Highly conserved CD4+ T-cell Hemagglutinin epitopes of seasonal flu and 2009 pandemic H1N1 viruses and their role in the infection dynamics

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Influenza and Other Respiratory Viruses (*In Press*)

Influenza A viruses

1. **Influenza A viruses are sub typed - surface glycoproteins, Hemagglutinin (HA) and Neuraminidase (NA)**

2. **HA1-16 and NA1-9 different subtypes were identified (birds) but H1,H2,H3 can able to infect the HUMAN population**

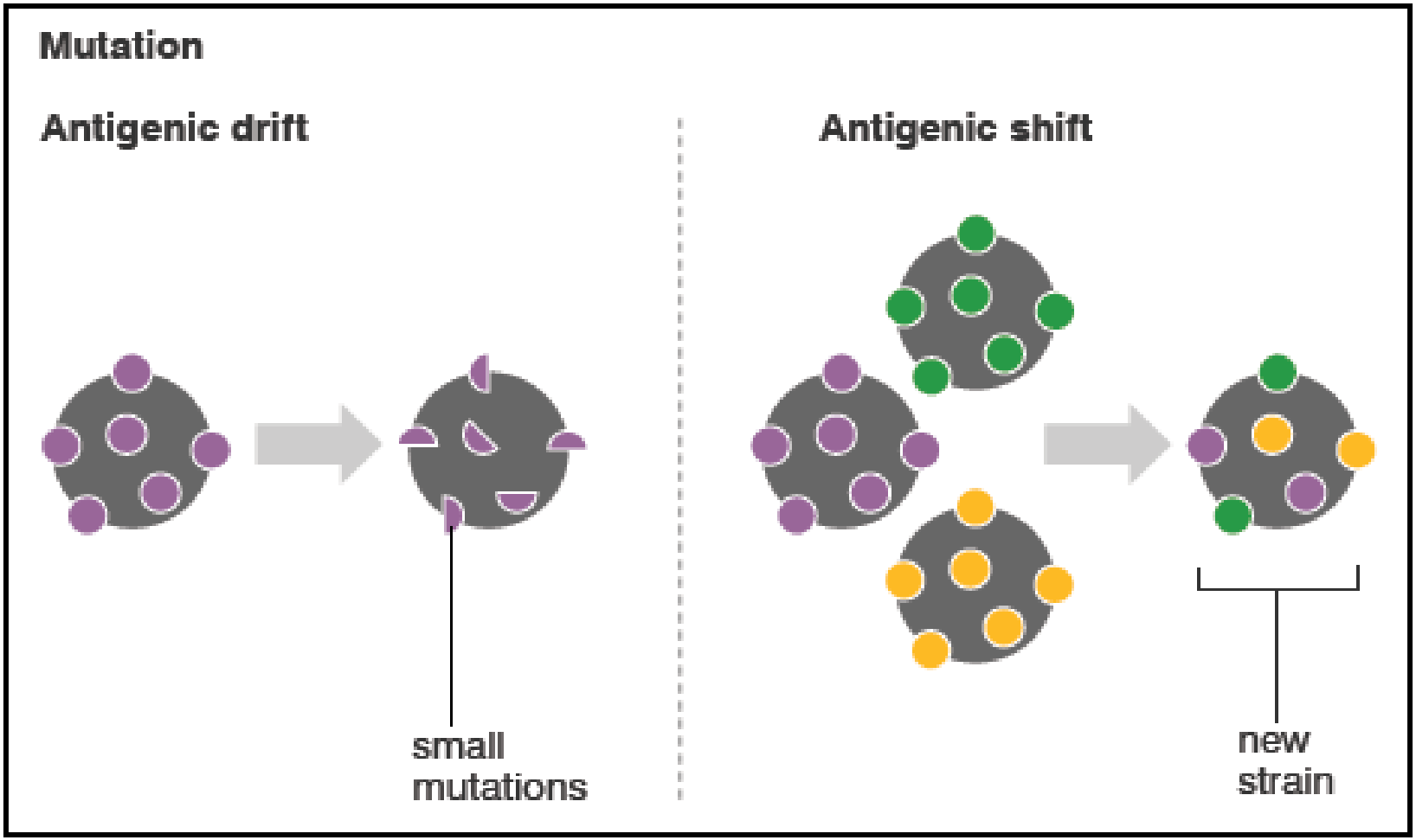
3. **Evolutionary mechanisms: Antigenic DRIFT and Antigenic SHIFT**

