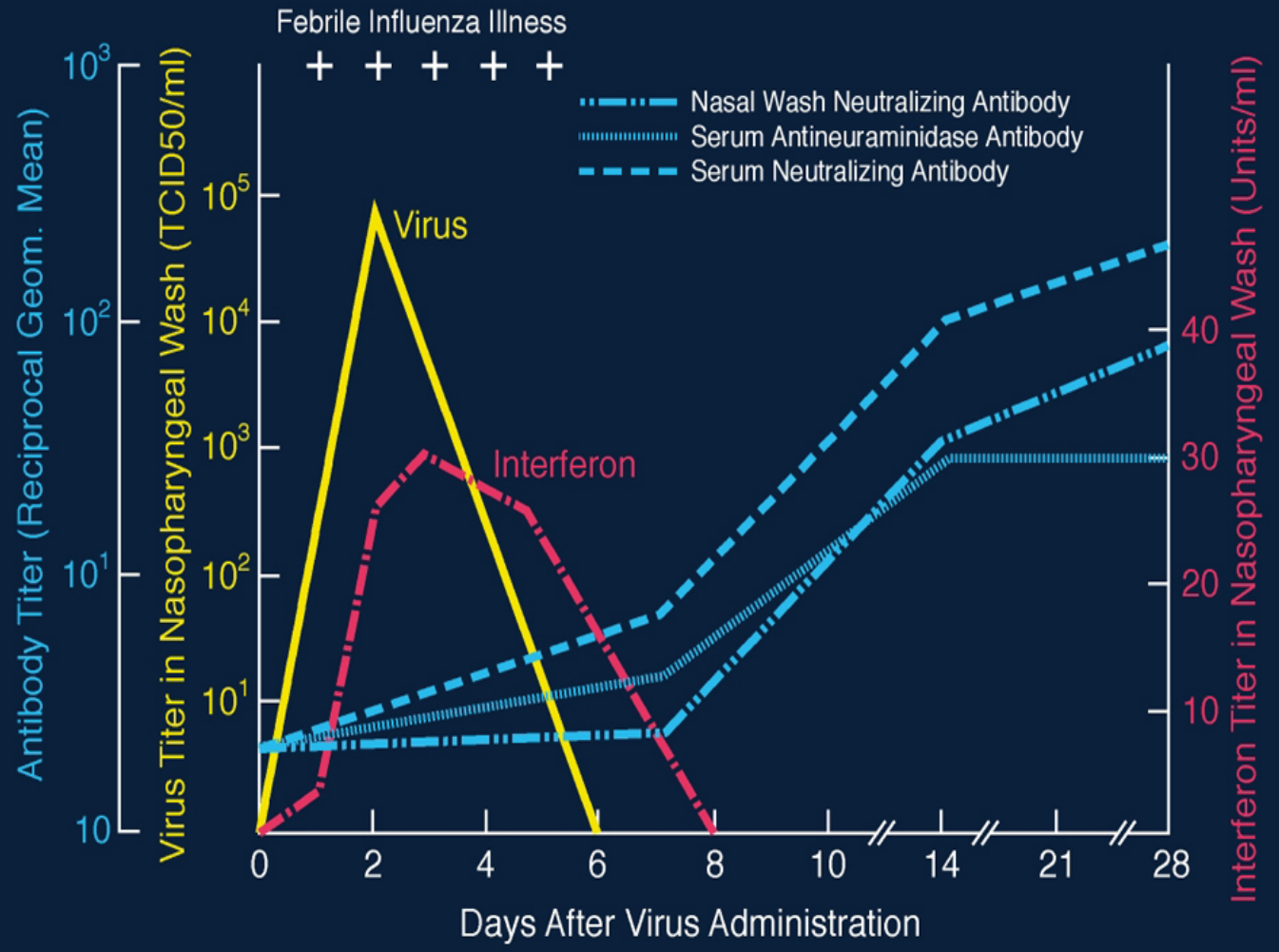
Immunity to Influenza A Virus in Humans

Brian Murphy

NIAID

Experimental Infection of Man with Influenza Wild Type Virus (H3N2)



Implications for the Rapid Rate of Replication in Humans

Facts/Observations

1- Single cycle growth curve = 8-10 hours.

2- Illness with titers >10^4 shed seen within 24 hrs after giving $10^{4.5}$ pfu of a Flu A H3N2 wt virus.

