by the University of Georgia Institutional Animal Care and Use Committee (IACUC) and conducted under AUP: #2016–02–011-Y3-A7 and in accordance with the Guide for the Care and Use of Laboratory Animals [2], Animal Welfare Act [3], and Biosafety in Microbiological and Biomedical Laboratories [4].

Prime-boost-boost vaccinations consisted of virus-like particles (VLPs) that expressed H1 hemagglutinin (HA) of human, swine or COBRA origin. All HA VLPs contained the same mismatched N3 neuraminidase from the avian-isolate A(H7N3)/mallard/Alberta/24/2001 and HIV GAG protein. Vaccine antigens were mixed in a 1:1 vol ratio with an MF59-like squalene oil-in-water adjuvant, and the vaccination was delivered in a volume of 100 μl. Mice were vaccinated in 4 week intervals, and serum samples, collected 54 days post-prime, were tested for HAI activity against a panel of H1 viruses.

Vaccinated mice were challenged with 5×10^4 plaque forming unit (PFU) ($10 \times 50\%$ lethal dose) A(H1N1)/California/07/2009 or 1×10^7 PFU A(H1N2)/swine/North Carolina/152,702/2015 in a volume of 50 μl. Mice were monitored daily for 14 days for weight loss (Fig. 1C), disease signs and death and were humanly euthanized if the cumulative clinical score exceeded 2 (lethargy=1, hunched posture=1, rough fur=1, weight loss 15%–20%=1, weight loss >20% of original body weight=3). Mice from each group ($n = 3$) were euthanized on day 3 post-infection for lung harvest. Lung tissue was snap frozen on dry ice, and stored at −80 °C for future viral titration.

2.2. Ferret infections

Immunological data of ferrets preimmunized through sub-lethal viral infection to A(H1N1)/California/07/2009, A(H1N1)/Brisbane/59/2007, or A(H1N1)/Singapore/6/1986 were obtained from previous publication [5, 6]. Briefly, fitch ferrets (*Mustela putorius furo*, female, 6 to 12 months, de-scented, Triple F Farms) were maintained and housed according to USDA guidelines. Ferrets ($n = 4$) were anesthetized and intranasally infected with one of the three H1N1 influenza viruses (10^6 PFU/ 1 ml). Animals were monitored daily during the infection for weight loss, loss of activity, nasal discharge, sneezing, and diarrhea and allowed to recover. All blood was harvested from anesthetized ferrets via the anterior vena cava at day 14 post-infection. Blood was transferred to a centrifuge tube and centrifuged at 6000 rpm. Clarified serum was collected, frozen at -20 ± 5 °C and used for HAI assays.

2.3. Hemagglutination inhibition (HAI) assay

The hemagglutination inhibition (HAI) assay was used to assess functional antibodies specific to the receptor binding site of the HA that inhibit the agglutination of turkey erythrocytes. The protocols were adapted from the WHO laboratory influenza surveillance manual [7] and were performed as previously described [8]. Sera was treated with receptor-destroying enzyme (RDE) (*Denka Seiken*, Co., Japan) resulting in a 1:10 dilution of sera. Briefly, three-parts RDE were added to one-part raw mouse or ferret sera and incubated overnight at 37 °C. After which, the RDE was inactivated by incubation at 56 °C for at least 30 min, followed by the addition of six-parts phosphate-buffered saline pH 7.2 (PBS, Gibco). For the HAI assay, RDE-treated sera were diluted in a series of two-fold dilutions in V-bottom microtiter plates. An equal volume of virus or virus-like particle, adjusted to approximately 8 hemagglutination units /50 μl, was added to each well. The plates were agitated, covered, and incubated at room temperature (RT) for 20 min. Then, 0.8% of turkey erythrocytes (RBCs; *Lampire Biologicals*, Pipersville, PA, USA) were added in a volume of 50 μl, agitated, and incubated at RT for 30 min. The HAI titer was recorded as the reciprocal dilution of the last well that agglutinated RBCs. Positive and negative serum controls were included for each antigen. All mice and ferrets were negative (HAI < 1:10) for preexisting antibodies to currently circulating human influenza viruses prior to vaccination or challenge. The limit of detection for $\log_2$ HAI titer was 3.32. If below the limit of detection, 2.32 $\log_2$ HAI titer was used for mathematical calculations. The distribution of the mouse and ferret HAI titers are shown in Fig. 1A, and Fig. 1D, respectively.