4. **Seasonal flu subtypes (H3N2, H1N1) are continuously evolving since 30 years and most of population could have been persistently exposed to them**

5. **Swine flu (2009 H1N1) is 1st pandemic in the 21st century and found to be a milder in clinical context**

3. Evolutionary mechanisms: Antigenic DRIFT and Antigenic SHIFT

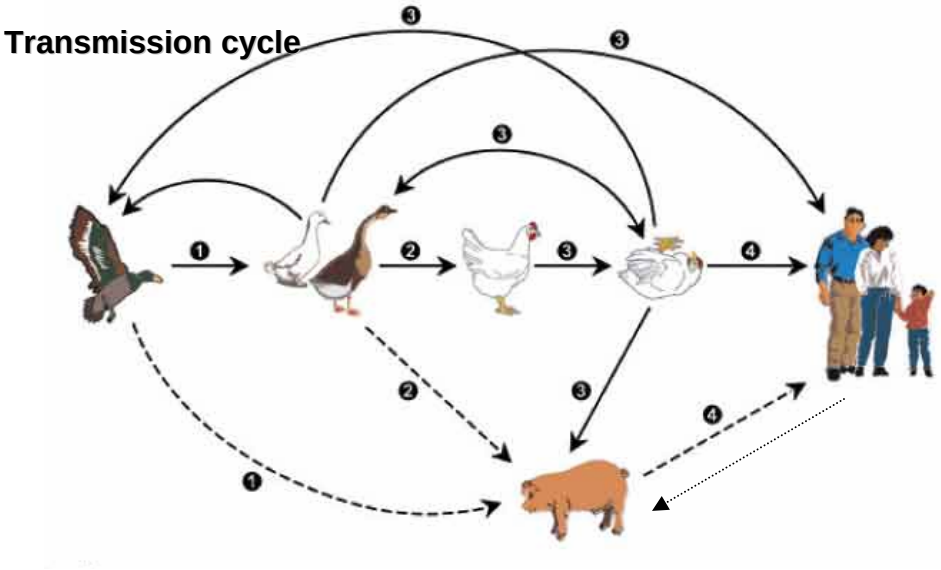
Evolutionary mechanisms

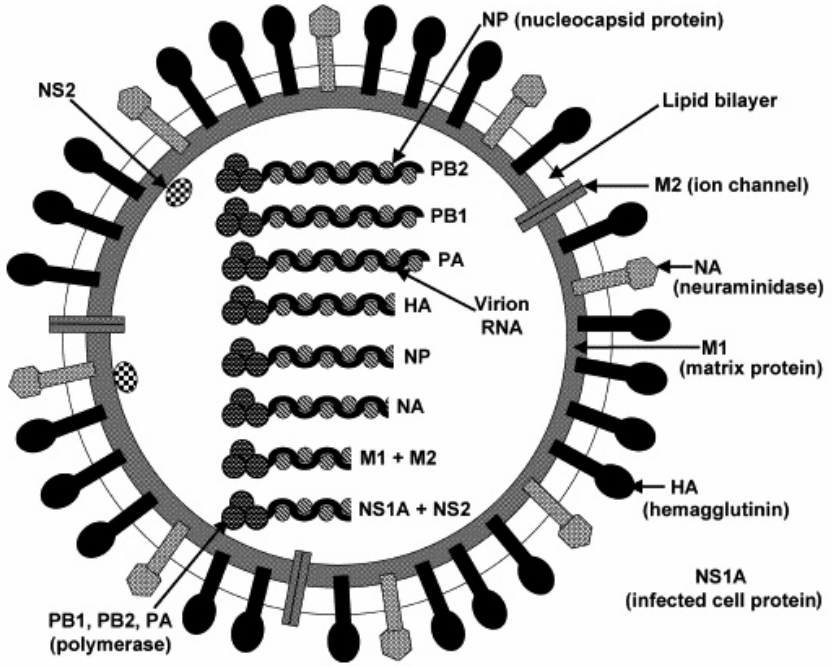


- HA and NA accumulate mutations RNA virus
- Immune response no longer protects fully
- Sporadic OUTBREAKS

- New HA or NA proteins
- Pre-existing antibodies do not protect
- May get PANDEMICS