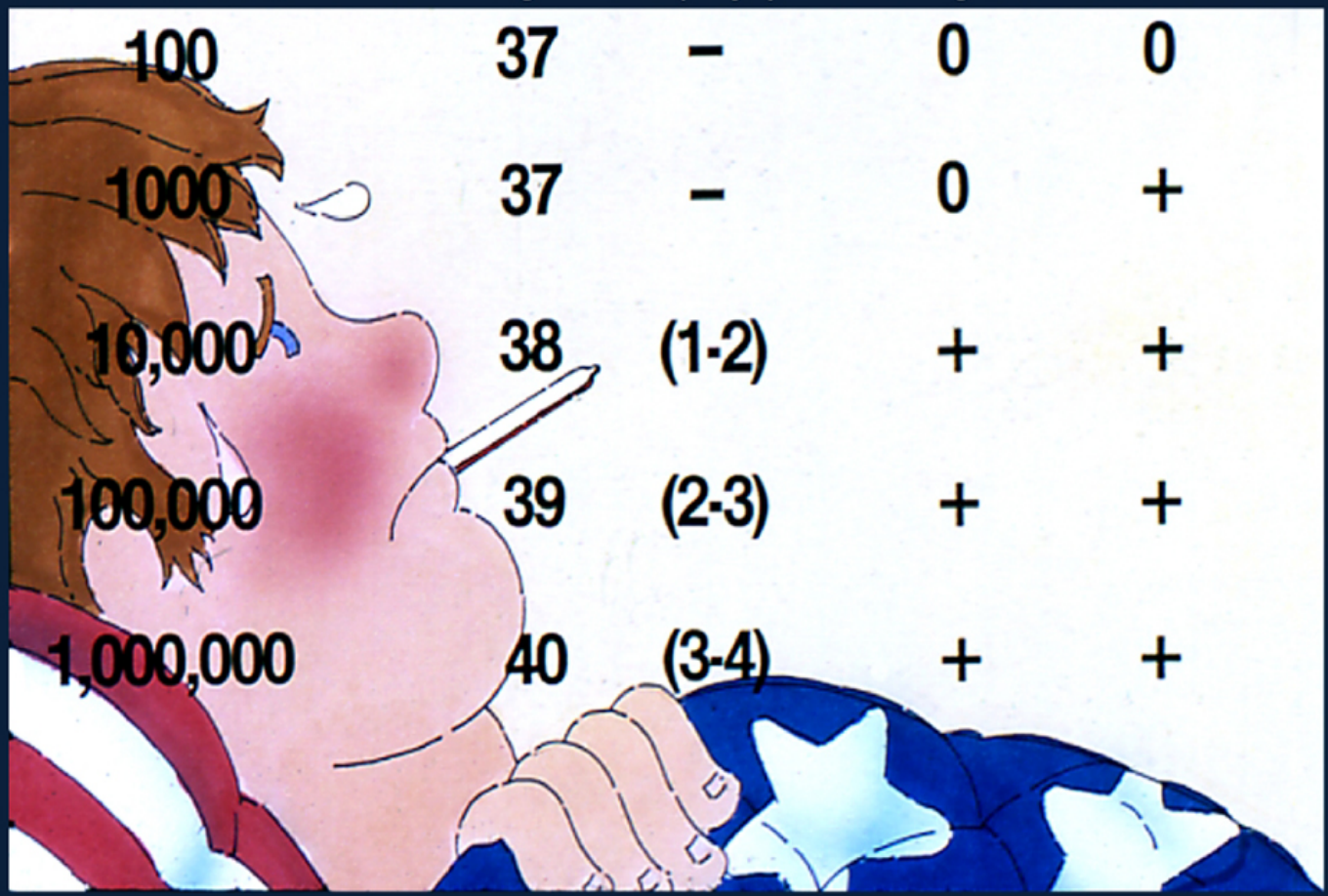
Implications

1- Immune mediators present at time of exposure are the major players in resistance

2- Immune factors, either cellular or humoral, generated from memory that require infection to be initiated and immune cells to be replicated and activated make minor contribution

Illness Experience

Number of Infectious Virus in Nose and Throat Secretions	Temperature Centigrade	Duration of Fever (Days)	Cough	Cold or Sore Throat
100	37	–	0	0
1000	37	–	0	+
10,000	38	(1-2)	+	+
100,000	39	(2-3)	+	+
1,000,000	40	(3-4)	+	+