2.4. Viral lung titers

Plaque assay was performed according to previously described protocols [9]. In brief, lungs were homogenized in 1 ml Dulbecco's Modified Eagle Medium (DMEM), and the supernatant was collected by spinning the homogenized samples at 2000 rpm for 5 min. Confluent Madin-Darby Canine Kidney (MDCK) in a 6-well plate were infected with different dilutions of lung supernatent in 100 μL of DMEM supplemented with penicillin-streptomycin. After 1 h incubation at RT,

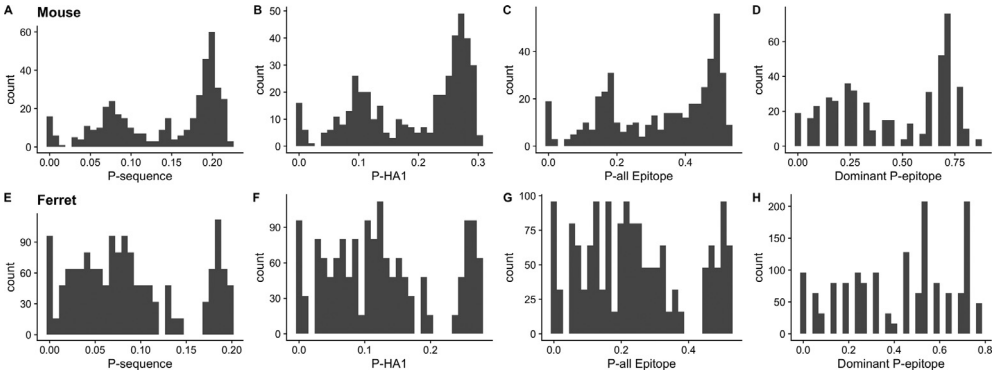

Fig. 2. Frequency distributions of the antigenic distance measures: $p_{sequence}$ (A), p_{HA1} (B), $p_{all\text{-}epitope}$ (C), and $p_{epitope}$ (D).

the medium was removed, and cells were washed. After the addition of 2 mL of Modified Eagle Medium (MEM) medium at 2 μg/mL TPCK-trypsin and 0.8% agarose (*Cambrex*, East Rutherford, NJ, USA), cells were incubated for 72 h at 37 °C with 5% CO_2. Agarose was removed, and the cells were fixed with 10% buffered formalin and stained with 1% crystal violet (*Fisher Science Education*) for 15 min to visualize the plaques. Each sample was plaqued in duplicate. Duplicates were then averaged and transformed by $\log_{10}$. The virus titer was analyzed as the average $\log_{10}$ PFU/lung for each individual mouse (Fig. 1B).

2.5. Amino acid based antigenic distance measure (ADM) calculation

The five antigenic sites used in the calculation of the H1 HA subtype antigenic distance were outlined previously and include residues predicted to be related to antibody neutralization [10, 11]. Pandemic H1 HAs contain an extra amino acid residue at site 130. Therefore, the A(H1N1)/California/04/2009 numbering scheme was used with a maximum length of 549 residues for the HA0 with a gap at 130 for the seasonal H1 HAs [11]. The numbering scheme begins after the seventeen amino acids in the H1 HA signal peptide [12-14]. The HA1 region was defined as amino acids 1–327. The following equations were used to calculate the HA antigenic distances between two virus strains using the sites defined previously [1]:

$$p_{sequence} = \frac{number\ of\ substitutions\ in\ entire\ HA\ sequence}{total\ number\ of\ amino\ acids\ in\ entire\ HA\ sequence}$$

$$p_{HA1} = \frac{number\ of\ substitutions\ in\ HA1}{total\ number\ of\ amino\ acids\ in\ HA1}$$

