LKS Faculty of Medicine
HKU-Pasteur Research Pole
香港大學-巴斯德研究中心

Immune response to emerging viruses: how can we be protected?



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Croucher Summer Courses
Emerging Viral Infections
18 July 2025

Lecture Outline

1. Basic concepts
   - Different mechanisms of protection

2. Understanding immunological correlates of protection against avian influenza virus (AIV)
   - Vaccination
   - Human studies

3. Understanding the role of immunological memory
   - Imprinting

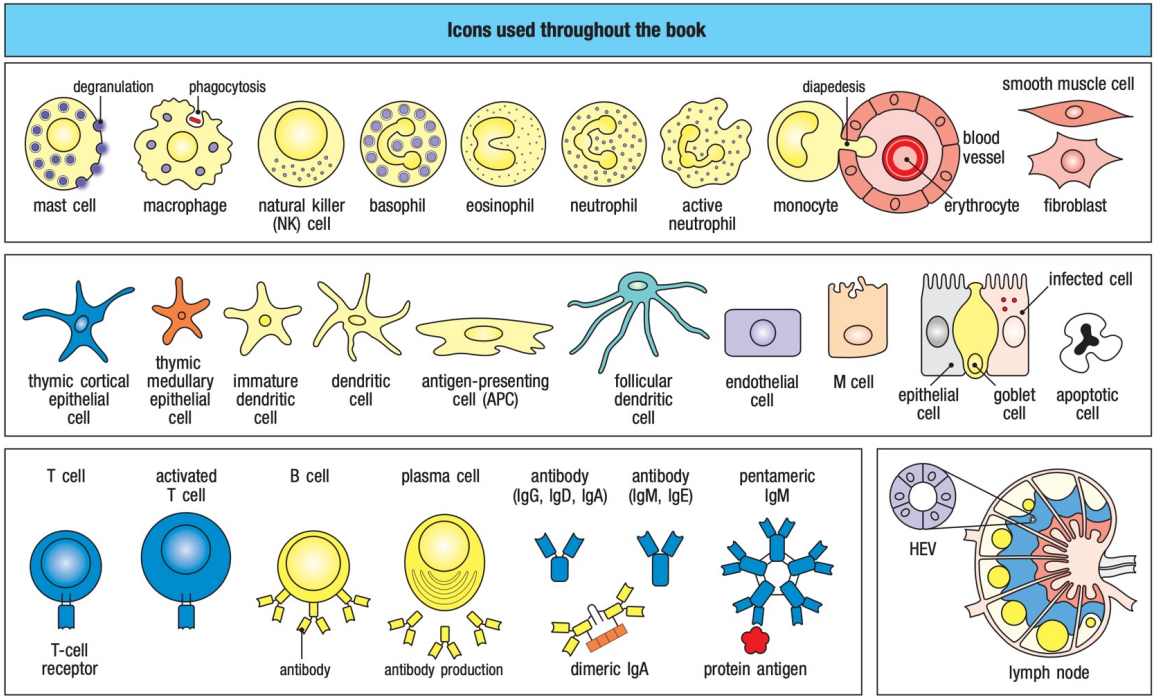
4. What is protecting us?
   - Occupational exposed individuals

Basic Concepts: Immunology

Innate

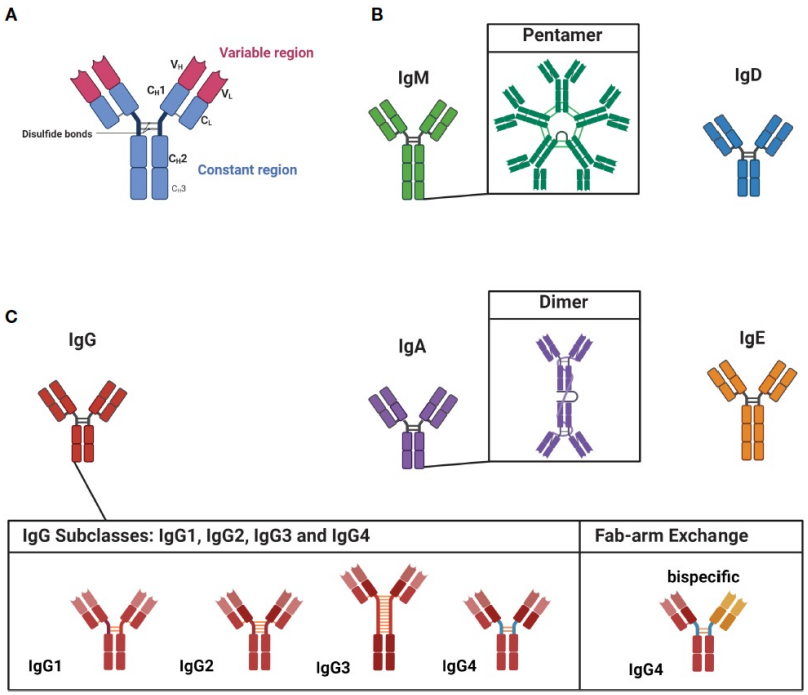
Supporting cells

Adaptive



Janeway 9th edition, Immunobiology

Immunoglobulins and Antibodies



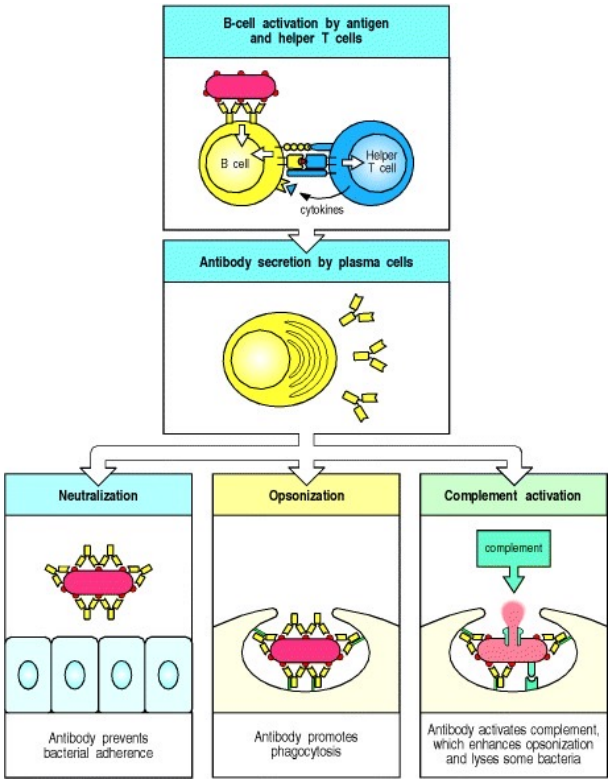
Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	+	–	++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activates complement system	+++	–	++	+	+++	–	+	–

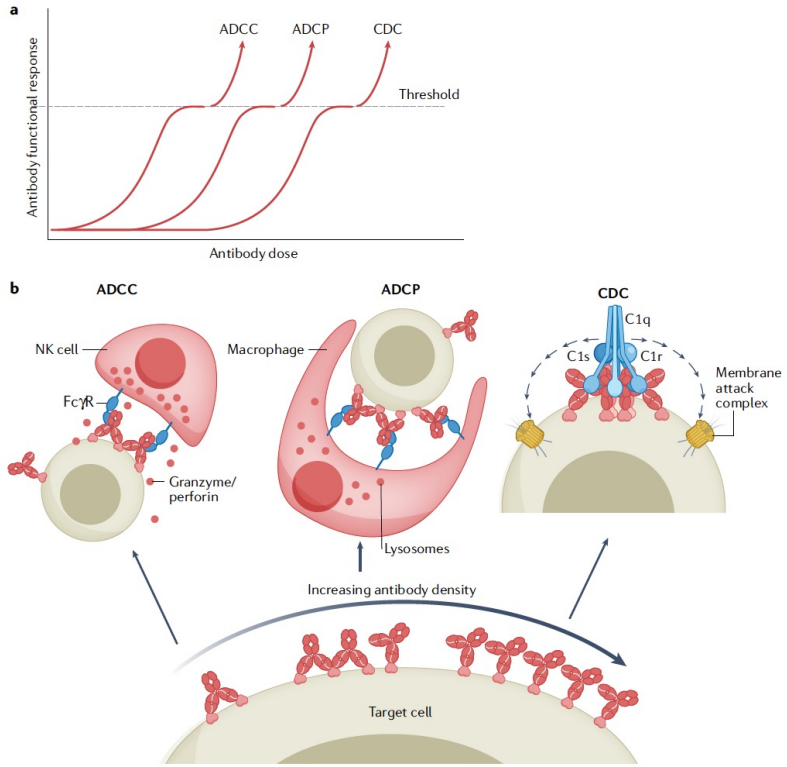
Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level ($mg \cdot ml^{-1}$)	1.5	0.04	9	3	1	0.5	2.1	3×10^{-5}

Exploring the depths of IgG4: insights into autoimmunity and novel treatments. Unlu et al., Frontiers Immunol, 2024

Janeway 9th edition, Immunobiology

How do antibodies protect during an infection?



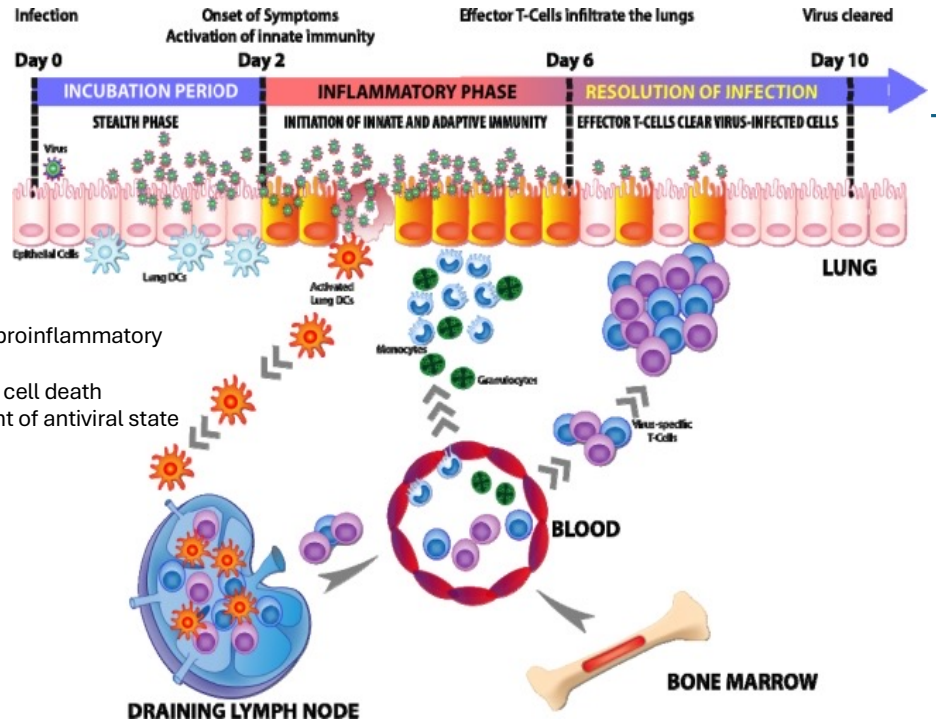


In general, induction of high affinity and avidity antibody responses are correlated with protection (SARS-CoV-2 studies).

Janeway 9th edition, Immunobiology

Oostindie et al., 2022, Nat Rev Drug Discovery

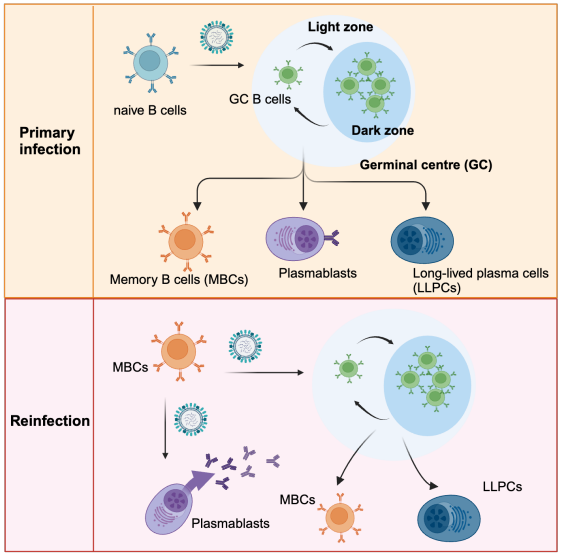
Immune response after virus infection



-Secretion of proinflammatory cytokines,
-Programmed cell death
-Establishment of antiviral state

Immune contraction of effector cells

Establishment of Immune Memory → **Determine susceptibility of future infections**



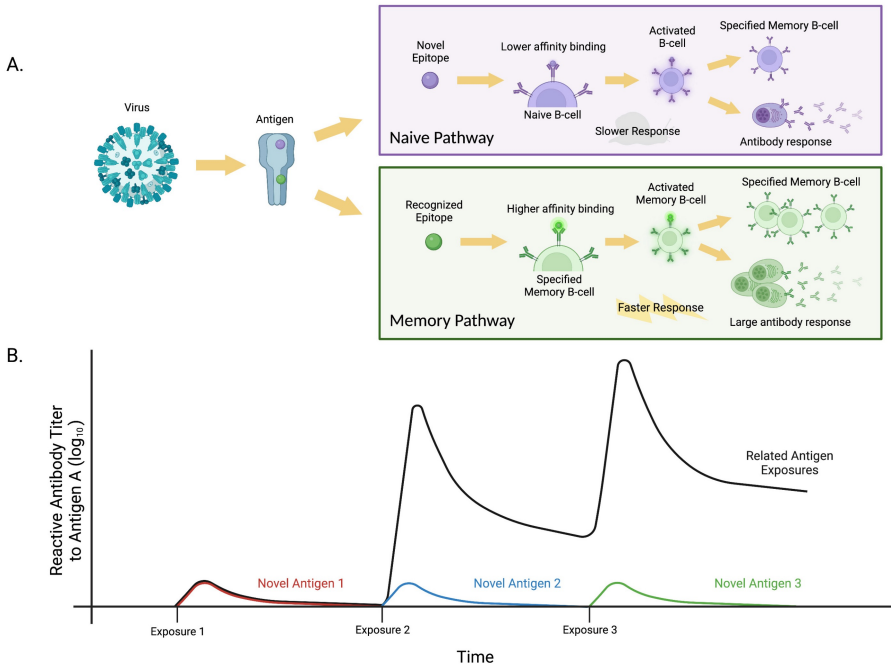
GC reaction is essential for production of high-affinity antibodies and memory B cells

Memory B cells are activated to conserved epitopes

Hermesh et al., Viruses, 2010

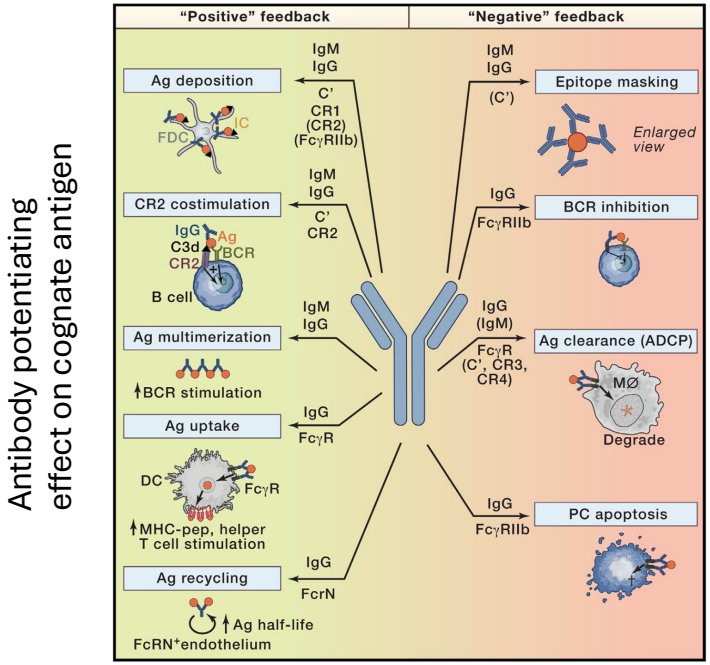
Immune imprinting and antibody feedback regulations

Imprinting is influence of first exposure



Zand et al., 2023, Pathogens 2023, 12(2), 169; https://doi.org/10.3390/pathogens12020169

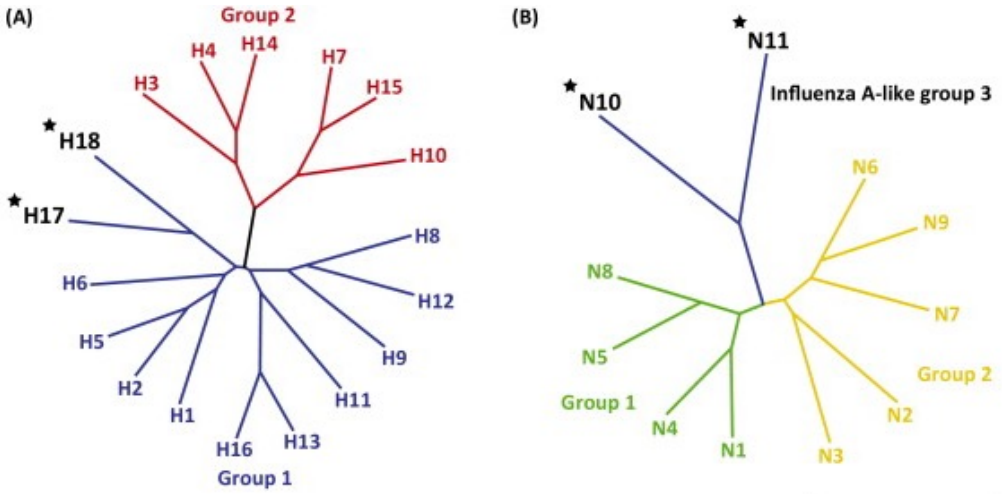
Antibody feedback regulation:



Cyster and Wilson, Immunity, 2024

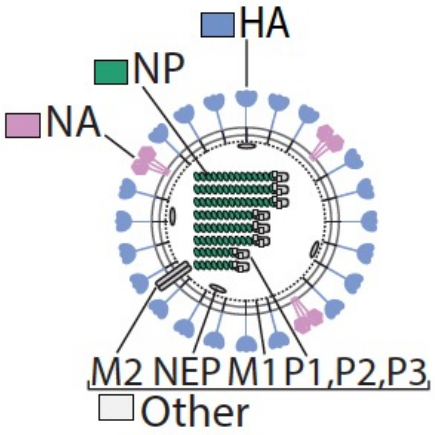
Influenza A virus classifications

Diversity of subtypes



Antibody Responses to Influenza Virus

Immunodominant antigens are viral surface glycoproteins



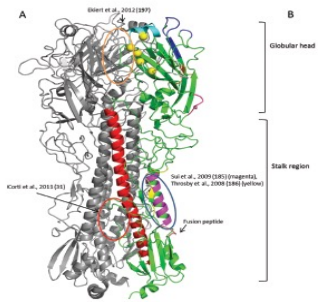
RNA virus = rapid evolution (antigenic drift)

Segmented genome = able to reassort (antigenic shifts)

Dugan et al, Sci Trans Med, 2020

HA

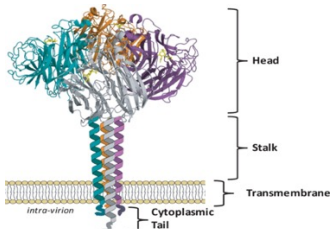
Detection assay



Hemagglutination-inhibition (HI)

ELISA, chimeric viruses

NA

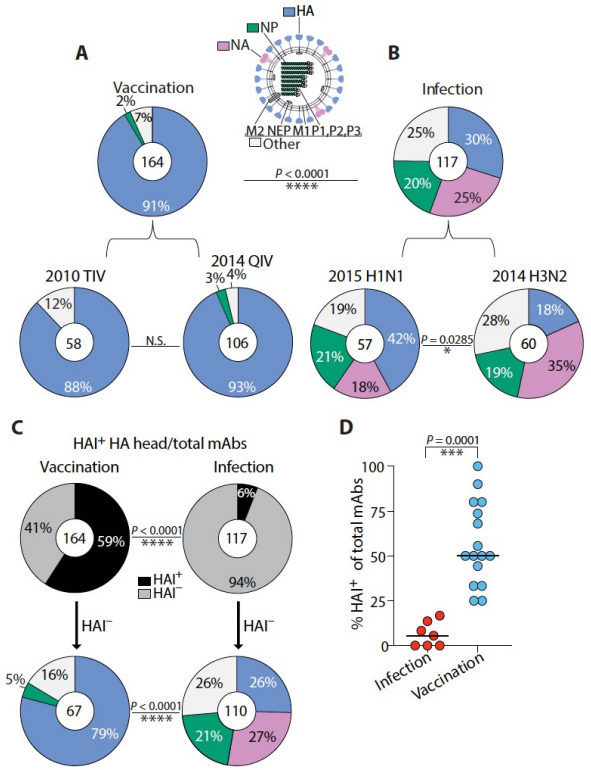


NA-inhibition: STAR/ MUNANA, Enzyme-linked lectin assay (ELLA)

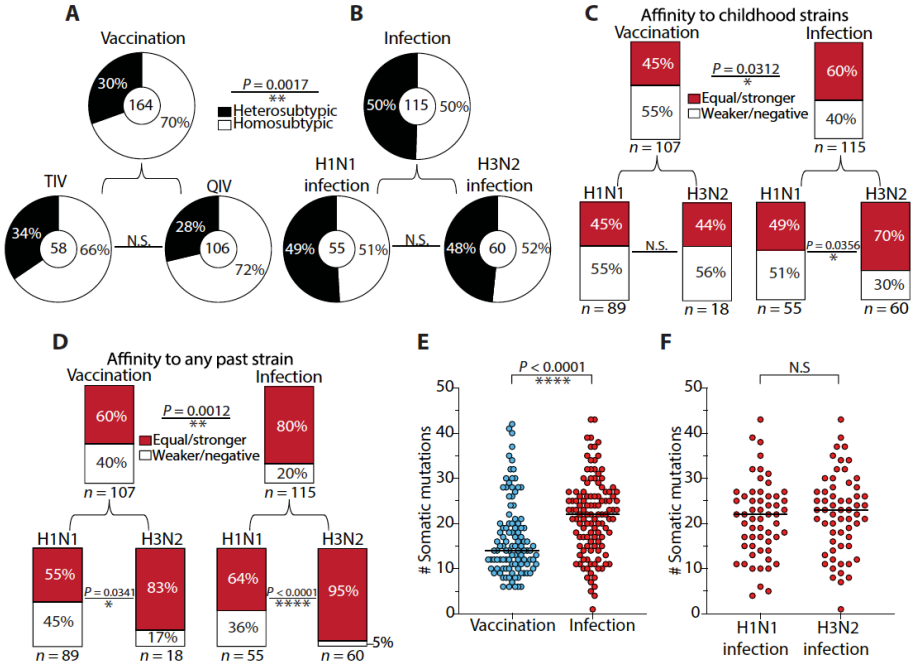
Other proteins: ELISA

Whole virus = virus neutralization assay

Antibody responses during infection vs vaccination



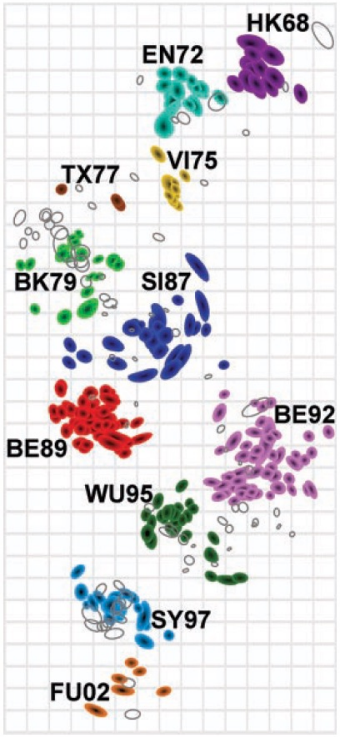
Infection results in diverse antibodies that are not dominated by HA/HAI responses

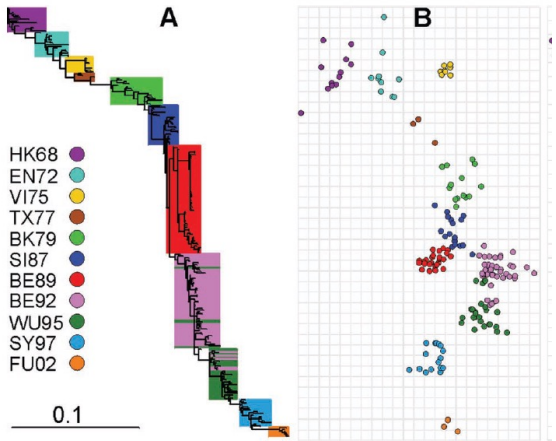


Infection preferentially recalls pre-existing B cells

Dugan et al., Sci Translational Medicine, 2020

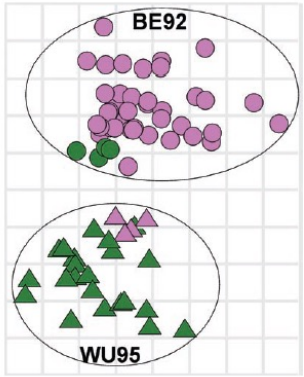
Genetic and antigenic evolution (variants)





Smith et al., Science 2004

Genetic changes do not always reflect antigenic changes



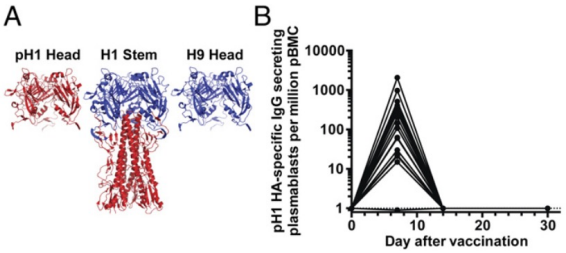
Antigenic Drift can be discordant between HA and NA

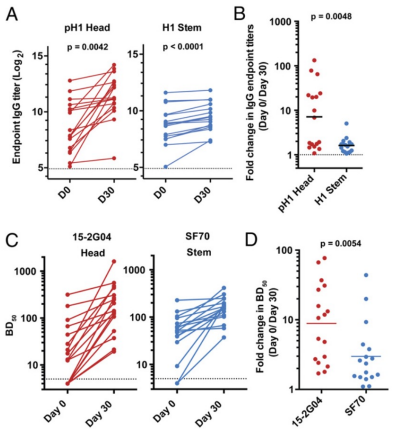
Antibodies targeting conserved epitopes can be preferentially recalled when immunodominant epitopes are novel

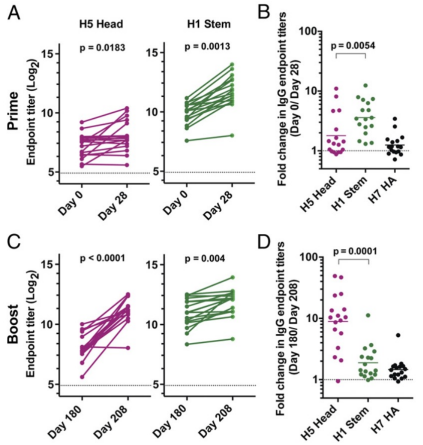
PNAS

Induction of broadly cross-reactive antibody responses to the influenza HA stem region following H5N1 vaccination in humans

Ali H. Ellebedy[a,b], Florian Krammer[c], Gui-Mei Li[a,b], Matthew S. Miller[c], Christopher Chiu[a,b,1], Jens Wrammert[a,d], Cathy Y. Chang[a,b], Carl W. Davis[a,b], Megan McCausland[a,b,2], Rivka Elbein[e], Srilatha Edupuganti[f], Paul Spearman[d], Sarah F. Andrews[g], Patrick C. Wilson[g], Adolfo García-Sastre[c,h,i], Mark J. Mulligan[f,j], Aneesh K. Mehta[e,f,j], Peter Palese[c,h], and Rafi Ahmed[a,b,3]







Antibodies can protect differently

HAI antibodies: prevent infection

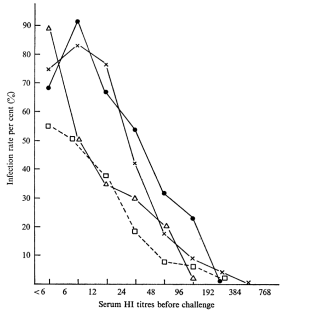


Fig. 6. The infection rate of volunteers challenged with various influenza viruses, in relation to their pre-challenge titres of homologous serum HI antibody.

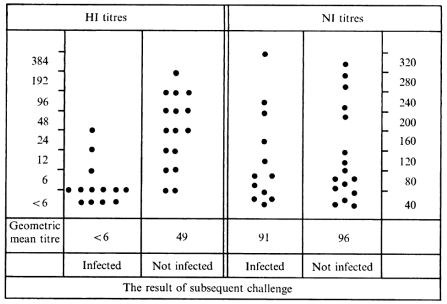
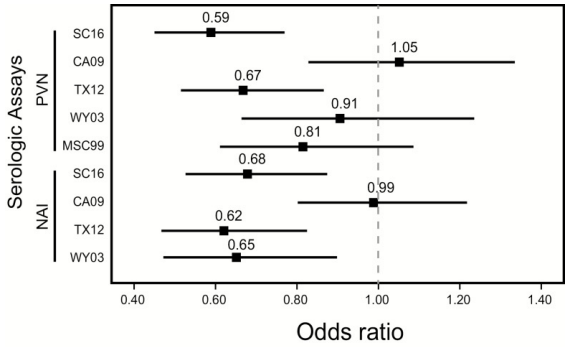


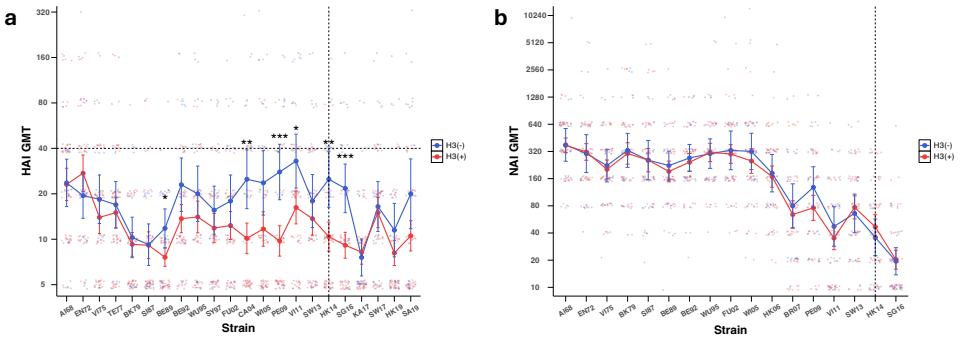
Fig. 4. The distribution of HI and NI serum antibodies in 29 volunteers before challenge infection with B/Eng/13/65 virus.

Hobson et al., 1972 J Hyg

NAI antibodies: prevent infection?

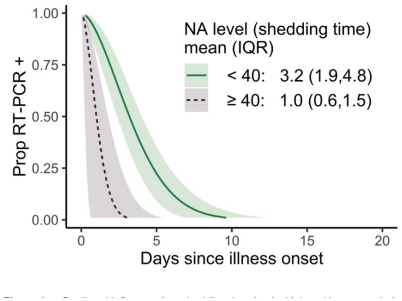


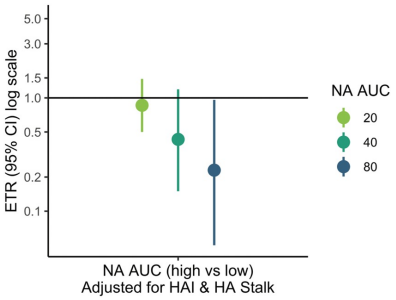
Weiss et al., CID, 2019



Issac Chow, Leung, Cowling

NAI antibodies: reduce viral shedding





Maier et al., CID, 2019

Role of T cells in protection

Influenza

ARTICLES

2012

nature medicine

Preexisting influenza-specific CD4$^+$ T cells correlate with disease protection against influenza challenge in humans

Tom M Wilkinson[1,6], Chris K F Li[2,6], Cecilia S C Chui[2], Arthur K Y Huang[2,3], Molly Perkins[4], Julia C Liebner[4], Rob Lambkin-Williams[5], Anthony Gilbert[5], John Oxford[5], Ben Nicholas[1], Karl J Staples[1], Tao Dong[2], Daniel C Douek[4], Andrew J McMichael[2] & Xiao-Ning Xu[2]

nature medicine

2013

Cellular immune correlates of protection against symptomatic pandemic influenza

Saranya Sridhar[1], Shaima Begom[1], Alison Bermingham[2], Katja Hoschler[2], Walt Adamson[3], William Carman[4], Thomas Bean[5], Wendy Barclay[6], Jonathan J Deeks[7] & Ajit Lalvani[1]

CD8+ T cells

Coronavirus (largely CD4 detected for technical reasons)

Article

Pre-existing polymerase-specific T cells expand in abortive seronegative SARS-CoV-2

https://doi.org/10.1038/s41586-021-04186-8
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Published online: 10 November 2021
Open access
Check for updates

Leo Swadling[1], Mariana O. Diniz[1,24], Nathalie M. Schmidt[1,24], Oliver E. Amin[1,24], Aneesh Chandran[1,24], Emily Shaw[1,24], Corinna Pade[2], Joseph M. Gibbons[2], Nina Le Bert[3], Anthony T. Tan[3], Anna Jeffery-Smith[1,2], Cedric C. S. Tan[4], Christine Y. L. Tham[3], Stephanie Kucykowicz[1], Gloryanne Aldoo-Micah[1], Joshua Rosenheim[1], Jessica Davies[1], Marina Johnson[5], Melanie P. Jensen[6,7], George Joy[6,8], Laura E. McCoy[1], Ana M. Valdes[9,10], Benjamin M. Chain[1], David Goldblatt[5], Daniel M. Altmann[11], Rosemary J. Boyton[12,13], Charlotte Manisty[6,8], Thomas A. Treibel[6,8], James C. Moon[6,8], COVIDsortium Investigators*, Lucy van Dorp[4], Francois Balloux[4], Áine McKnight[2], Mahdad Noursadeghi[1,24], Antonio Bertoletti[3,14,24] & Mala K. Maini[1]

CORONAVIRUS

Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans

Jose Mateus[1], Alba Grifoni[1], Alison Tarke[1], John Sidney[1], Sydney I. Ramirez[1,3], Jennifer M. Dan[1,3], Zoe C. Burger[3], Stephen A. Rawlings[3], Davey M. Smith[3], Elizabeth Phillips[2], Simon Mallal[2], Marshall Lammers[1], Paul Rubiro[1], Lorenzo Quiambao[1], Aaron Sutherland[1], Esther Dawen Yu[1], Ricardo da Silva Antunes[1], Jason Greenbaum[1], April Frazier[1], Alena J. Markmann[4], Lakshmanane Premkumar[5], Aravinda de Silva[5], Bjoern Peters[1,3], Shane Crotty[1,3], Alessandro Sette[1,3]*†, Daniela Weiskopf[1]*†

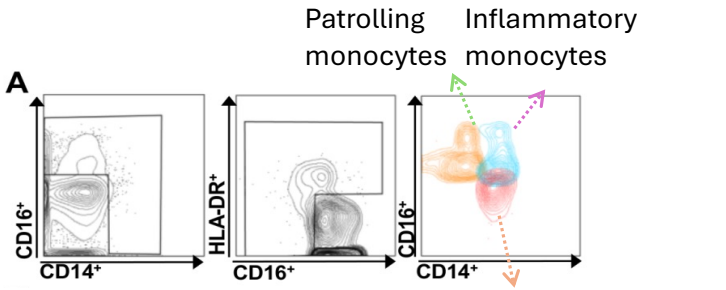
Correlates of protection to severe influenza (seasonal)