Implications on the Correlation Between Level of Virus Replication and Clinical Response

Observations

1- Illness correlates with peak virus titer

2- Titers of 10^1 to 10^3 = asymptomatic or URI

3- Titers of 10^6 to 10^7 = 104-105°F fever

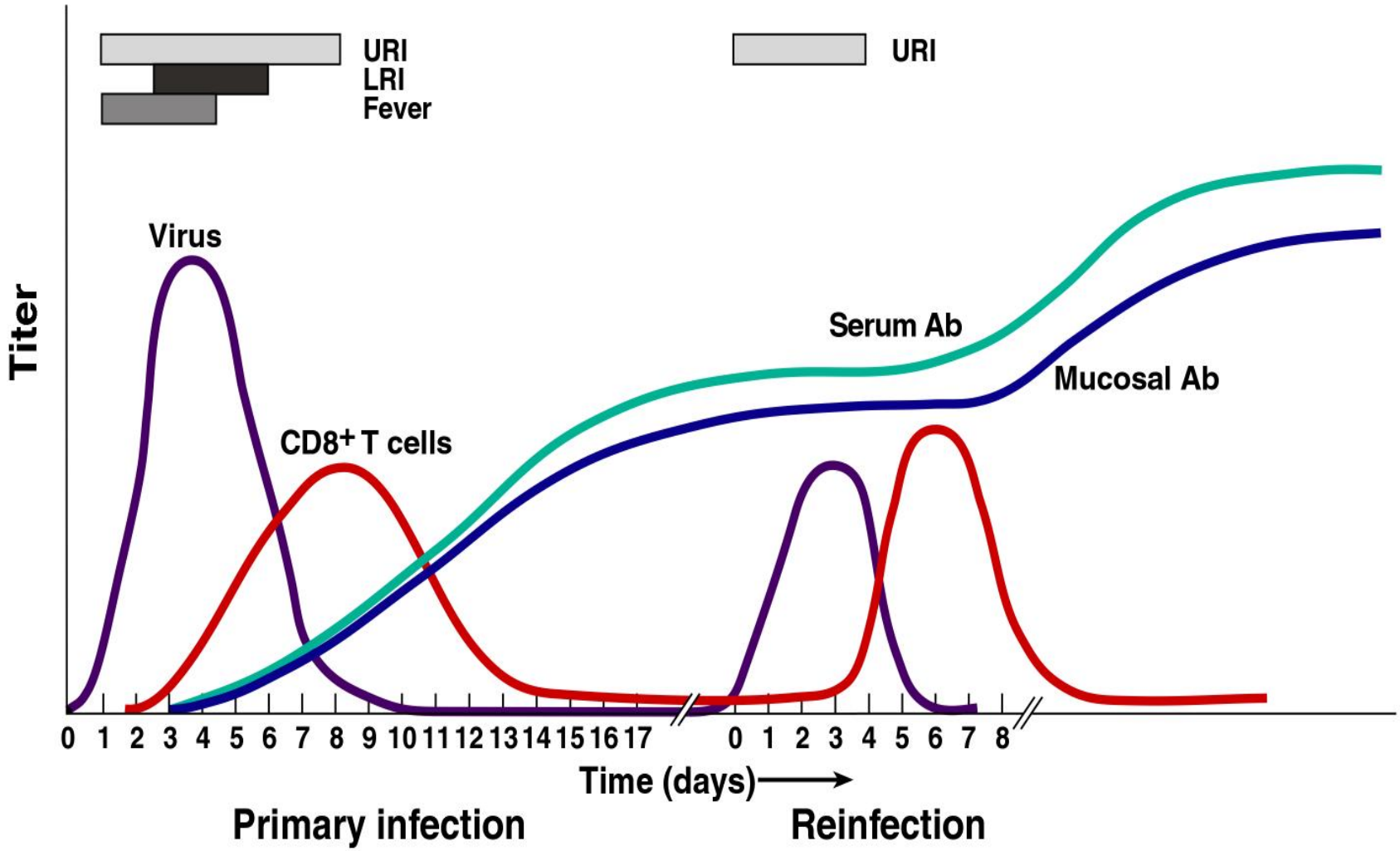
4- Peak titer achieved early after infection