2009 H1N1 Genome

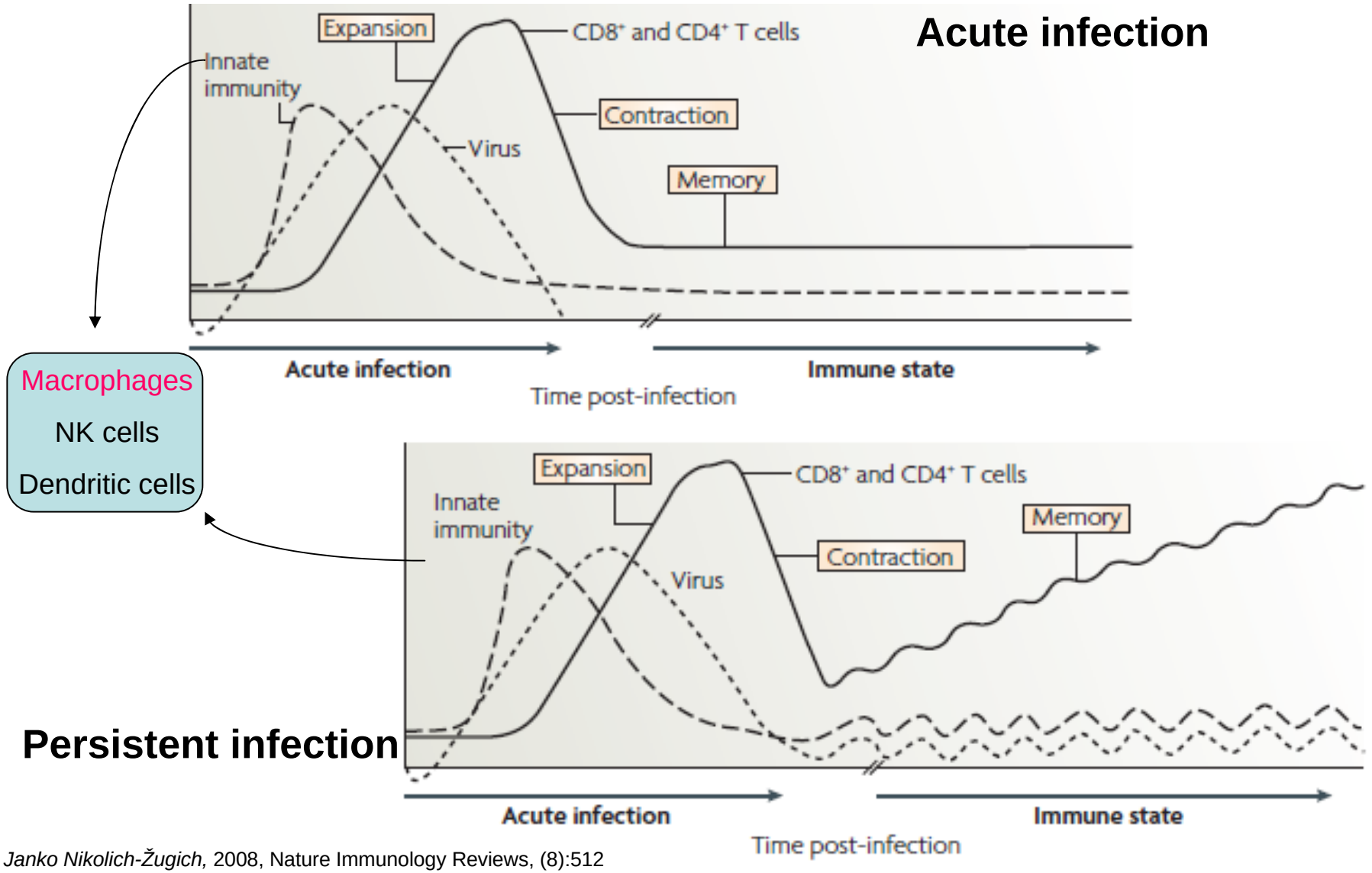




	Gene	Source	Origin
PB2	polymerase	avian	North American
PB1	polymerase	human	H3N2
PA	polymerase	avian	North American
HA	hemagglutinin	swine	North American
NP	nucleoprotein	swine	North American
NS	nonstructural	swine	North American
NA	neuraminidase	swine	Eurasian
M	M protein	swine	Eurasian

4. Seasonal flu subtypes (H3N2, H1N1) are continuously evolving since 30 years and most of population could have persistently exposed to them

Immunological Memory to Influenza Infection



Janko Nikolich-Žugich, 2008, Nature Immunology Reviews, (8):512

5. Swine flu (2009 H1N1- 1st pandemic in the 21st century and found to be a milder