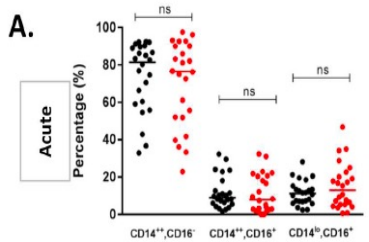
Acute Respiratory Illness (ARI) (N=24)
General Practitioners (GP)

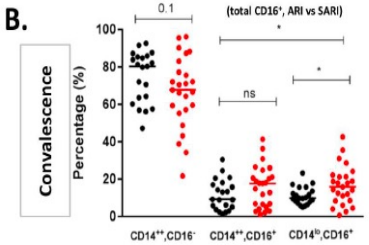
VS

Severe Acute Respiratory Illness (SARI) (N=25)

Auckland Hospitals

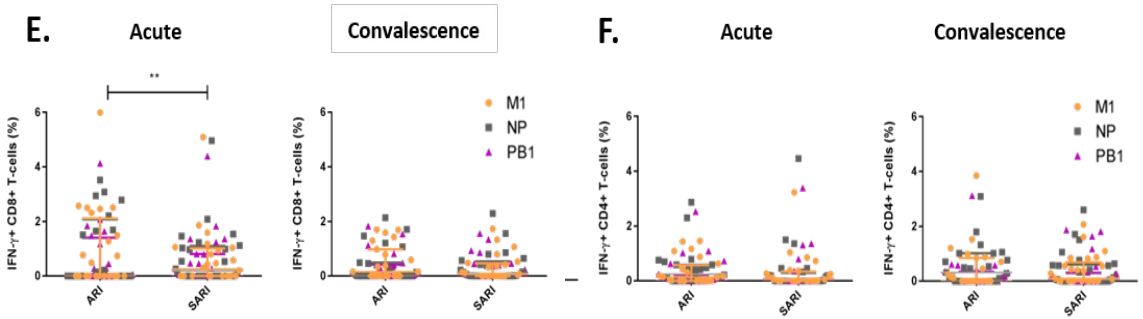






Severe cases have elevated CD16+ monocytes during recovery

Severe cases:
Does not return to baseline even a month after infection



Severe Influenza is characterized by prolonged immune activation: results from the SHIVERS Cohort Study. Wong et al., JID 2017

Severe cases have less antigen-specific CD8+ T-cells in the acute phase

Key takeaway

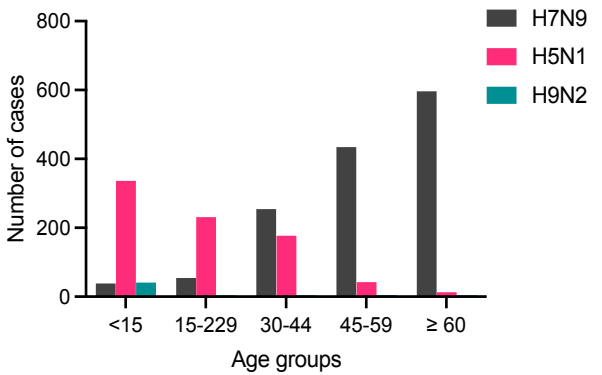
1. Efficient clearance of infection and recovery require coordinated engagement of all immune arms.
   - Early control reduces viral load. Uncontrolled replication overwhelms the host
   - Allows for adaptive immune system to kick in
2. Dysregulation of response causes pathogenesis.
   - Host factors: genetic susceptibility (IFITM genes)
   - Viral factors: viral proteins that inhibit IFN-pathways (NS1, ORF proteins)
3. Response during primary infection is straightforward but can get complicated during secondary exposure.
4. Different immune arms can mediate different infection outcome.
   1. Neutralizing antibodies target pathogen surface protein.
      - Potent ones can block infection (sterilizing immunity)

   2. T cell targets are often conserved internal peptides.
      - Need cells to be infected

What are the correlates of protection for avian influenza viruses?

Disease epidemiology for avian influenza virus

Subtype	Year of First Human Identification	Country with highest number of cases	No. Deaths/Cases	%
H7N7	1996	Netherlands	1/93	1.1
H5N1	1997	Egypt	462/881	52.4
H9N2	1998	China	1/58	1.7
H7N2	2002	United States	0/8	0
H7N3	2004	Canada	0/5	0
H10N7	2004	Egypt/Australia	0/4	0
H7N9	2013	China	616/1568	39.3
H6N1	2013	Taiwan	0/1	0
H10N8	2013	China	2/3	66.7
H5N6	2014	China	14/22	63.6
H7N4	2018	China	0/1	0



H7N9

1. H7N9 emerged in China in 2013.
2. Caused over 1500 cases between 2013 to 2019.
3. Approximately 30% CFR, mostly male, mean age: 55 years old

H5N1

1. 907 cases between 1997-2015, approximately 50% CFR.
2. Median age: 19 years, (IQR: 5 to 32) 41.2%: <15 yo, 80.3% : <35 yo

H9N2

1. >70 cases between 1998-2015,
2. >80% of clinical cases are < 8 yo

Ferret immunization and challenge studies



- Gold standard animal model

Vaccine 32 (2014) 4571–4577

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

A single dose of whole inactivated H7N9 influenza vaccine confers protection from severe disease but not infection in ferrets

Sook-San Wong[a], Trushar Jeevan[a], Lisa Kercher[b], Sun-Woo Yoon[a,c], Atanaska-Marinova Petkova[a], Jeri-Carol Crumpton[a], John Franks[a], Jennifer Debeauchamp[a], Adam Rubrum[a], Patrick Seiler[a], Scott Krauss[a], Robert Webster[a], Richard J. Webby[a,*]

MAJOR ARTICLE

npj | Vaccines

www.nature.com/n

ARTICLE OPEN

H5N1 influenza vaccine induces a less robust neutralizing antibody response than seasonal trivalent and H7N9 influenza vaccines

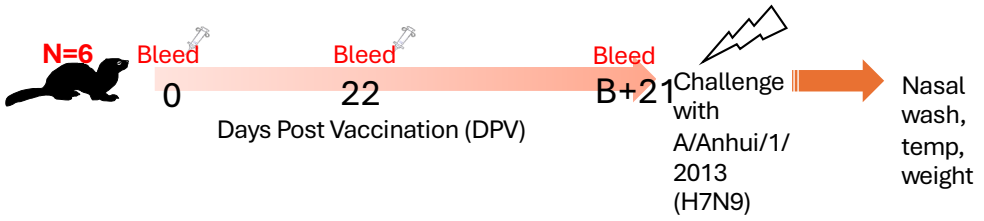
Sook-San Wong[1], Jennifer DeBeauchamp[1], Mark Zanin[1], Yilun Sun[2], Li Tang[2] and Richard Webby[1]

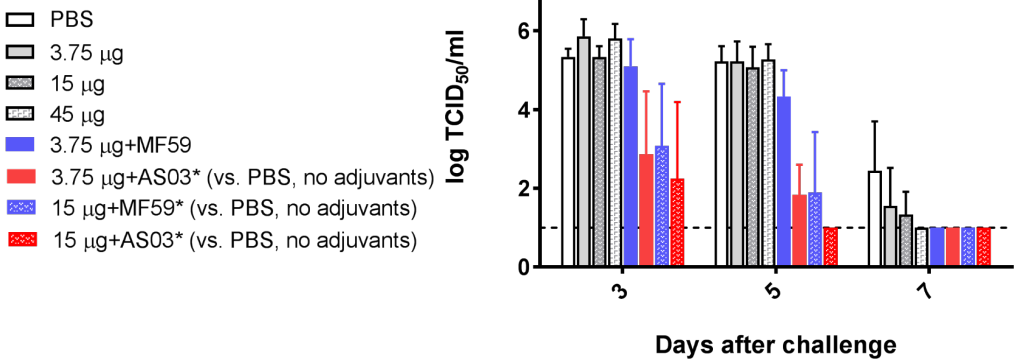
Impact of Adjuvants on the Immunogenicity and Efficacy of Split-Virion H7N9 Vaccine in Ferrets

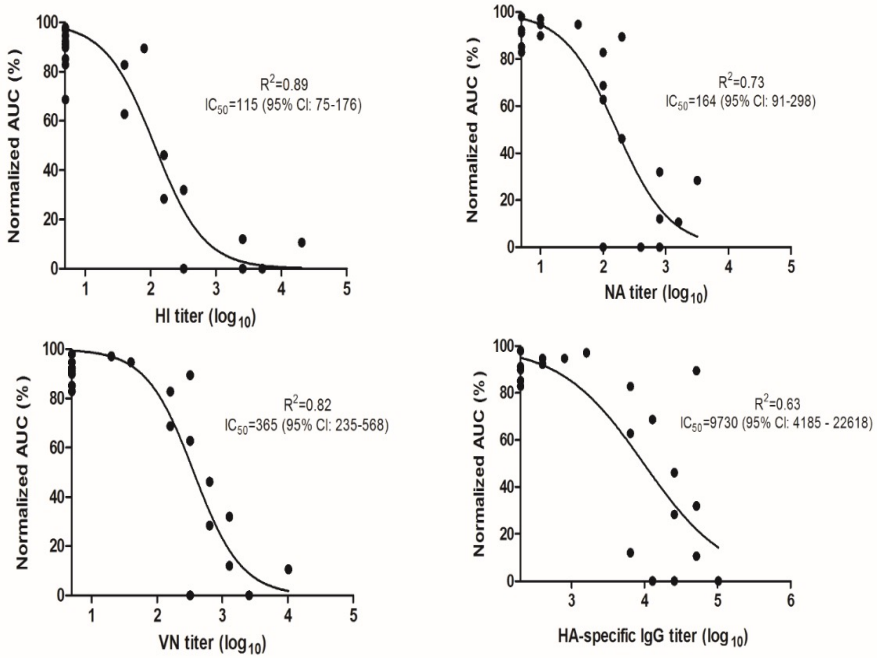
Sook-San Wong,[1] Bryan Kaplan,[1] Mark Zanin,[1] Jennifer Debeauchamp,[1] Lisa Kercher,[2] Jeri-Carol Crumpton,[1] Patrick Seiler,[1] Yilun Sun,[3] Li Tang,[3] Scott Krauss,[1] Robert Webster,[1] and Richard J. Webby[1]

[1]Department of Infectious Diseases, [2]Animal Resource Center, and [3]Department of Biostatistics, St Jude Children's Research Hospital, Memphis, Tennessee

Immunological correlates of protection against H7N9







IC_{50}:

HAI=NAI

VN:~ 2x HAI, NAI

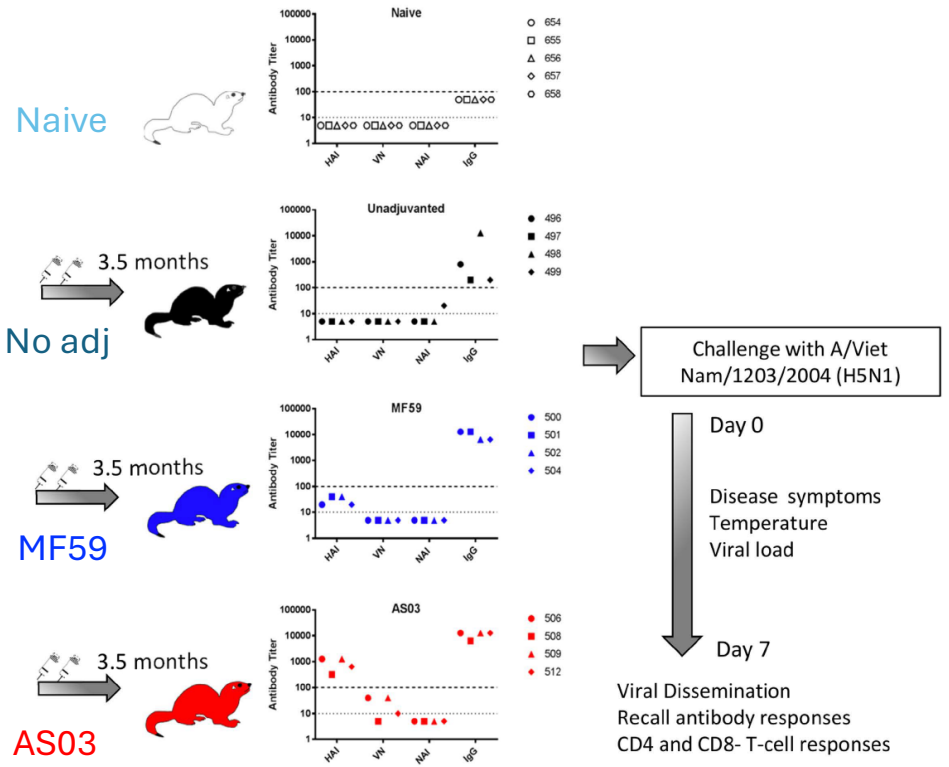
HA-IgG*= 10x HAI (IgG titers-poorest R^2)- does not reliably correlate with viral clearance in URT

Wong et al., J Inf Dis 2015

Influenza disease dynamics: animal model- HPAI H5N1 disease model

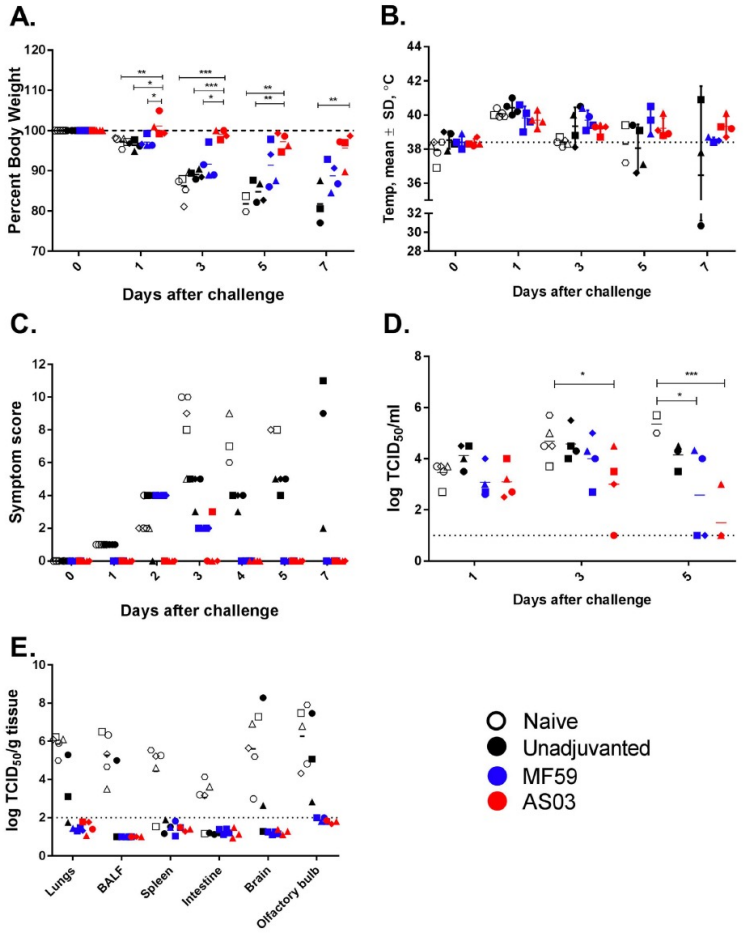
- H5N1- outbreaks in Asia in 1997, 2004 (Clade 1)
- 30% mortality
- Highly pathogenic in ferrets; neurological involvement; BSL3+ agent
- Conducted immunogenicity experiment with stockpiled vaccine

Vaccination regimen	HAI-titer group	HAI titer (GMT, 95%CI)	VN titer (GMT, 95%CI)	Flu-specific IgG titer (GMT, 95%CI)	NAI titer (GMT, 95%CI)
None	Naïve	5 (5)	5(5)	50(50)	5(5)
Unadjuvanted	No titer	5 (5)	5(5)	800 (35.4-18102)	7.1 (2.4-21.3)
MF59	Low	28.3 (15.0-53.5)	5(5)	9051 (4788-17108)	5(5)
AS03	High titer	761.1 (264.8-2188)	16.8 (3.2-87.9)	10763 (6201-18682)	5(5)



Wong et al., NPJ Vaccine, 2017, Sci Report, 2018

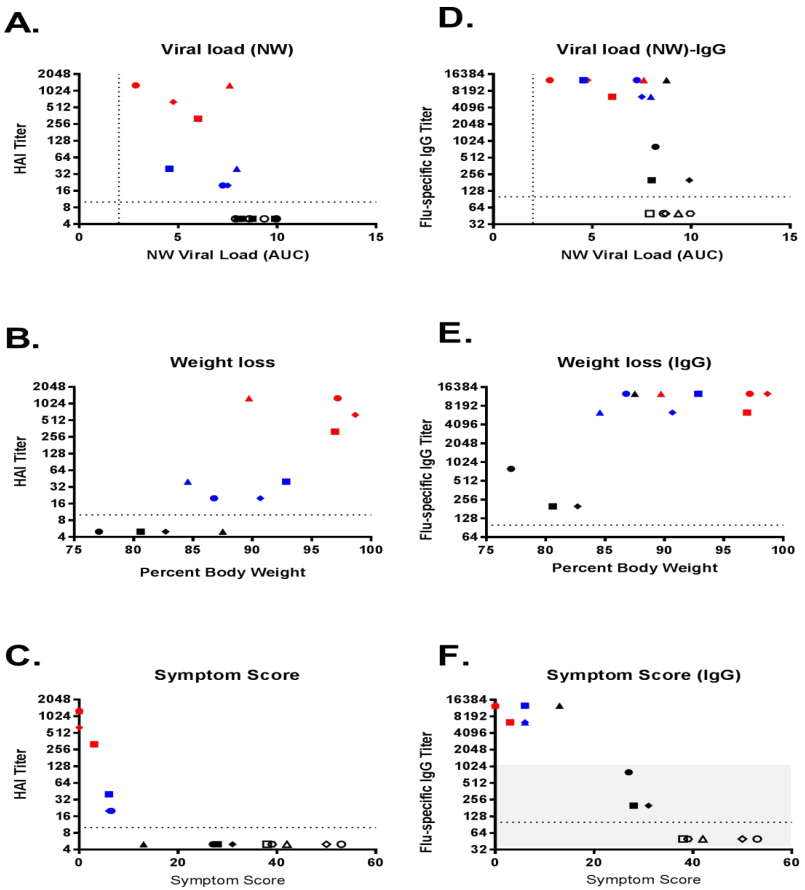
Dose dependent outcome of infection



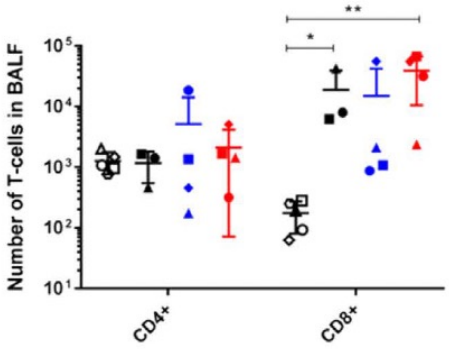
High levels of systemic immunity did not provide sterilizing immunity against infection- but it prevented extrapulmonary spread.