Implications

1- Job of immune system is to keep peak titer < 10^3

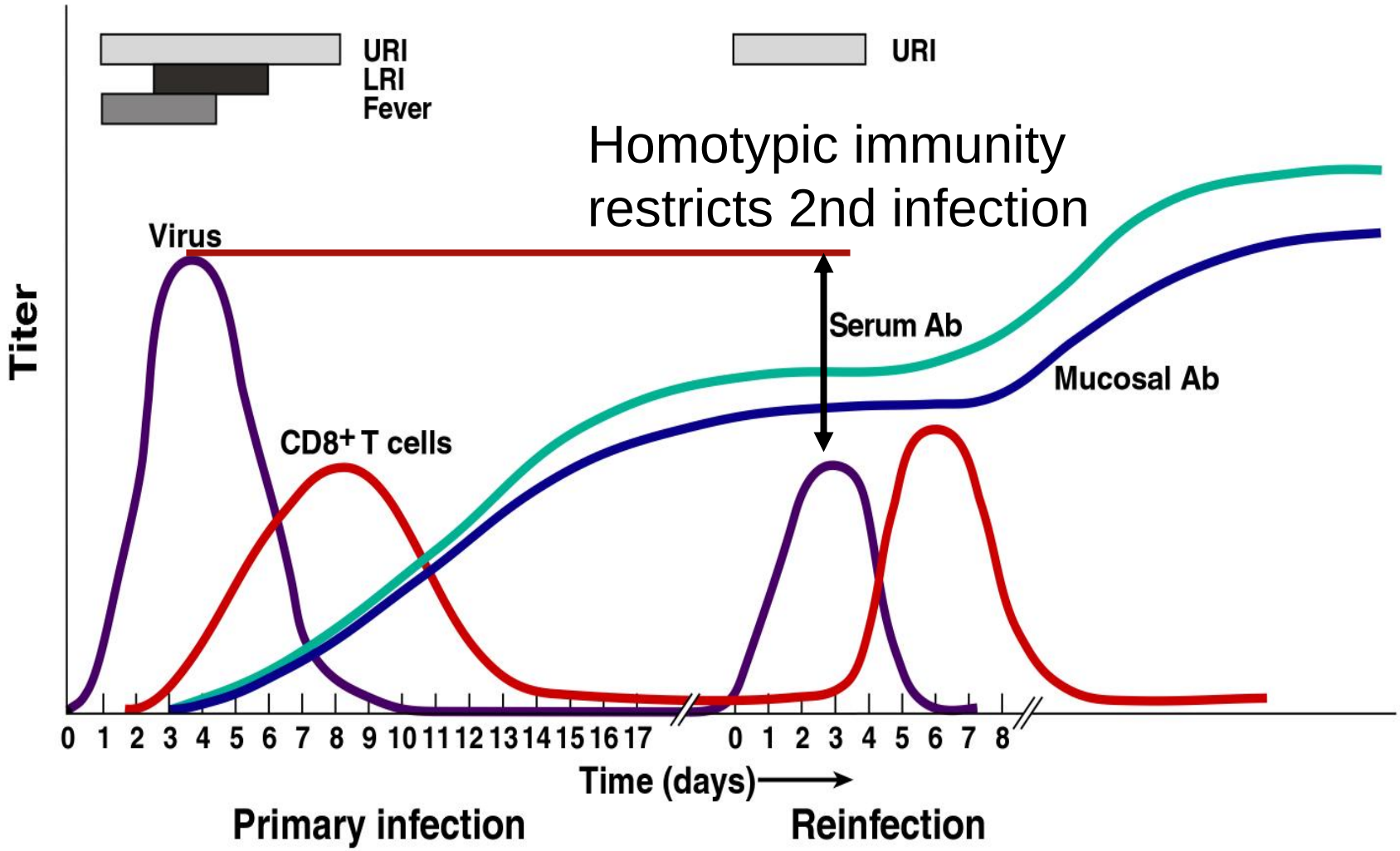
Note: Live *att* vaccines replicate to < 10^3

Course of Immune Response During Influenza Infection



Source: Subbarao et al. *Immunity* 24, 5-9 (2006)

Course of Immune Response During Influenza Infection



Source: Subbarao et al. *Immunity* 24, 5-9 (2006)

Lessons Learned From Experiments of Nature

1- 1977 H1N1 – *Long duration of HA/NA specific immunity seen in those > 20-25 years of age.*