Immune responses during the course of 2009 H1N1 infection

1. **Antibody (B-cell) can able to see WHOLE virus – Protective Immunity**
2. **T-cells (T helper and CTLs) can only see the PROCESSED ANTIGEN (small fragments) in context of MHC) – Probability of memory HIGH**

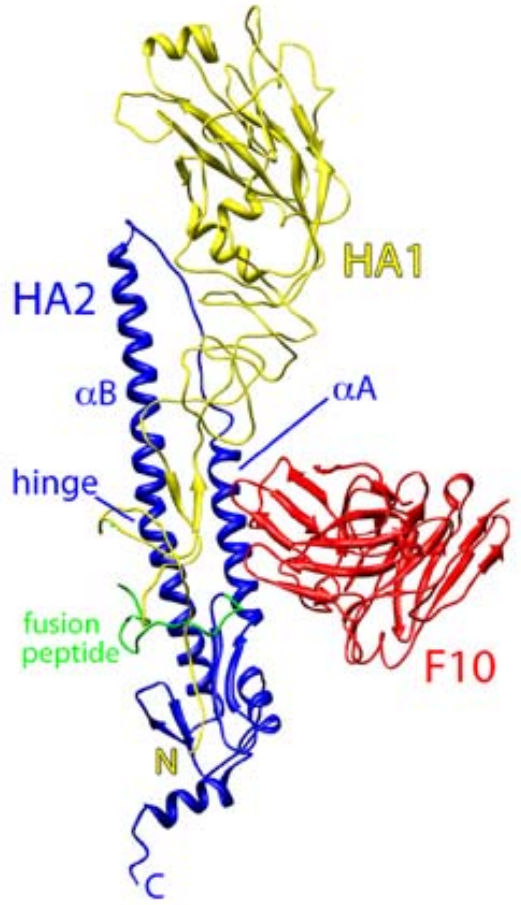
Surface protein - Hemagglutinin

- helps the virus to enter into the host system
- determines the subtype, and its novelty, host specificity, transmission efficiency of virus and immunity

HA1 being a major target of neutralizing antibodies is under immunological pressure to accumulate mutations