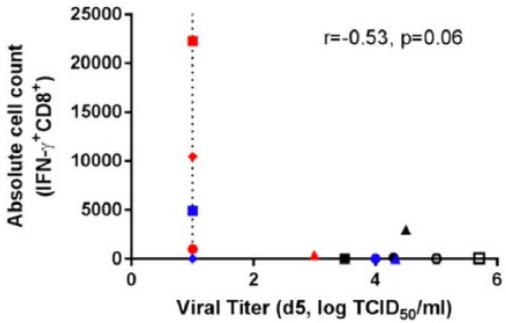
High igG can be indicative of ability to have rapid induction of protective antibodies.

Quantitative determination of protective antibody titer in the ferret model (H5N1)

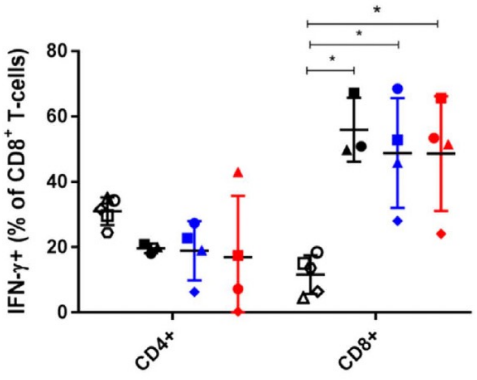


Role of T cells

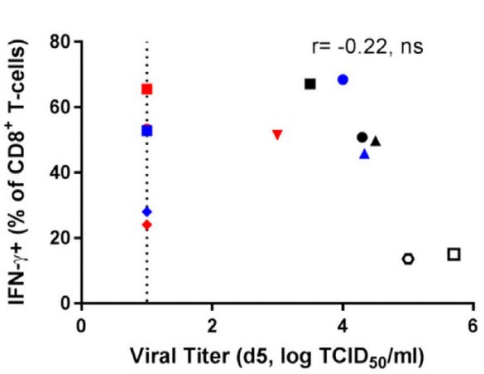




More infiltration of T cells in vaccinated ferrets into the airways, correlated with viral clearance



F.



More activated CD8+ T cells in vaccinated ferrets, no correlation with viral load

No correlation with peripheral blood T cells

Critical role in understanding immunity at site of infection

Summary

Vaccine Group	Pre-challenge			Post-challenge										
	Antibody response[a]			Clinical Manifestations				Viral Burden			Antibody response		Cellular Immunity	
	HAI	Flu-specific IgG	NAI	Weight Loss[b]	Fever > 5 days	Neurological symptoms[c]	Diarrhea[c]	Death[c]	Nasal cavity[d]	Extrapulmonary spread	HAI	NAI	Activated CD4+/CD8+ in BALF	Activated CD4+/ CD8+ in blood
Naïve	n.d	n.d	n.d	+++	One	100%	80%	100%	High	Widespread	n.d	low	n.d	n.d
No Titer	n.d	+	Low	+++	Yes	25%	100%	25%	High	Brain/Olfactory Bulb	none-moderate	Moderate-High	CD4 < CD8	CD4 < CD8
Low Titer	Moderate	++	n.d	++	No	0%	100%	0%	50% ferrets cleared	None	High	Low-moderate	CD4 < CD8	CD4 < CD8
High Titer	High	+++	n.d	+	No	0%	25%	0%	Mostly cleared	None	High	Low-moderate	CD4 < CD8	CD4 < CD8

1. High systemic neutralizing titers does not prevent shedding in the URT but reduces disease severity (also seen with COVID-19 vaccines).

1. Local CD8 T cells is important for viral clearance.
   - T cell assays are hard to standardize
   - Limitation: lack of ferret immunological reagent

2. Pre-existing immunity can modulate outcome of disease, but consider sensitivity of assays.

What about vaccines against current H5N1 Clade 2.3.4.4b?

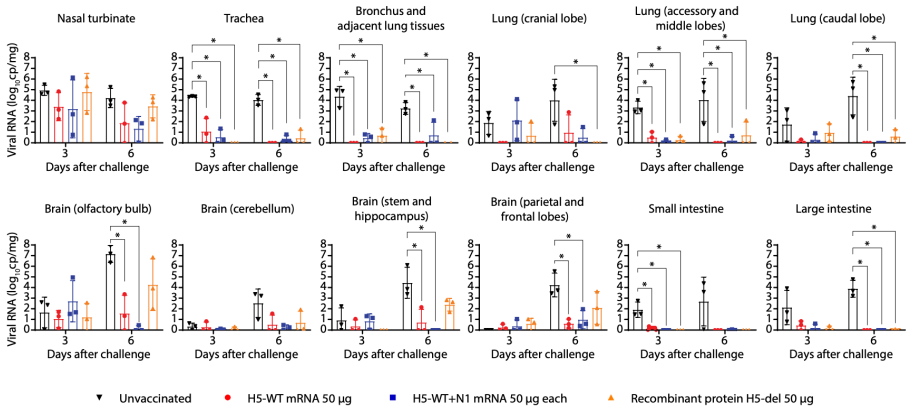
mRNA vaccine

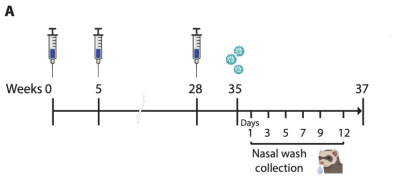
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

INFLUENZA

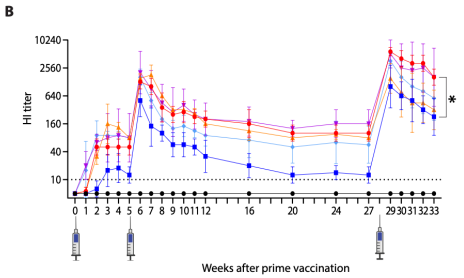
An influenza mRNA vaccine protects ferrets from lethal infection with highly pathogenic avian influenza A(H5N1) virus

Masato Hatta[1]†, Yasuko Hatta[1]†, Angela Choi[2]†, Jaber Hossain[1], Chenchen Feng[1], Matthew W. Keller[1], Jana M. Ritter[3], Ying Huang[1], Emma Fang[1,4], Elizabeth A. Pusch[1], Thomas Rowe[1], Juan A. De La Cruz[1], Monique C. Johnson[1], Jimma Liddell[1], Nannan Jiang[1], Daniel Stadlbauer[2], Li Liu[2], Arindam K. Bhattacharjee[2], Joseph R. Rouse[1], Michael Currier[1], Li Wang[1], Min Z. Levine[1], Marie K. Kirby[1], John Steel[1], Han Di[1], John R. Barnes[1], Carole Henry[2], C. Todd Davis[1], Raffael Nachbagauer[2], David E. Wentworth[1], Bin Zhou[1]*



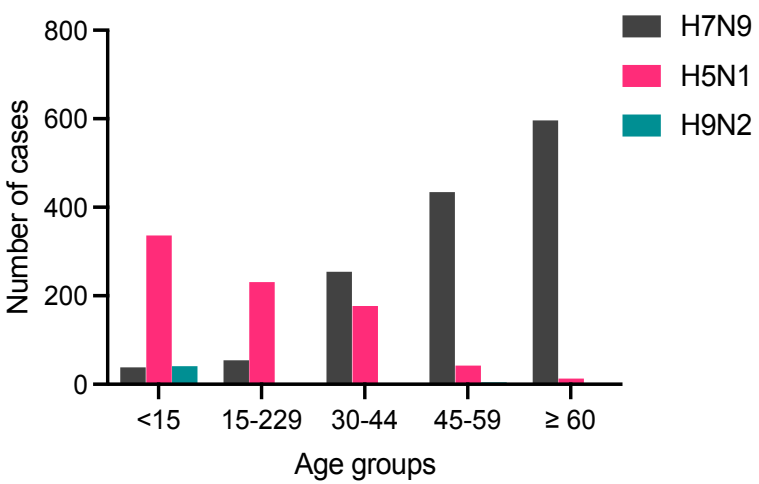


Group	Vaccine	HA	NA	Dose	Group size (*n*)
1	Unvaccinated	-	-	-	3
2	Moderna mRNA LNP	H5-WT	-	50 µg	3
3	Moderna mRNA LNP	H5-del	-	50 µg	3
4	Moderna mRNA LNP	H5-del	N1	50 µg each	3
5	Moderna mRNA LNP	H5-del	N1	100 µg each	3
6	Recombinant protein + AddaVax	H5-del	-	50µg	3



The association of A(H7N9) antibody responses with disease severity

Human cases of avian influenza virus infections show age-specific susceptibility



H7N9

1. H7N9 emerged in China in 2013.
2. Caused over 1500 cases between 2013 to 2019.
3. Approximately 30% CFR, mostly male, mean age: 55 years old

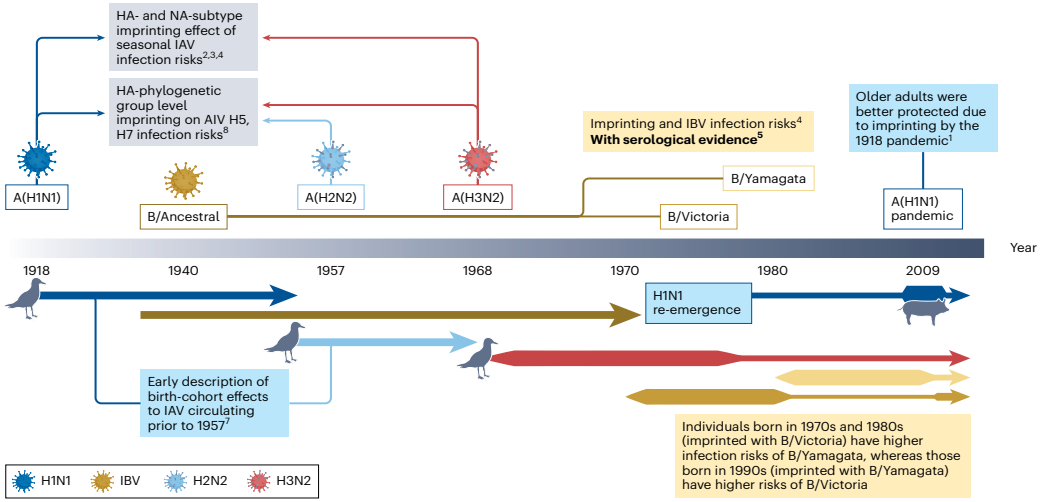
H5N1

1. 907 cases between 1997-2015, approximately 50% CFR.
2. Median age: 19 years, (IQR: 5 to 32) 41.2%: <15 yo, 80.3% : <35 yo

H9N2

1. >70 cases between 1998-2015,
2. >80% of clinical cases are < 8 yo

Immune imprinting and infection risks



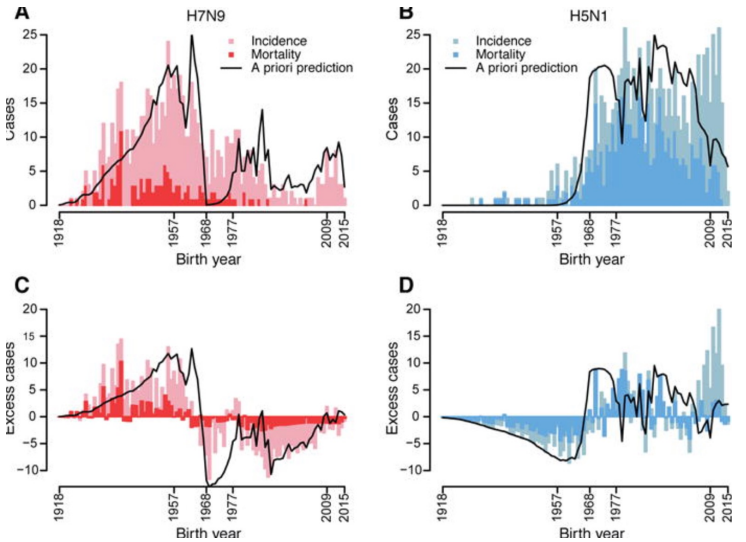
Chow and Wong, Nat Immunol. 2024

RESEARCH ARTICLE

Potent protection against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting

KATELYN M. GOSTIC, MONIQUE AMBROSE, MICHAEL WOROBEY, AND JAMES O. LLOYD-SMITH Authors Info & Affiliations

SCIENCE · 11 Nov 2016 · Vol 354, Issue 6313 · pp. 722-726 · DOI: 10.1126/science.aag1322



Influenza disease dynamics in humans: individual immunity to H7N9

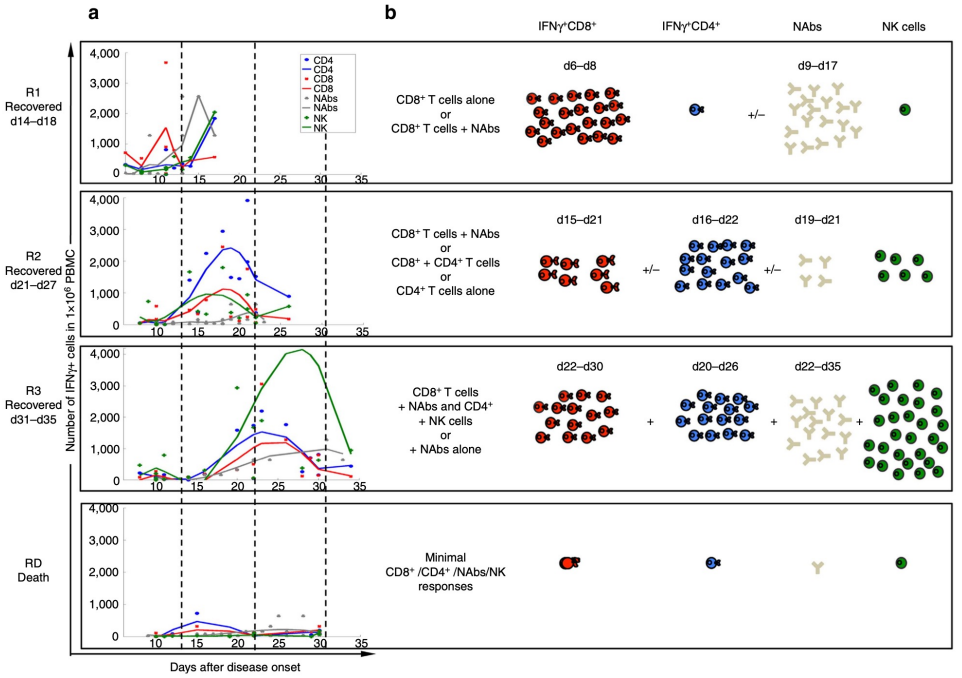
ARTICLE

Received 24 Aug 2014 | Accepted 2 Mar 2015 | Published 13 May 2015

DOI: 10.1038/ncomms7833 OPEN

Recovery from severe H7N9 disease is associated with diverse response mechanisms dominated by CD8$^+$ T cells

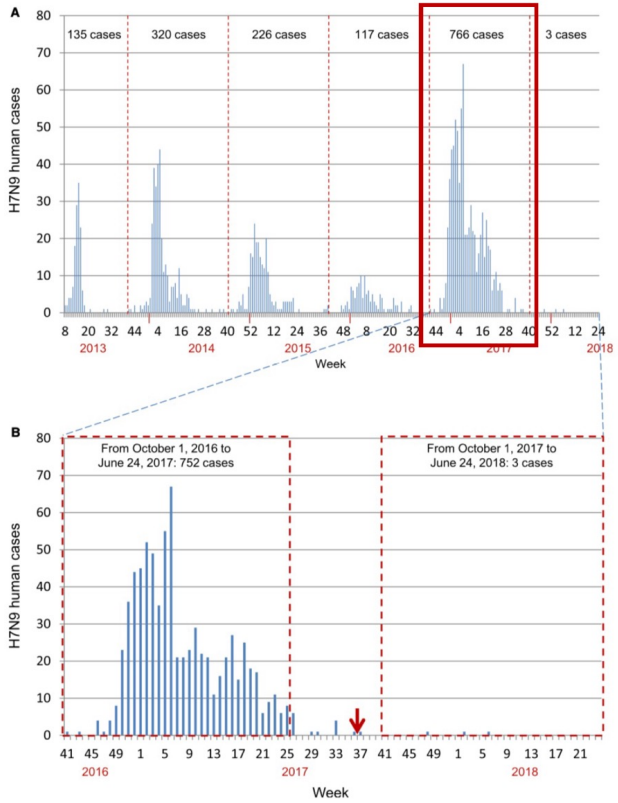
Zhongfang Wang[1,2,*], Yanmin Wan[1,*], Chenli Qiu[1], Sergio Quiñones-Parra[2], Zhaoqin Zhu[1], Liyen Loh[2], Di Tian[1], Yanqin Ren[1], Yunwen Hu[1], Xiaoyan Zhang[1], Paul G. Thomas[3], Michael Inouye[2,4], Peter C. Doherty[2,3], Katherine Kedzierska[2,†] & Jianqing Xu[1,†]



In the absence of functional antibody responses, do pre-existing cross-reactive antibodies matter?