2- Antigenic shift and drift – drift and shift viruses selected based on ability to escape neutralizing antibody to HA
Thus - Neut antibody to HA important for immunity

3-1957/1968 - H3N2 (68) epidemic in US milder than H2N2 (57)
- *Immunity to N2 NA likely played a role in resistance to H3N2*

4- Severe epidemics in 1957 and 1968 despite heterosubtypic immunity - *heterosubtypic immunity is weak in humans*

Mediators of Immunity to Influenza Virus

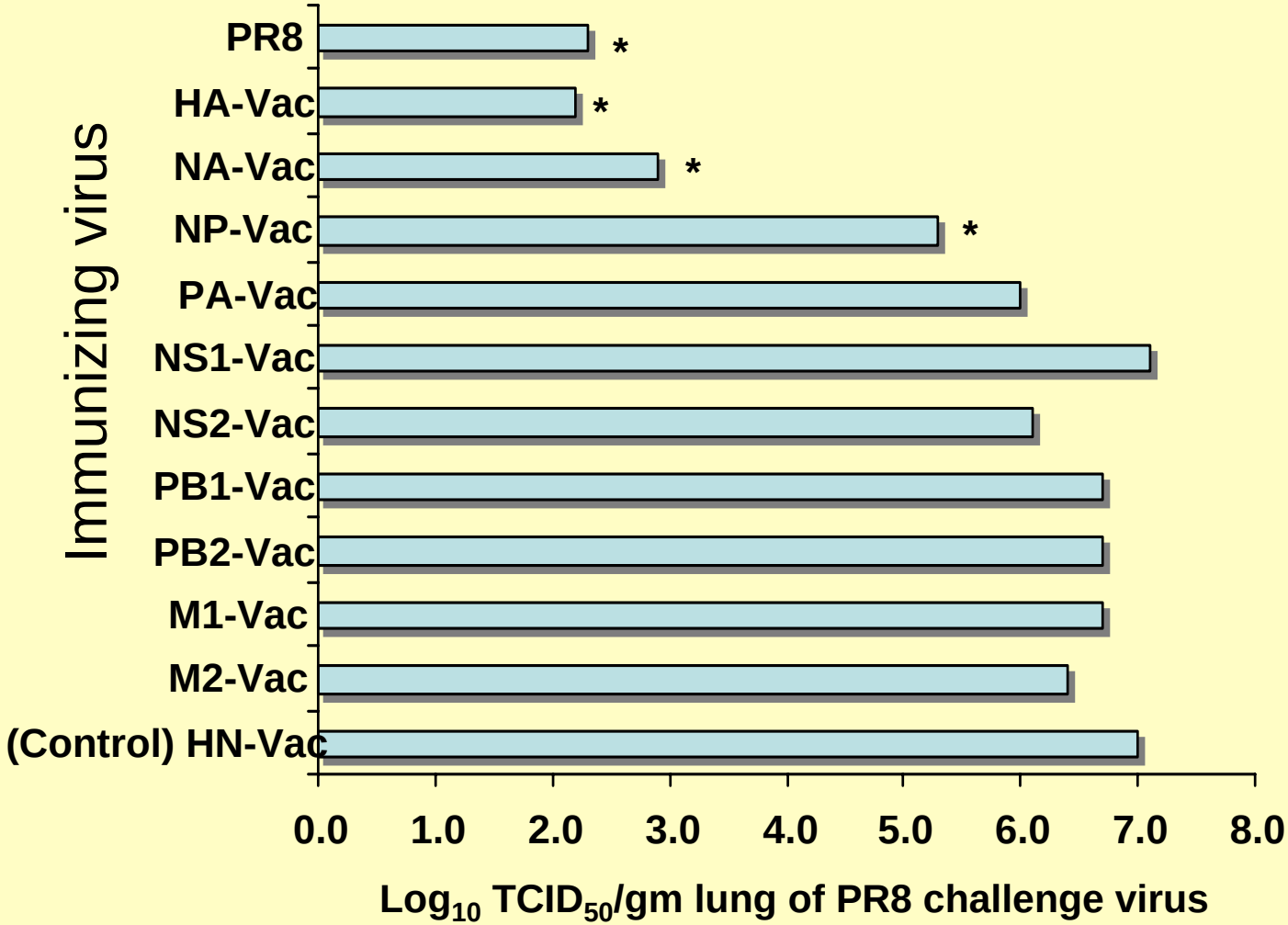
1- Protective antigens
- animal studies
- human studies

2- Evidence for a role for serum antibodies to HA and NA

3- Evidence for a role for mucosal antibodies to HA and NA

Protective Antigens - Animal Studies

The HA and NA genes of influenza A virus are the primary determinants of immunity in mice



Level of replication of A/PR8 challenge virus in mice 30 days after immunization with a vaccinia recombinant virus.