HA2 highly conserved and helps in membrane fusion mechanism

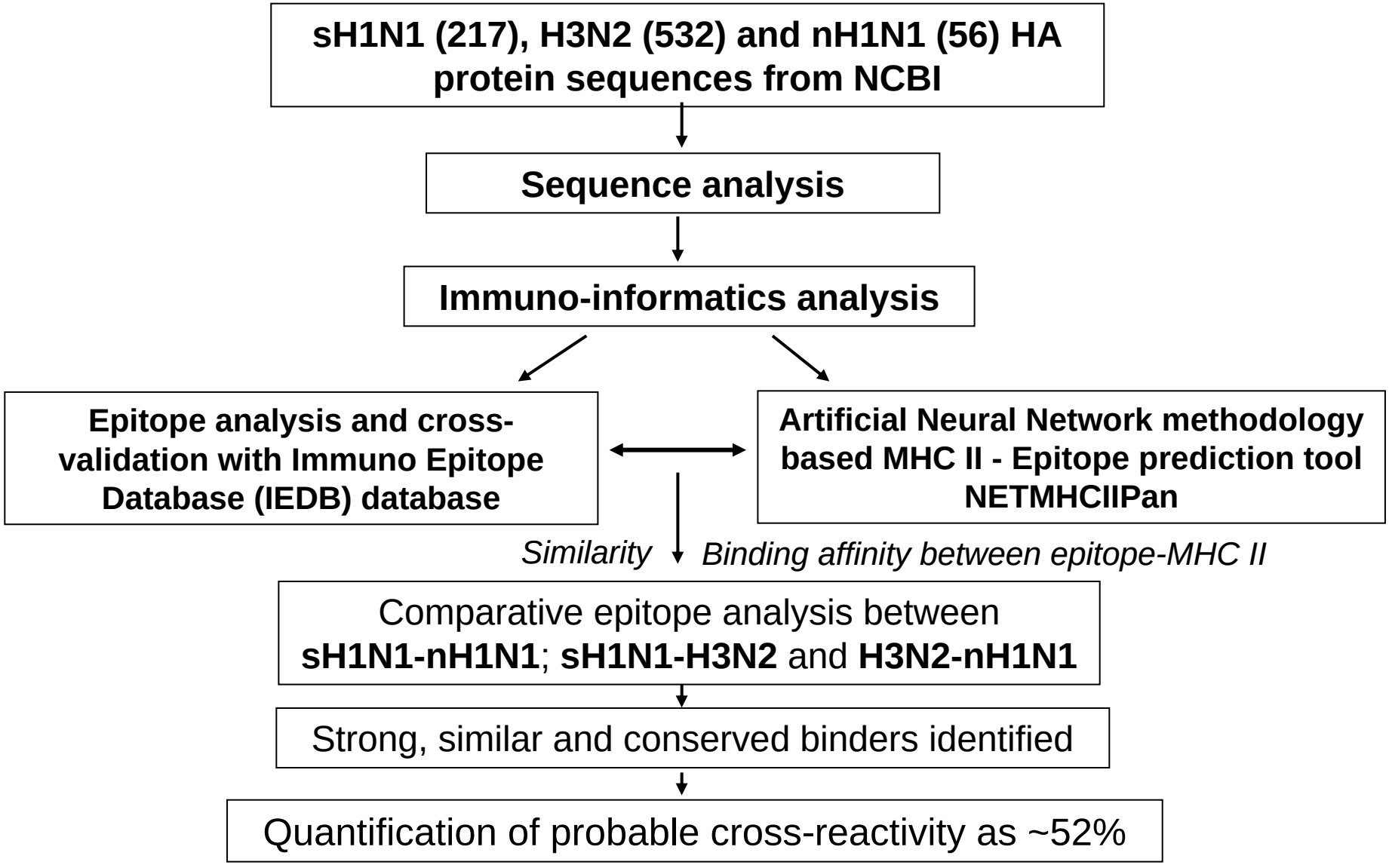
HA2 has been shown to induce strong CD4+ T-cell responses