$$p_{all-epitope} = \frac{number\ of\ substitutions\ in\ all\ epitopes}{total\ number\ of\ amino\ acids\ in\ all\ epitopes}$$

$$p_{epitope\ x} = \frac{number\ of\ substitutions\ in\ epitope\ x}{total\ number\ of\ amino\ acids\ in\ epitope\ x}$$

$$p_{epitope} = max\ p_{epitope\ x}$$

ADM were calculated from alignments of amino acid sequences. Alignments were done using Geneious software (v11.1.5). The HA0 sequences were aligned by global alignment with free end gaps and a Blosum62 cost matrix with two refinement iterations, and these alignments were used for the $p_{sequence}$ ADMs. From the HA0 alignment, the HA1 domains (1–327AA) were extracted and used for the p_{HA1} ADMs. From the HA1 region, all or individual antigenic sites were

extracted and the $p_{all\text{-}epitope}$ and $p_{epitope}$ were calculated from the antigenic site alignments. The ADMs were determined for all pairs of vaccine and challenge combinations possible. Fig. 2 contains the frequency distribution of the various ADM.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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References

[1] A.L. Skarlupka, S.O. Owino, L.P. Suzuki-Williams, C.J. Crevar, D.M. Carter, T.M Ross, Computationally optimized broadly reactive vaccine based upon swine H1N1 influenza hemagglutinin sequences protects against both swine and human isolated viruses, Hum. Vaccin. Immunother. 15 (2019) 2013–2029.
[2] N.R. Council, Guide for the Care and Use of Laboratory Animals, 8 ed., The National Academies Press, Washington, DC, 2011.
[3] U. Congress, Transportation, sale, and handling of certain animals, Agriculture USDo, USDA, United States, 2015 editor. United States Code.
[4] J. Richmond, R. McKinney, D.C. Washington (Ed.), Government Printing Office, U.S., 1993.
[5] D.M. Carter, C.A. Darby, S.K. Johnson, M.A. Carlock, G.A. Kirchenbaum, J.D. Allen, et al., Elicitation of protective antibodies against a broad panel of H1N1 viruses in ferrets preimmune to historical H1N1 influenza viruses, J. Virol. 91 (2017).
[6] D.M. Carter, C.E. Bloom, E.J. Nascimento, E.T. Marques, J.K. Craigo, J.L. Cherry, et al., Sequential seasonal H1N1 influenza virus infections protect ferrets against novel 2009 H1N1 influenza virus, J. Virol. 87 (2013) 1400–1410.
[7] Organization W.H., Network W.G.I.S. Manual for the laboratory diagnosis and virological surveillance of influenza: world health organization; 2011.
[8] D.M. Carter, C.A. Darby, B.C. Lefoley, C.J. Crevar, T. Alefantis, R. Oomen, et al., Design and characterization of a computationally optimized broadly reactive hemagglutinin vaccine for H1N1 influenza viruses, J. Virol. 90 (2016) 4720–4734.
[9] C.R. Gaush, T.F. Smith, Replication and plaque assay of influenza virus in an established line of canine kidney cells, Appl. Microbiol. 16 (1968) 588–594.
[10] M.W. Deem, K. Pan, The epitope regions of H1-subtype influenza A, with application to vaccine efficacy, Protein Eng. Des. Sel. 22 (2009) 543–546.
[11] M.W. Deem, K.Y. Pan, The epitope regions of H1-subtype influenza A, with application to vaccine efficacy, Protein Eng. Des. Sel. 22 (2009) 543–546.
[12] G. Winter, S. Fields, G.G Brownlee, Nucleotide sequence of the haemagglutinin gene of a human influenza virus H1 subtype, Nature 292 (1981) 72–75.
[13] E. Nobusawa, T. Aoyama, H. Kato, Y. Suzuki, Y. Tateno, K Nakajima, Comparison of complete amino-acid-sequences and receptor-binding properties among 13 serotypes of hemagglutinins of influenza a-viruses, Virology 182 (1991) 475–485.
[14] D.F. Burke, D.J. Smith, A recommended numbering scheme for influenza A HA subtypes, PLoS ONE 9 (2014) e112302.