H7N9 patient cohort from Southern China

H7N9 has caused 5 infection waves between 2013-2017

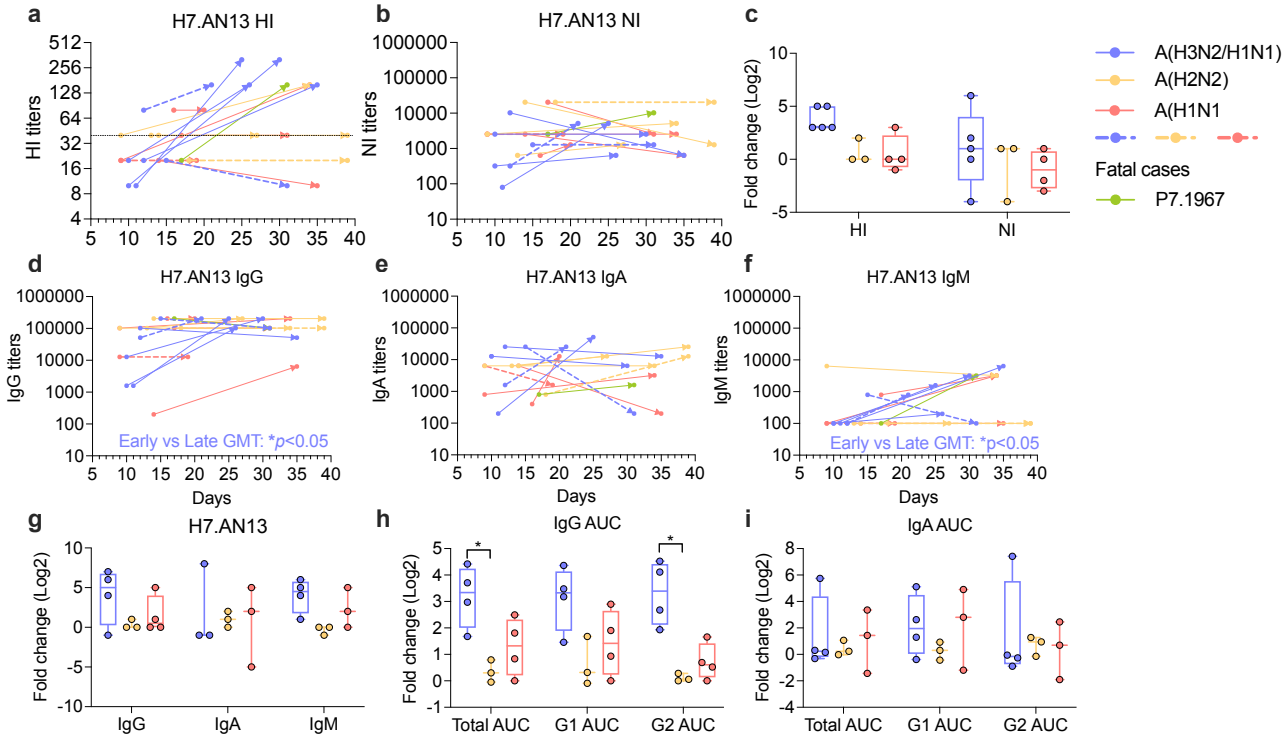


1568 cases

Mortality rate: 39% (616/1568)

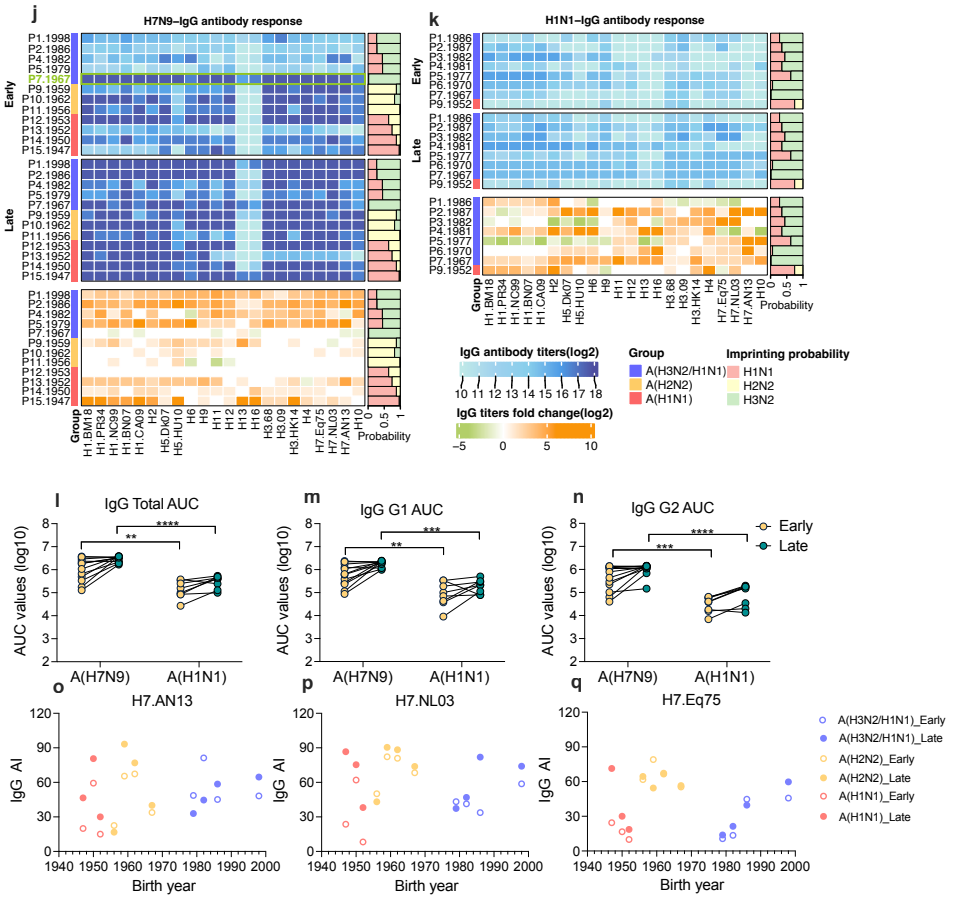
Patient no.	Age at time of infection	The year of Birth	Gender	Early	Late	Sampling year	Infection wave	Clinical Outcome	Duration of hospitalization (days)	ICU stay (days)
P1	17	1998	F	10	30	2015	3rd	Discharged	25	20
P2	28	1986	M	11	25	2014	2nd	Discharged	20	15
P3	31	1983	F	12	21	2014	2nd	Deceased	86	84
P4	33	1982	M	12	35	2015	3rd	Discharged	39	24
P5	35	1979	F	10	26	2014	2nd	Discharged	81	61
P6	39	1974	M	15	31	2013	1st	Deceased	26	24
P7	47	1967	M	17	31	2014	2nd	Discharged	21	16
P8	56	1961	M	18	34	2017	5th	Deceased	36	36
P9	59	1959	F	9	39	2018	5th	Discharged	101	57
P10	51	1962	F	14	39	2013	1st	Discharged	38	24
P11	58	1956	M	13	27	2014	2nd	Discharged	26	12
P12	64	1953	M	16	20	2017	5th	Discharged	22	18
P13	62	1952	M	14	35	2014	2nd	Discharged	53	35
P14	67	1950	M	17	31	2017	5th	Discharged	65	38
P15	66	1947	F	9	34	2013	1st	Discharged	44	38
P16	74	1943	F	9	19	2017	5th	Deceased	26	20

Antibody response to H7N9



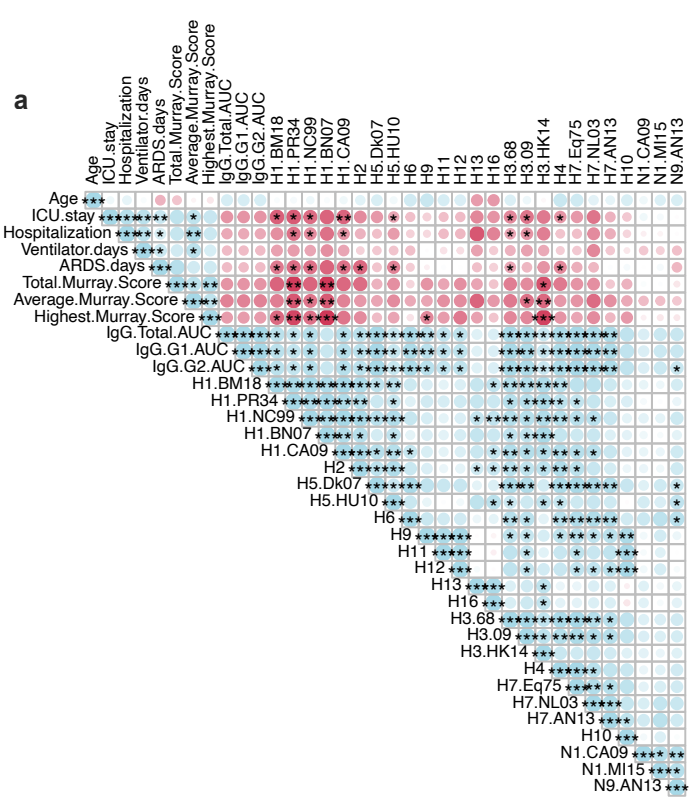
Younger patients exhibit primary-like antibody response: lower baseline and a detectable IgM response

Binding antibody landscapes

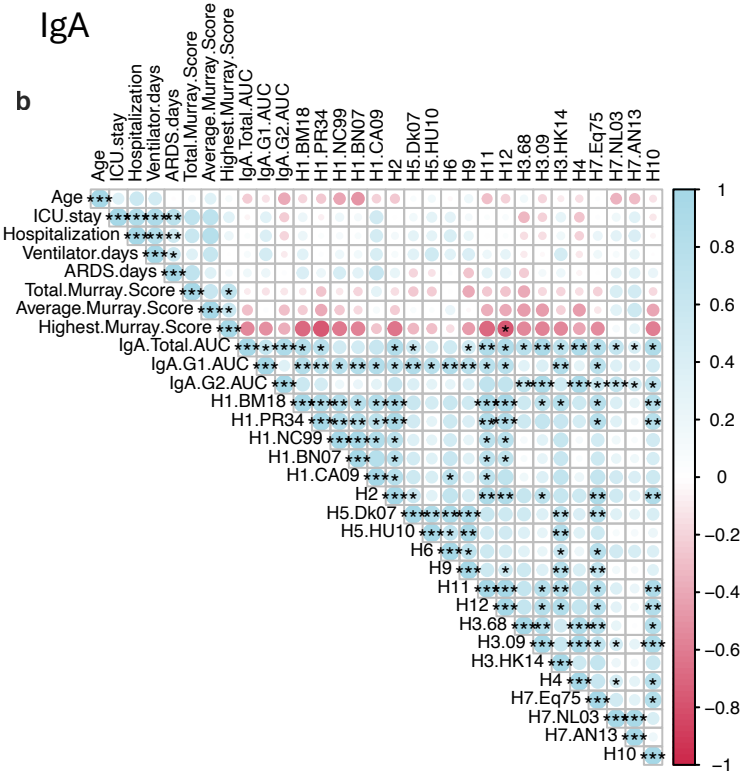


Correlation to clinical outcome

IgG



IgA



Role of NA-Immunity

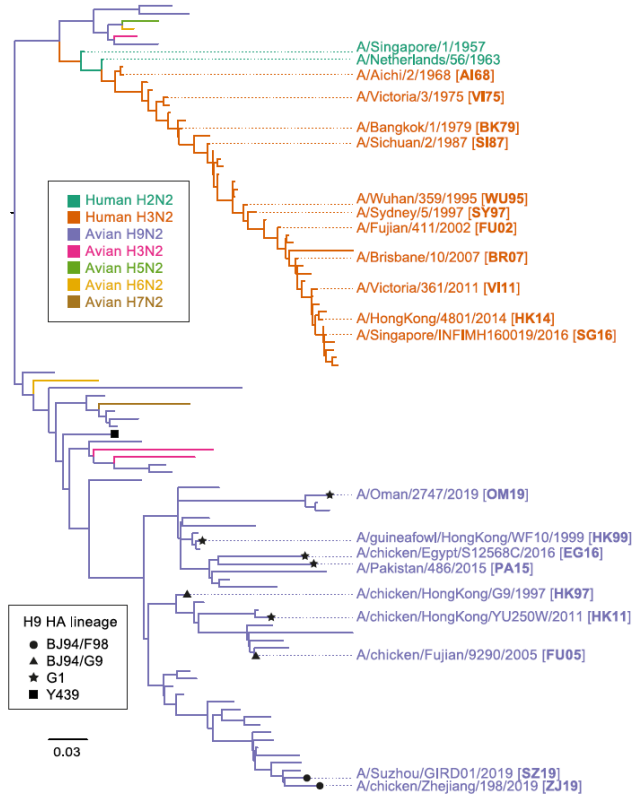
NA-Immunity

- NA antibodies have broader cross-reactivity compared to HA antibodies *(Chen et al., Cell, 2018; Sandbulte et al., Plos Med, 2007)*.

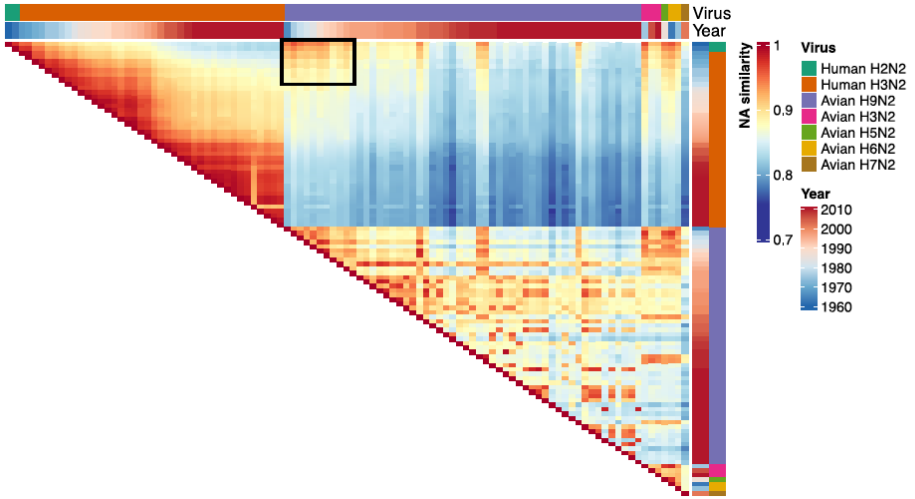
- Seasonal H3N2 and H9N2 share the same NA subtype!

Does immunity to NA explain the trend of age-susceptibility observed for human cases of H9N2 virus infections?

The early human N2 strains share high sequence similarity to avian N2 strains



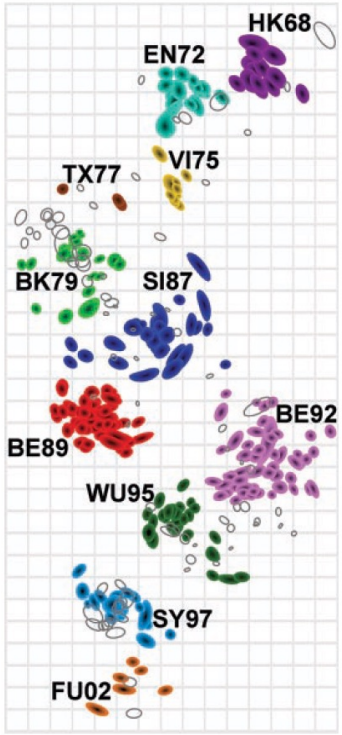
Vijay Dhanasekaran

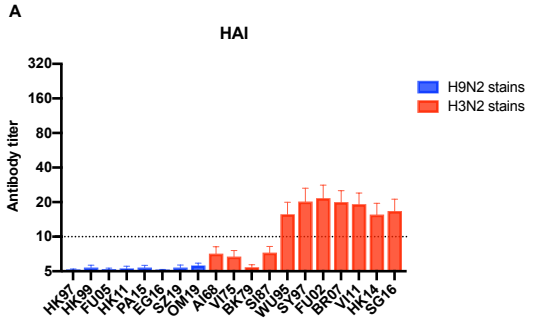


Evolutionary relationship between human and avian N2 strains since 1957 to 2019. (A) Phylogenetic tree of N2 representative human influenza viruses H3N2 and avian influenza viruses H9N2. (B) Pairwise comparison of amino acid sequence similarities for human and avian N2 strains between 1957 to 2019.