Unique characteristics of 2009 H1N1

- **Lack of considerable antibody protection due to its unique genome composition**
- **However, nH1N1 has shown milder severity - WHY**
- **Cellular level immunity acquired from the existing circulating seasonal flu strains, H3N2 and sH1N1**
- **Almost all age groups are persistently exposed to seasonal flu strains since 30 years**

Bioinformatics



= conserved strong binders of both strains (119) /Total common binders of both strains (227)

Note: sH1N1 (1985, 1989, 1990, 1992, 1993, 1994, 1997, 1998, 1999, 2004) sequences and H3N2 (1979, 1981, 1982, 1984, 1987, 1989, 1991, 1992) sequences are not available in NCBI Influenza database. Hence, are not reflected in the figures. Mutated epitopes within same year are represented as year followed by a,b,c so on. Accesssion numbers are given in Table S8

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Comparative Epitope Conservancy

Epitopes mapped on HA protein

Results from Bioinformatics

- 25 common and strong MHC II epitopes were identified between sH1N1 and nH1N1
- 85% – 100% similarity observed between 25 epitopes of sH1N1 and nH1N1
- 35% to 66.6% similarity observed between epitopes of H3N2 and nH1N1
- Quantified the probable cross-reactivity ~52% (sH1N1 and nH1N1)

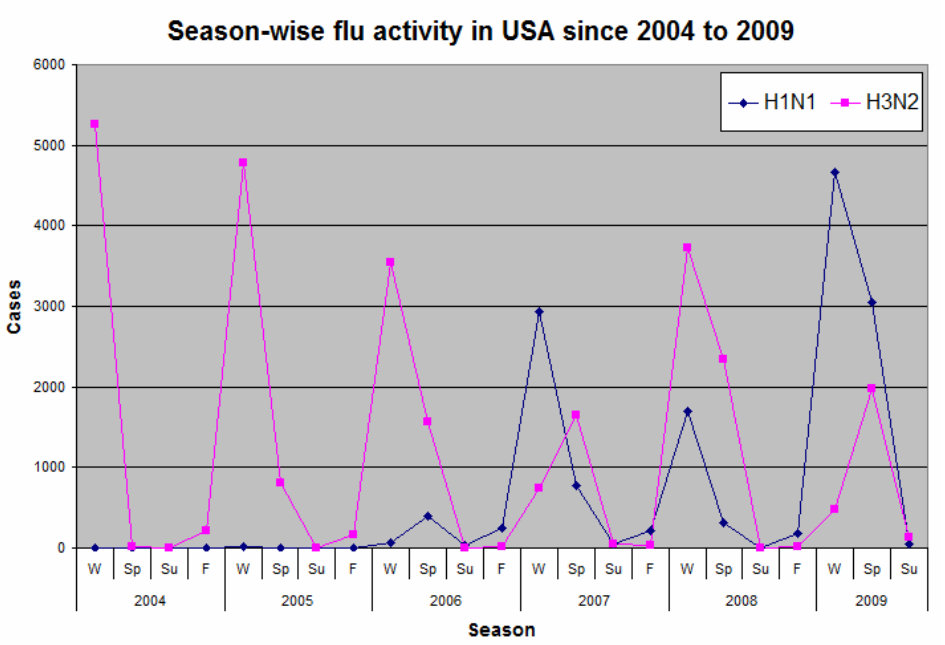
Cellular level (CD4+) T-cell memory might be the reason for mild severity of nH1N1