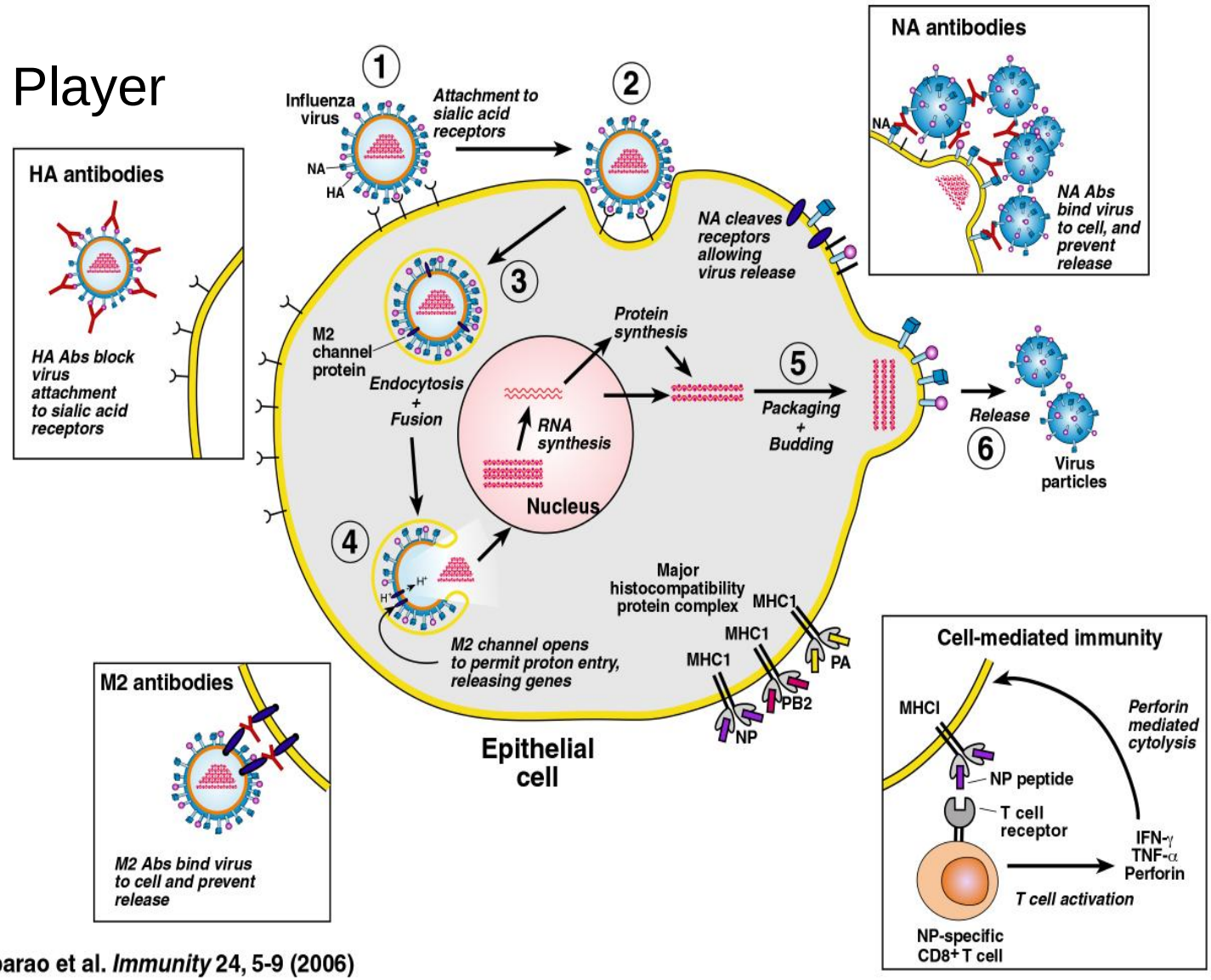
*Significantly reduced

Epstein et al., J Immun; 13:5484, 1993

Summary of Observation from Natural Infections and Animal Studies

Adaptive Immune Response During Influenza Infection

Major Player