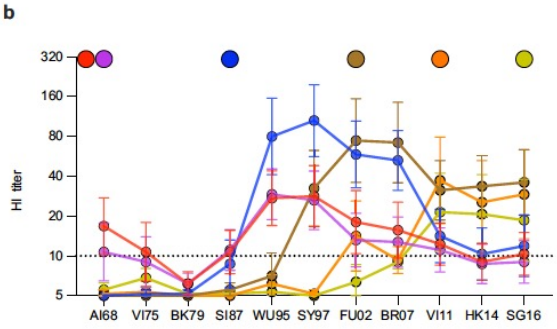
Liang et al., Nat Comm, 2024

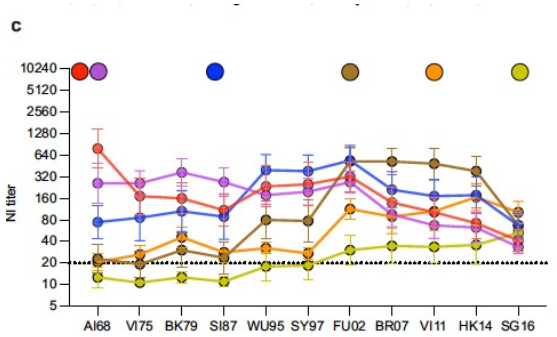
Human serological study



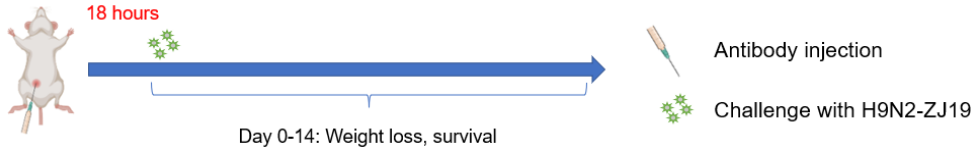


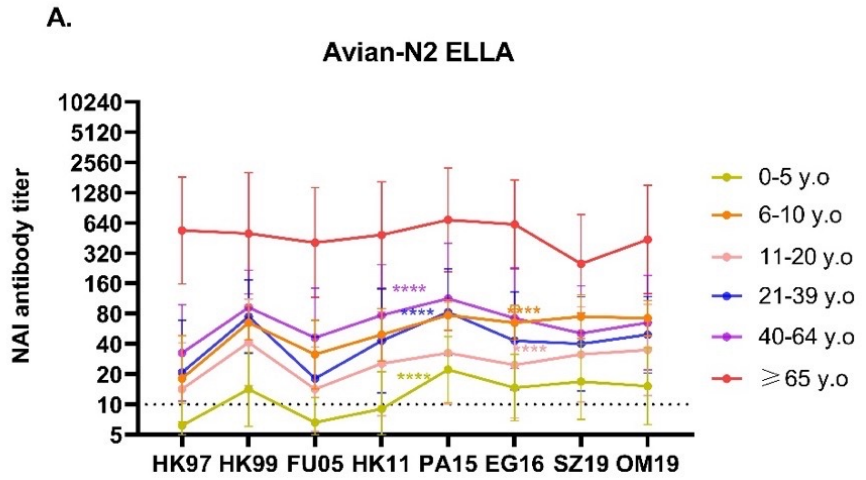


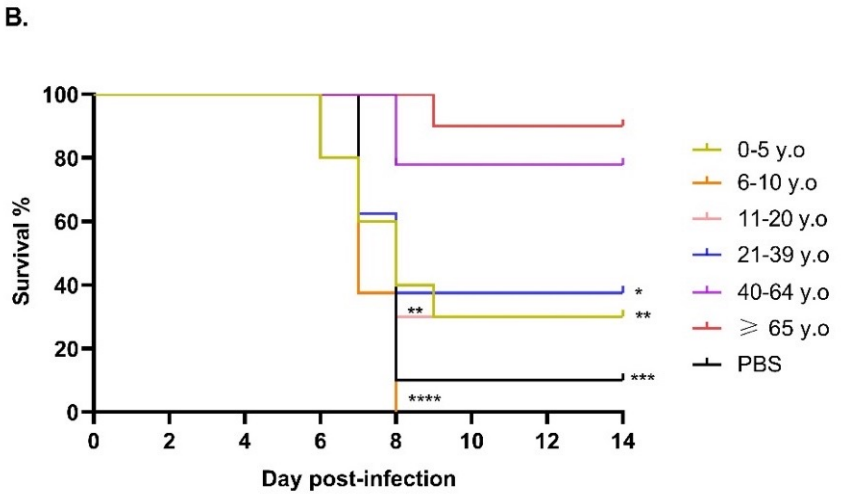




Avian N2 antibodies are present in an age-dependent manner in the human population

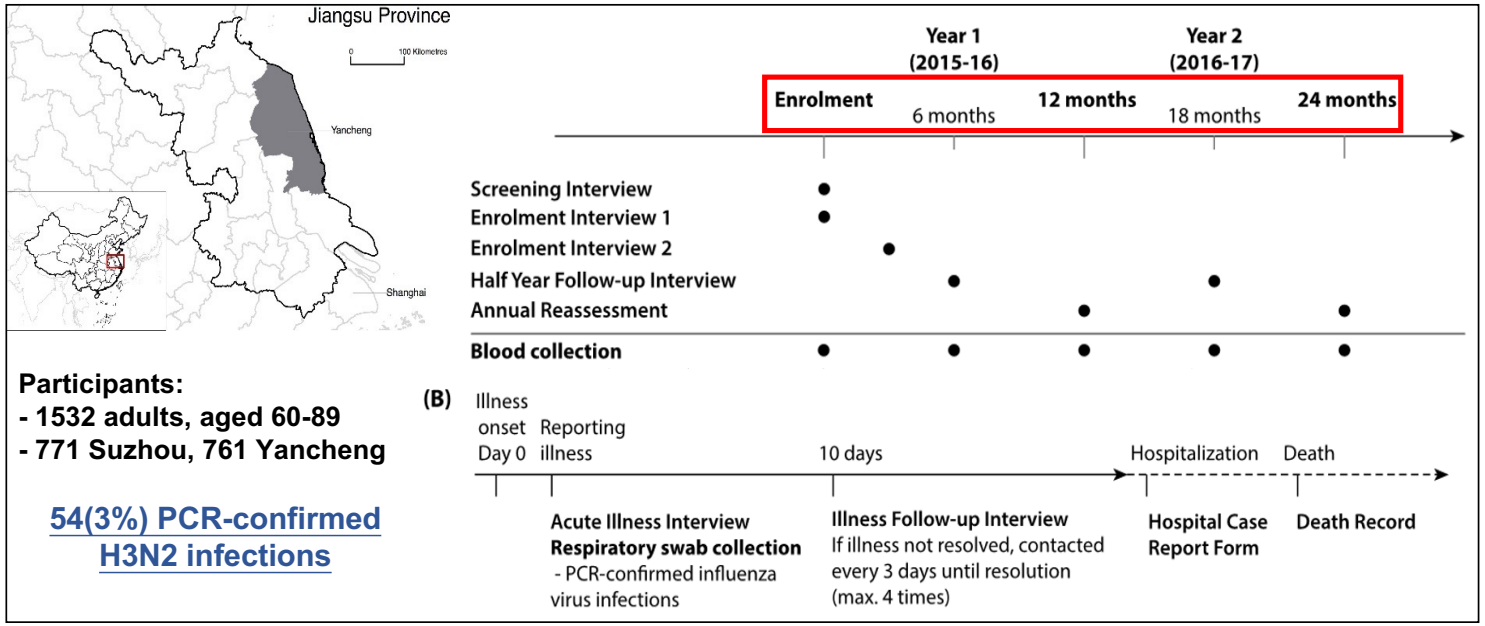






Can this response be recalled after seasonal influenza A (H3N2) infection?

China Ageing REspiratory infections Study (CARES)



Participants:
- **1532 adults, aged 60-89**
- **771 Suzhou, 761 Yancheng**

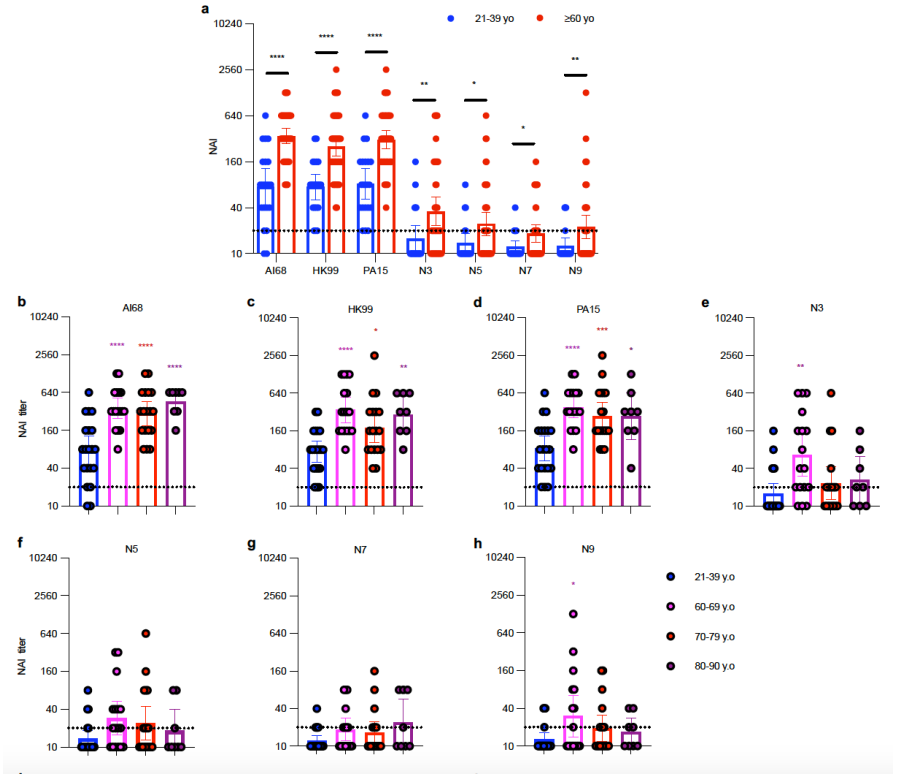
54(3%) PCR-confirmed H3N2 infections

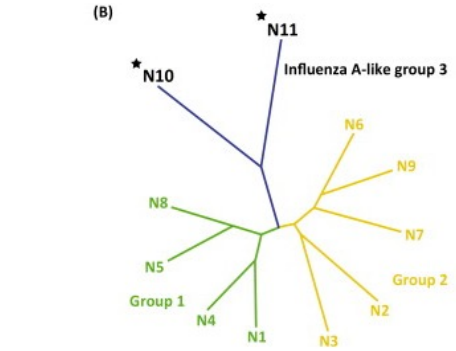
Paired sera from 45 A(H3N2) infections : 2016-2017

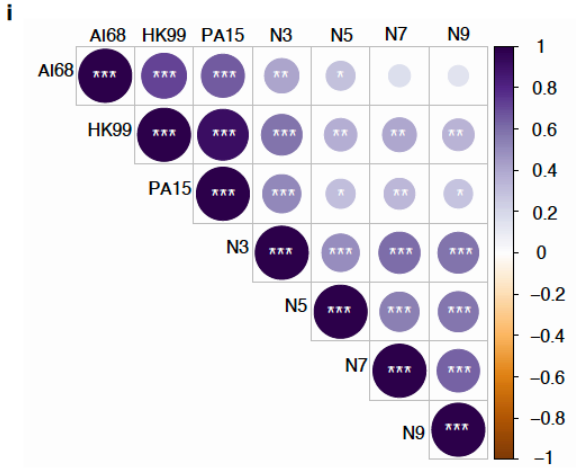
Majority of isolates belonged to clade 3c.2a

Ben Cowling, Nancy Leung, HKU
Funded by US CDC

Cross-reactivity at baseline to avian NA subtypes





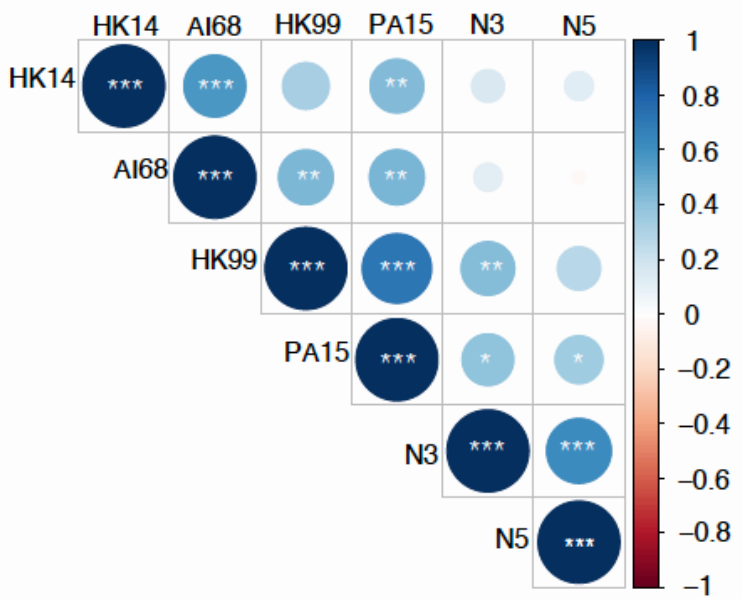


Seasonal H3N2 infection can recall cross-reactive NA antibodies in some participants

Table 2 | Seroconversion rates to the different NA subtypes among the 43 A(H3N2)-infected participants in CARES cohort.

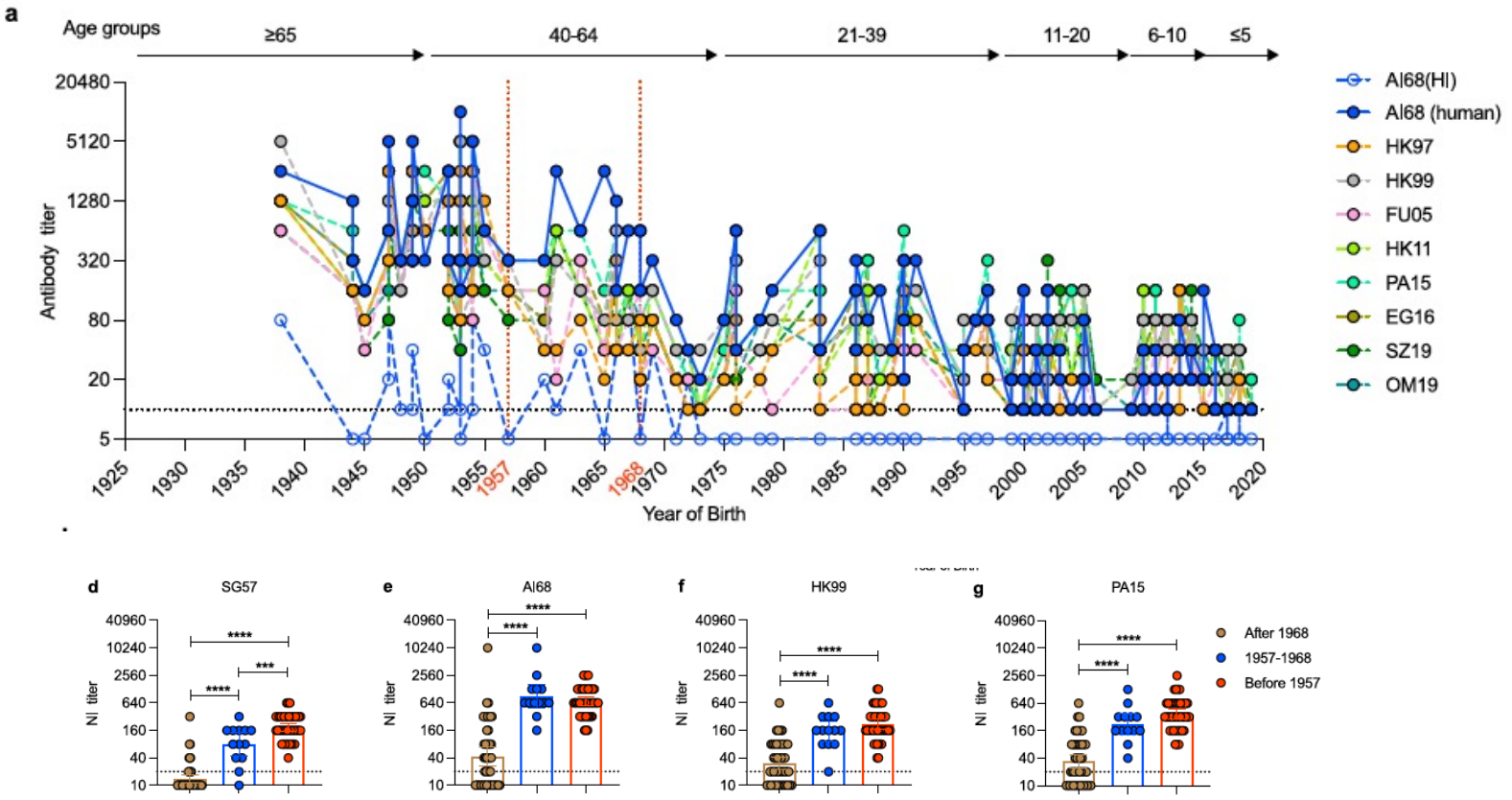
NA Group	Group 1	Group 2						
Subtype	N5	Human N2		Avian N2		N3	N7	N9
Strain	GD08[b]	HK14[c]	AI68[d]	HK99[e]	PA15[f]	GD96[g]	SX10[h]	AH13[i]
Seroconversion[a] No. (%)	5(11.6)	35(81.4)	17(39.5)	8(18.6)	11(25.6)	8(18.6)	1(2.3)	2(4.7)

[a] Seroconversion was defined as four-fold increase in neuraminidase inhibition (NI) antibody titers in paired serum samples
[b] H6N5-A/duck/Guangdong/wy11/2008 (H5N5)
[c] H6N2_A/Hong Kong/4801/2014 (H3N2)
[d] H6N2_A/Aichi/2/1968 (H3N2)
[e] H6N2_A/Guineafowl/Hong Kong/WF10/99 (H9N2)
[f] H6N2_A/Pakistan/486/2015 (H9N2)
[g] H6N3_A/Duck/Guangdong/1/1996 (H7N3)
[h] H6N7_A/duck/Shanxi/3180/2010 (H10N7)
[i] H6N9_A/Anhui/1/2013 (H7N9)