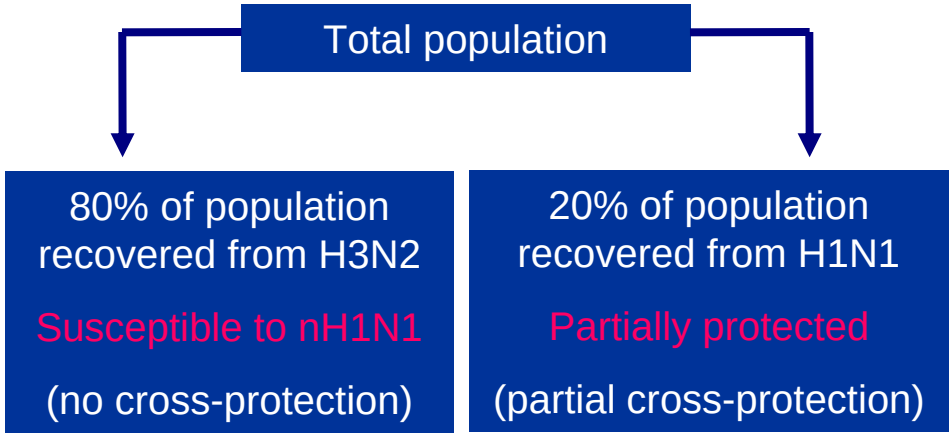
Stochastic dynamic model

(Markov-Chain Monte-Carlo method)

- To examine the implications of cross-reactivity on the transmission dynamics of nH1N1, **Stochastic dynamic model** was developed.

- Model formulation: Of both seasonal flu strains (H3N2 and H1N1), H3N2 has been the dominant (80%) circulating strain based on the past 32 influenza seasons, (1976/77 to 2003/2004 from Grenfell et al, *Science*, 2006 and 2004/2005 to 2008/2009 our analysis)





Model

(*Rs*) Individuals susceptible to nH1N1 infection with prior exposure to H3N2 and with prior exposure to sH1N1 (*Rp*); exposed to nH1N1 (*Es*, *Ep*), asymptomatically infected with nH1N1 (*As*, *Ap*), symptomatically infected with nH1N1 (*Is*, *Ip*), and recovered/immune to nH1N1 infection

Parameter values extracted from the published literature

Parameter	Baseline values
R_0	1.4 (range: 1.25–1.8)
δ_p	0.48 from our analysis
δ_A	0.5
γ_s	1.5 $(\text{days})^{-1}$
γ_p	1.5, 2.6, 4.3, 6 $(\text{days})^{-1}$
p_s	0.6
p_p	0.3
η_s, η_p	4.1 $(\text{days})^{-1}$
α_s	5 $(\text{days})^{-1}$
α_p	7 $(\text{days})^{-1}$
β	variable

N is the total population; 2000

β is the baseline transmission rate;

δ_p is the reduction factor in transmissibility of nH1N1 due to prior exposure to sH1N1

δ_A is the reduction in infectiousness of asymptomatic infection;

$1/\gamma_s$ and $1/\gamma_p$ are the incubation periods;

p_s and p_p are the probabilities of developing symptomatic infection;

η_s and η_p are recovery rates from asymptomatic infections;

α_s and α_p are the recovery rates from symptomatic infections

Stochastic simulations for the time-courses of epidemics with different incubation periods

Infection curves correspond to incubation periods of 1.5 days (black curves), 2.6 days (blue curves), 4.3 days (red curves), and 6 days (green curves), with no cross-protection in (a) and 52% cross-protection in (b)

Assumptions from Literature: Cross-immunity could result in prolonged incubation period, reduced severity of the disease, reduced infectiousness, and a longer delay in the illness peak of the epidemic.

Summary

- Study highlights the role of pre-existing T cell memory responses against an emerging influenza virus.
- **Model results corroborates with the assumptions** that there could be heterotypic immune response viz. **cellular level cross-protection.**
- Cellular level cross protection can dampen the "transmission dynamics" even in the absence of measurable antibody protection.
- A further practical implication of these findings is towards the development of epitope based vaccines for influenza.

Thank You