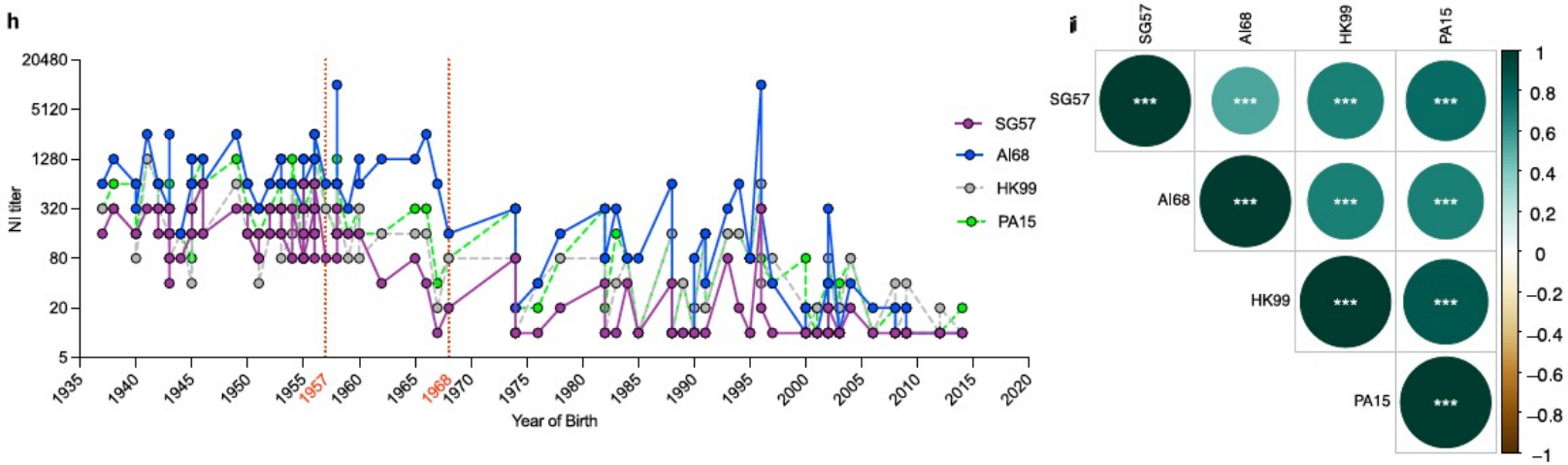


Recall pattern seems to be human vs avian

Cross-reactivity stratified by year of birth



Role of pandemic exposure-1957 N2

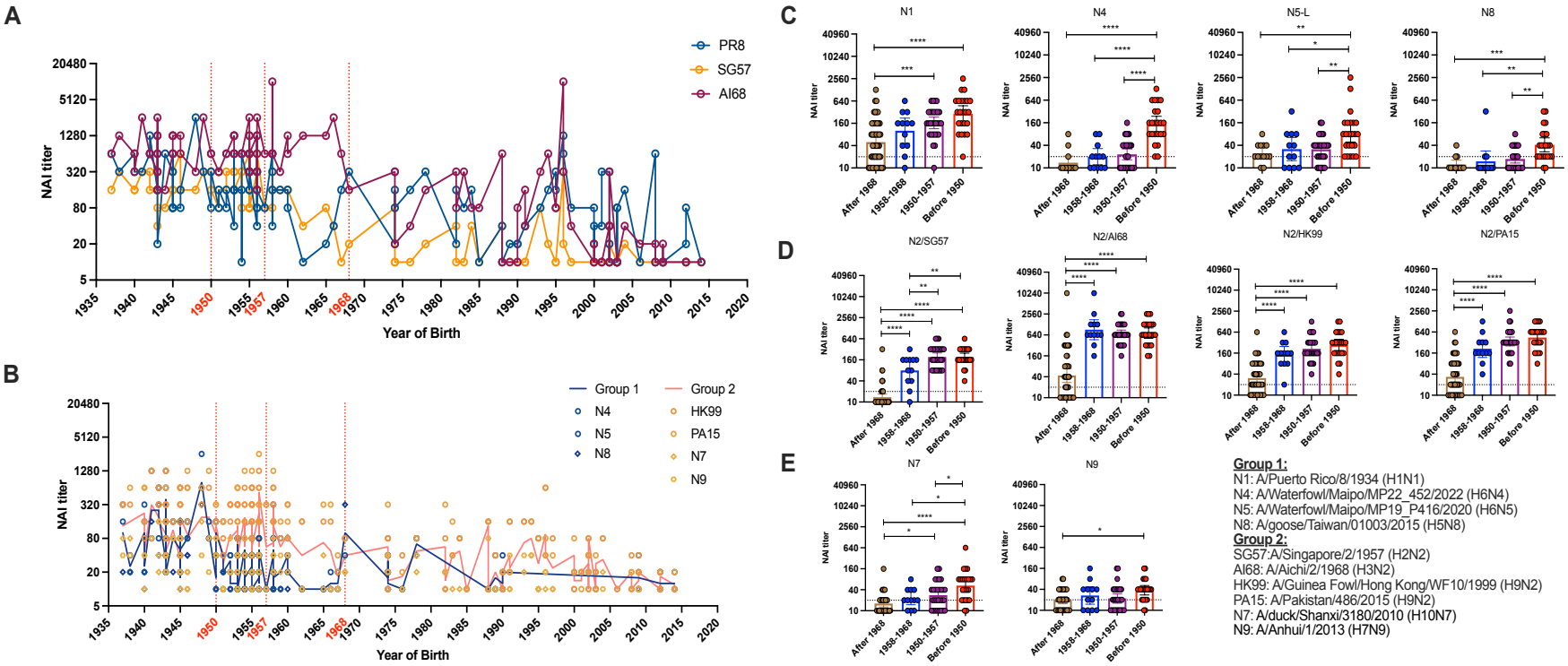


•Imprinting occurs in first decade of life:
- H1N1 in circulation: YOB <1950: cross-reactive to Group 1 NA
- H2N2 in circulation: YOB 1950-1957: cross-reactive to Group 2 avian
- H3N2pdm: YOB >1957: avian N2 but not N3 cross-reactivity

•Similar observation for N1: cross-reacts with avian N1 (Sandbulte et al. Plos Med, 2007, Daulagala et al., EMI, 2024).

What about Group 1 NA?

Older adults born prior to 1950 have cross-reactive antibodies to Group 1 NA



Group 1:
N1: A/Puerto Rico/8/1934 (H1N1)
N4: A/Waterfowl/Maipo/MP22_452/2022 (H6N4)
N5: A/Waterfowl/Maipo/MP19_P416/2020 (H6N5)
N8: A/goose/Taiwan/01003/2015 (H5N8)

Group 2:
SG57:A/Singapore/2/1957 (H2N2)
AI68: A/Aichi/2/1968 (H3N2)
HK99: A/Guinea Fowl/Hong Kong/WF10/1999 (H9N2)
PA15: A/Pakistan/486/2015 (H9N2)
N7: A/duck/Shanxi/3180/2010 (H10N7)
N9: A/Anhui/1/2013 (H7N9)

What about N1?

DISPATCHES

Avian Influenza A(H5N1) Neuraminidase Inhibition Antibodies in Healthy Adults after Exposure to Influenza A(H1N1)pdm09

Pavithra Daulagala, Samuel M.S. Cheng, Alex Chin, Leo L.H. Luk, Kathy Leung, Joseph T. Wu, Leo L.M. Poon, Malik Peiris,[1] Hui-Ling Yen[1]

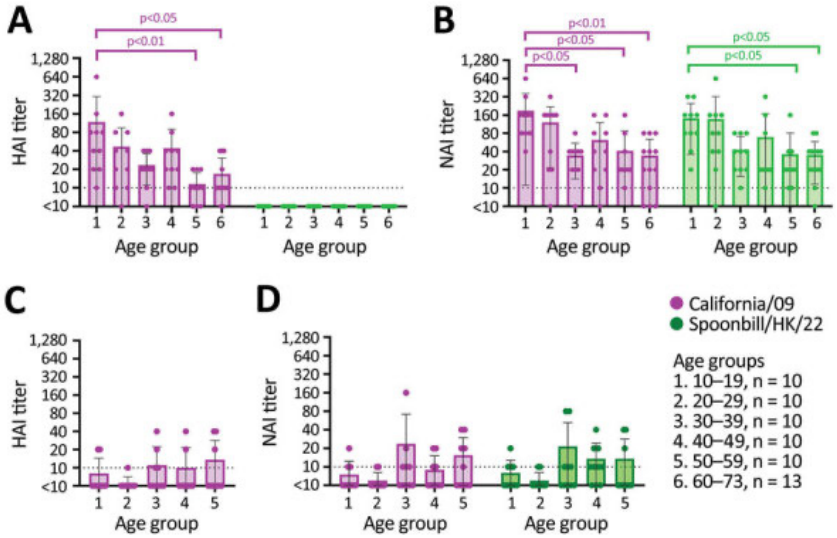


Table 1. NAI antibody titers detected in postinfection ferret antiserum against influenza A(H1N1), A(H1N1)pdm09, and A(H5N1) viruses, Hong Kong, China

Postinfection ferret antiserum†	NAI antibody titers*		
	Homologous virus	California/09 A (H1N1)pdm09	Spoonbill/HK/22 A(H5N1)
A/USSR/90/1977	320	<10	<10
A/Chile/01/1983	320	<10	<10
A/Singapore/06/1986	320	<10	<10
A/Texas/36/1991	320	<10	<10
A/Brisbane/59/2007	1,280	<10	<10
A/California/04/2009	2,560	2,560	320–640

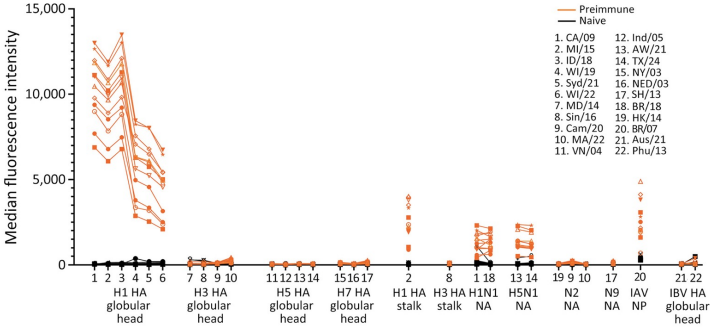
*NAI antibody response was determined using recombinant A(H6N1) viruses carrying the N1 proteins derived from A(H1N1), A(H1N1)pdm09, or A(H5N1) viruses that were generated as described previously (*10*). Although we used recombinant H6N1 viruses in ELLA assays to avoid interference of hemagglutinin-reactive antibodies, we might not completely prevent the interference of cross-reactive antibodies that bind to the stalk of H6 protein and inhibit neuraminidase activity through stearic hindrance. NAI, neuraminidase inhibition.
†One ferret antiserum against each of the influenza A(H1N1) viruses and 6 ferret antiserum against the influenza A(H1N1)pdm09 were used.

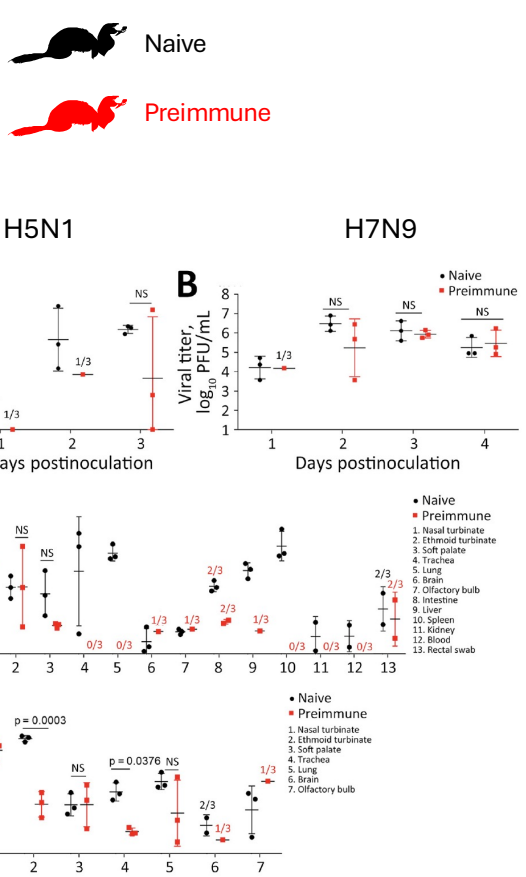
Daulagala t al., Emerging Infct Dis. 2024 Jan;30(1):168-171.

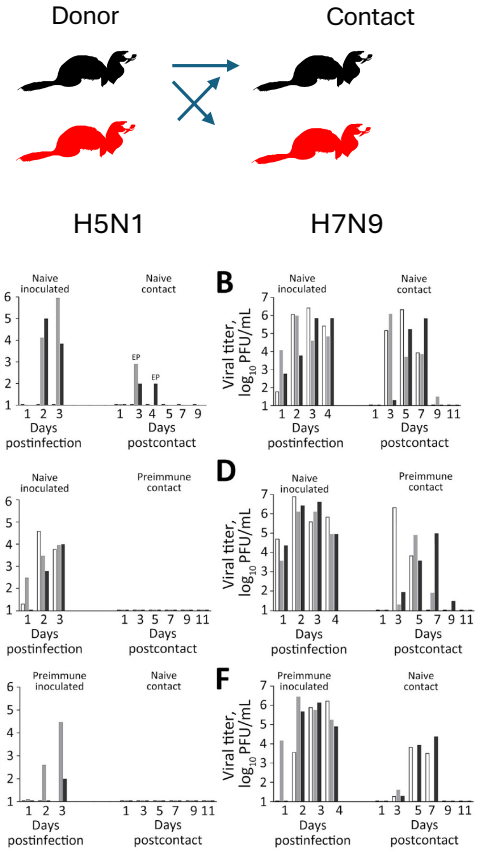
PdmN1 and Group 1 HA-stalk antibodies are protective against H5N1 Clade 2.3.4.4b challenge and transmission

Effect of Prior Influenza A(H1N1)pdm09 Virus Infection on Pathogenesis and Transmission of Influenza A(H5N1) Clade 2.3.4.4b Virus in Ferret Model

Xiangjie Sun, Jessica A. Belser, Zhu-Nan Li, Nicole Brock, Joanna A. Pulit-Penaloza, Troy J. Kieran, Claudia Pappas, Hui Zeng, Jessie C. Chang, Paul J. Carney, Brandon L. Bradley-Ferrell, James Stevens, Terrence M. Tumpey, Min Z. Levine, Taronna R. Maines



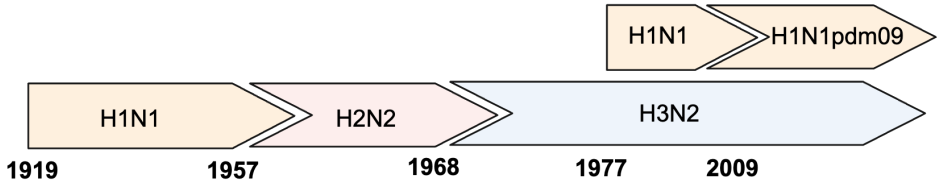


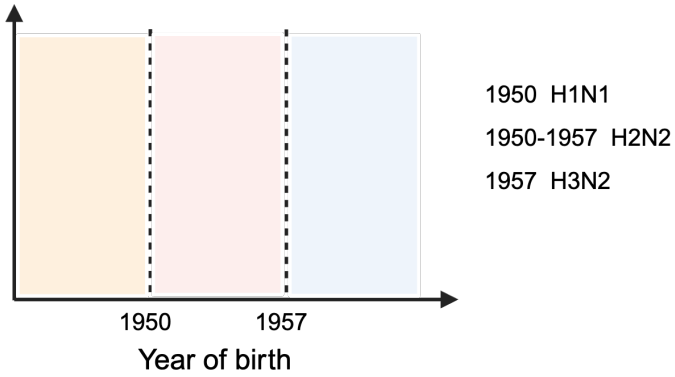


Sun X, et al., Emerg Infect Dis. 2025;31(3):458-466.
https://doi.org/10.3201/eid3103.241489

What about memory B cells?

Memory B cells are “first responders”
retains more plasticity to recognize variants



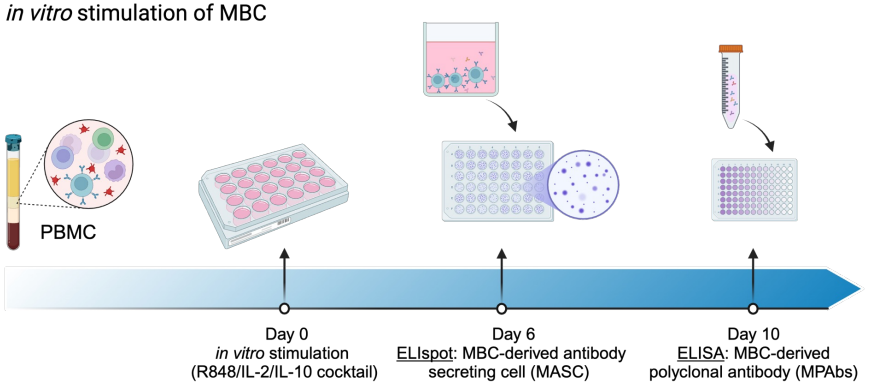


1950 H1N1

1950-1957 H2N2

1957 H3N2

in vitro stimulation of MBC



Hong Kong Red Cross Blood Transfusion Service

Memory B cells reflect imprint profile

Human

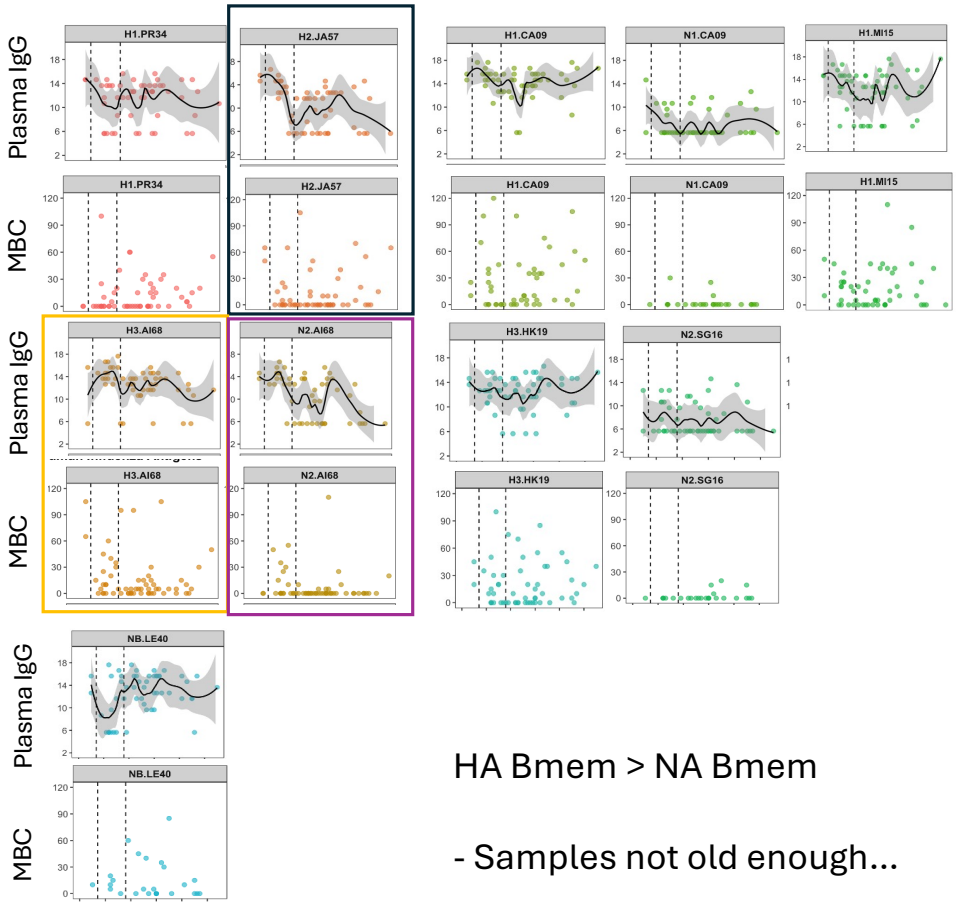
Avian

Group 1

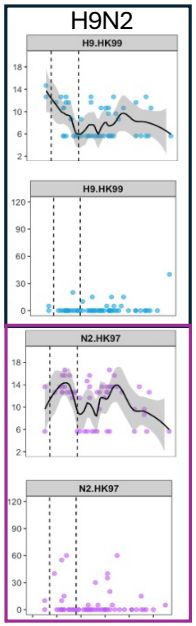
Group 2

Plasma IgG

MBC



H9N2

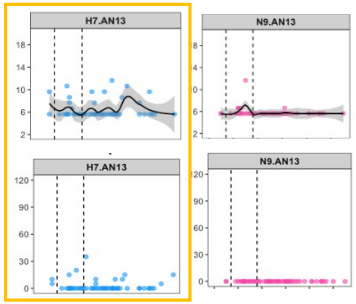


Cross-reactivity between H2/H9 that is not reflected in the MBC

Cross-reactivity between H3/H7 that is reflected in the MBC

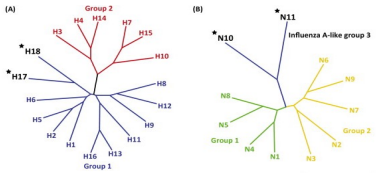
Cross-reactivity between N2 that is reflected in the plasma and MBC

H7N9



HA Bmem > NA Bmem

- Samples not old enough...

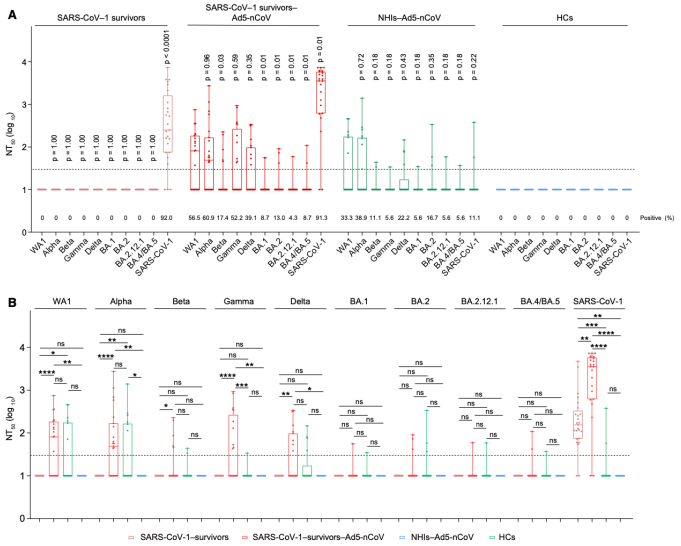


What about coronaviruses?

Cell Reports

Report

SARS-CoV-2 vaccine-induced antibody and T cell response in SARS-CoV-1 survivors



SARS-CoV-2 vaccine boosted SARS-CoV-1 T cell responses

Duan et al., 2022, Cell Reports 40, 111284

ScienceAdvances | AAAS
SIGNIFICANT RESEARCH. GLOBAL IMPACT

Sci Adv. 2024 Feb 28;10(9):eadk6425. doi: 10.1126/sciadv.adk6425

Rise in broadly cross-reactive adaptive immunity against human β-coronaviruses in MERS-recovered patients during the COVID-19 pandemic

So-Hee Kim [1,2,†], Yuri Kim [1,2,3,†], Sangeun Jeon [4,†], Uni Park [1,2], Ju-Il Kang [3], Kyeongseok Jeon [1,2], Hye-Ran Kim [1,2], Songhyeok Oh [1,2], Ji-Young Rhee [5], Jae-Phil Choi [6], Wan Beom Park [7], Sang Won Park [7], Jeong-Sun Yang [8], Joo-Yeon Lee [8], Jihye Kang [9], Hyoung-Shik Shin [10], Yeonjae Kim [11], Seungtaek Kim [4,*], Yeon-Sook Kim [12,*], Dong-Gyun Lim [9,*], Nam-Hyuk Cho [1,2,3,13,*]

Summary

1. Imprinting with avian influenza viruses during past influenza pandemics have elicited durable, cross-reactive antibodies to avian influenza NA.

2. This imprint profile is not clear for HA.
   - Could be an effect of boosting

3. Infection with zoonotic strains may elicit cross-reactive antibodies against other zoonotic strains.

What is protecting us?

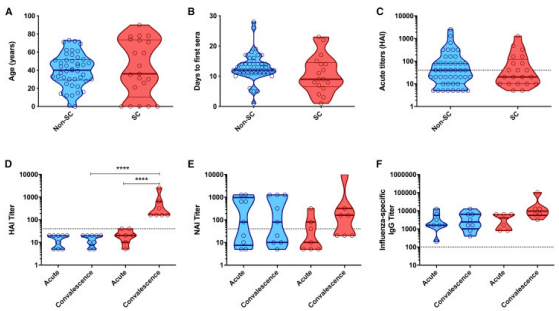
- do occupational exposed individuals show distinct immune profiles?

Infection without seroconversion- seasonal influenza

Cell Reports
Medicine

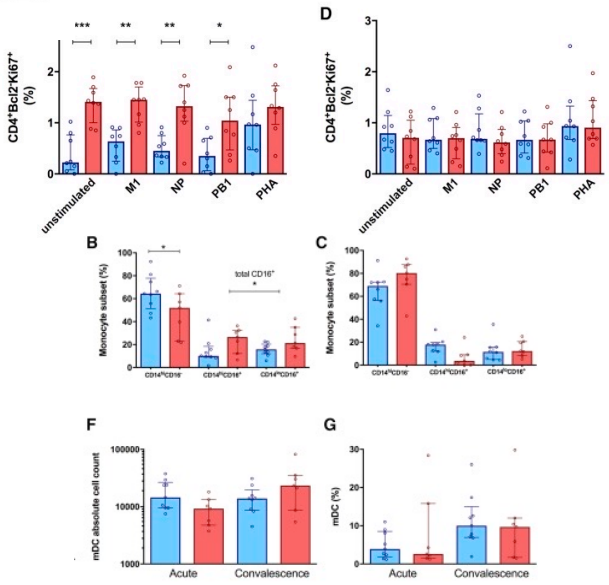
Report

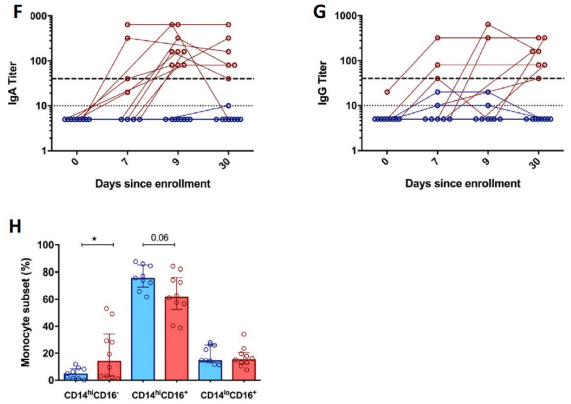
Activated CD4⁺ T cells and CD14^{hi}CD16⁺ monocytes correlate with antibody response following influenza virus infection in humans



Hospitalization was associated with more robust responses, with SARI participants more likely to seroconvert than ILI participants (86% versus 14%, respectively, p = 0.0012)

Of those with PCR-confirmed infection (N = 66), 21 (32%) participants met the definition of HAI seroconversion.





There is a cellular basis for non-seroconversion

Shown for COVID-19: early studies show that mild/asymptomatic disease does not always lead to SC

MERS: Camel workers show elevated T cell response

High Prevalence of MERS-CoV Infection in Camel Workers in Saudi Arabia

Abeer N. Alshukairi,[a] Jian Zheng,[b] Jingxian Zhao,[c] Atef Nehdi,[d] Salim A. Baharoon,[e] Laila Layqah,[d] Ahmad Bokhari,[f] Sameera M. Al Johani,[g] Nosaibah Samman,[d] Mohamad Boudjelal,[d] Patrick Ten Eyck,[h] Maha A. Al-Mozaini,[i] Jincun Zhao,[c,j] Stanley Perlman,[b,c] Abdulaziz N. Alagaili[k]

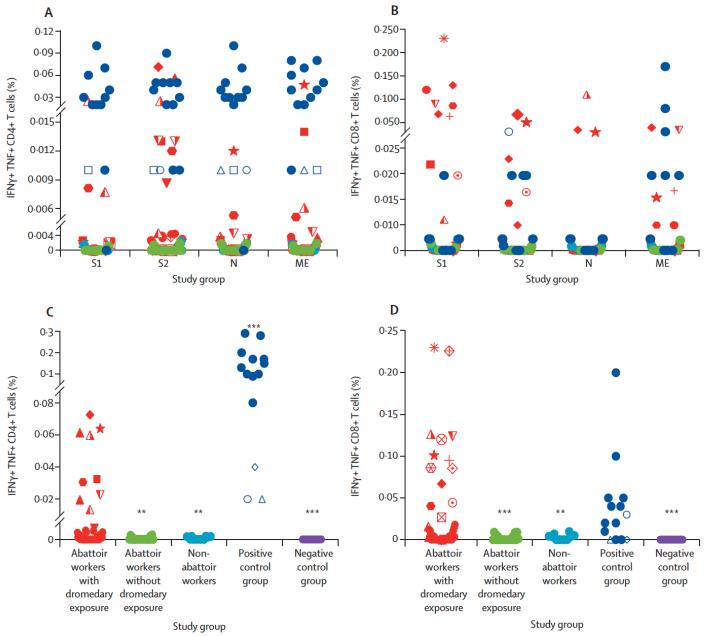
"Unlike prior reports, we found that a high percentage of CWs had been previously infected with the virus, although most, if not all, had no clinical disease consistent with MERS".

N=30

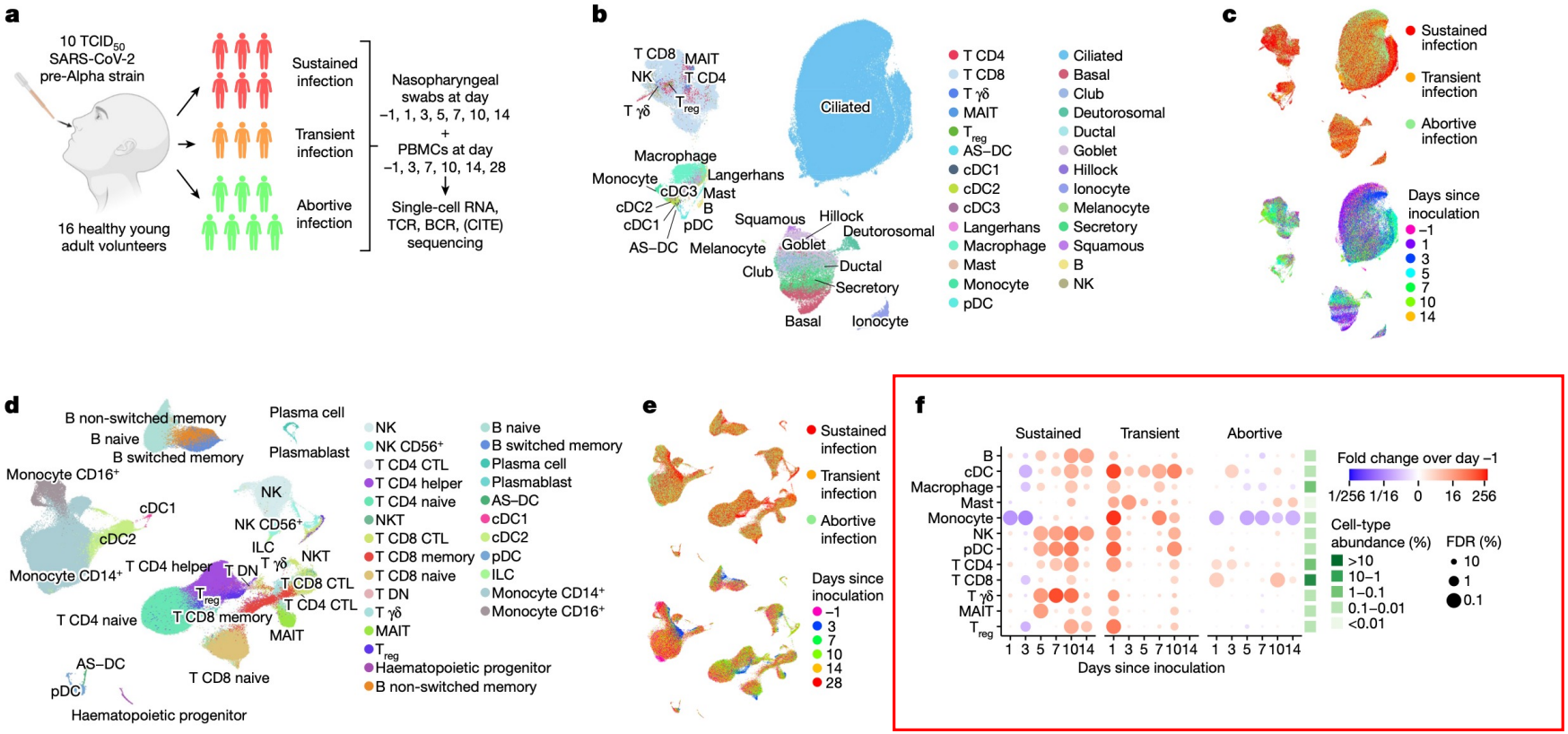
T-cell responses to MERS coronavirus infection in people with occupational exposure to dromedary camels in Nigeria: an observational cohort study

Chris Ka Pun Mok, Airu Zhu*, Jingxian Zhao*, Eric H Y Lau, Junxiang Wang, Zhao Chen, Zhen Zhuang, Yanqun Wang, Abeer N Alshukairi, Salim A Baharoon, Wenling Wang, Wenjie Tan, Weiwen Liang, Jamiu O Oladipo, Ranawaka A P M Perera, Sulyman A Kuranga, Malik Peiris, Jincun Zhao*

- No neutralizing antibodies detected



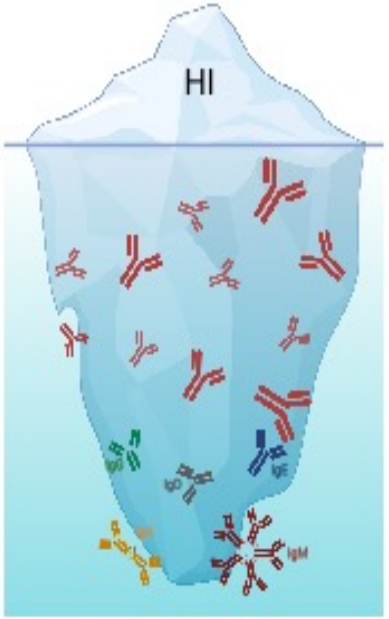
What immune profile distinguishes sustained, transient and abortive infection?



Lindeboom et al. Nature. 2024 Jul;631(8019):189-198.

Key takeaway

1. Neutralizing antibodies are strongest indicator of protection but are just tip of the immunological iceberg.
2. Population immunity is linked to virus evolution and vice versa- need to understand both.
3. ”Protection” and “Infection” needs to be contextualized.
4. Understanding antigenic cross-reactivity with zoonotic strains might provide new vaccine strategy for against emergence of novel strains.
5. Think beyond traditional targets; but this is often decided by reagent/technical resources.
6. Immune correlates of protection for respiratory pathogens may differ from blood-borne viruses and for RNA vs DNA viruses.



Neutralizing antibodies are just the tip of the iceberg




Questions?

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Co-I: Mark Zanin

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