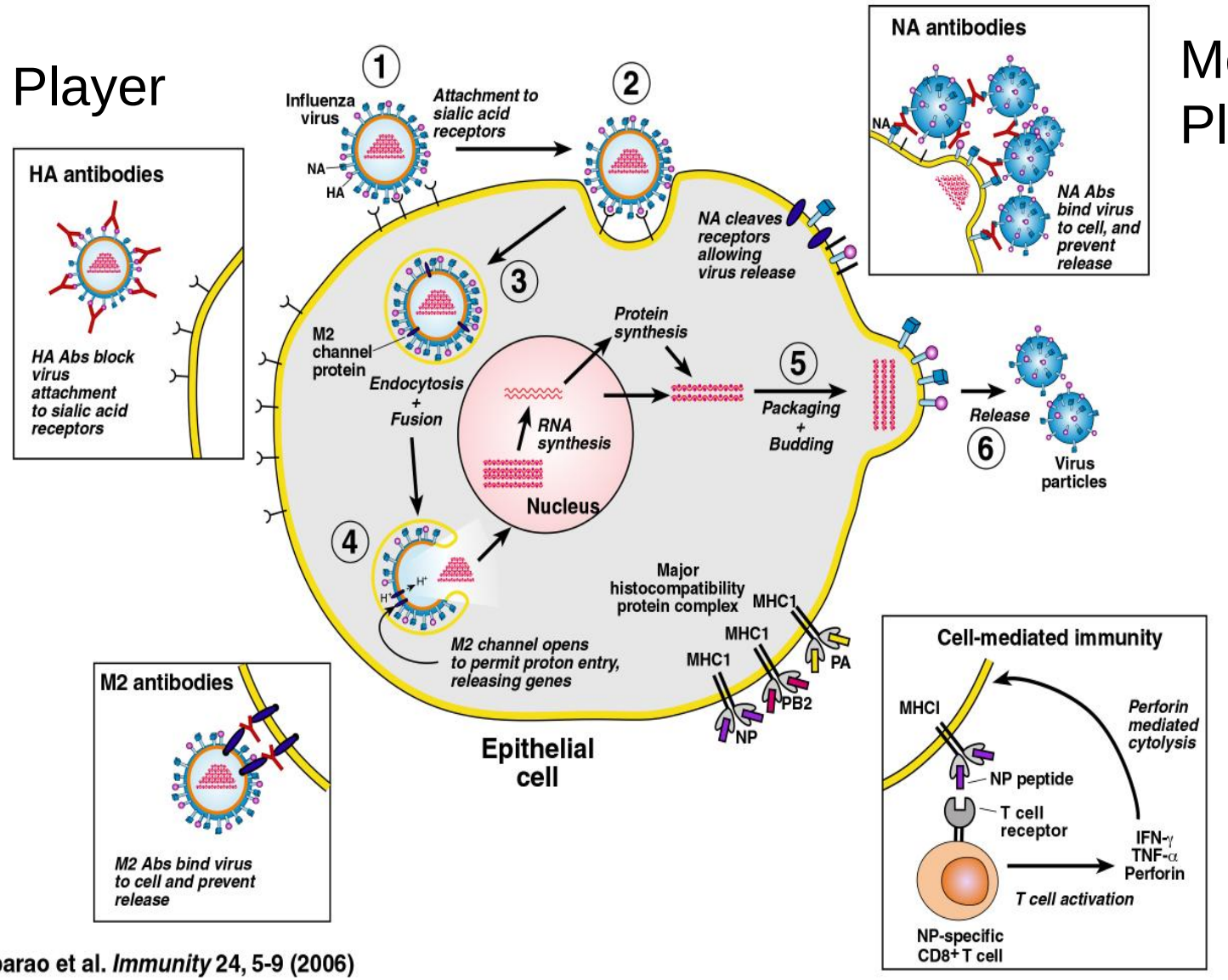


Source: Subbarao et al. *Immunity* 24, 5-9 (2006)

Adaptive Immune Response During Influenza Infection

Major Player

Moderate Player



Source: Subbarao et al. *Immunity* 24, 5-9 (2006)

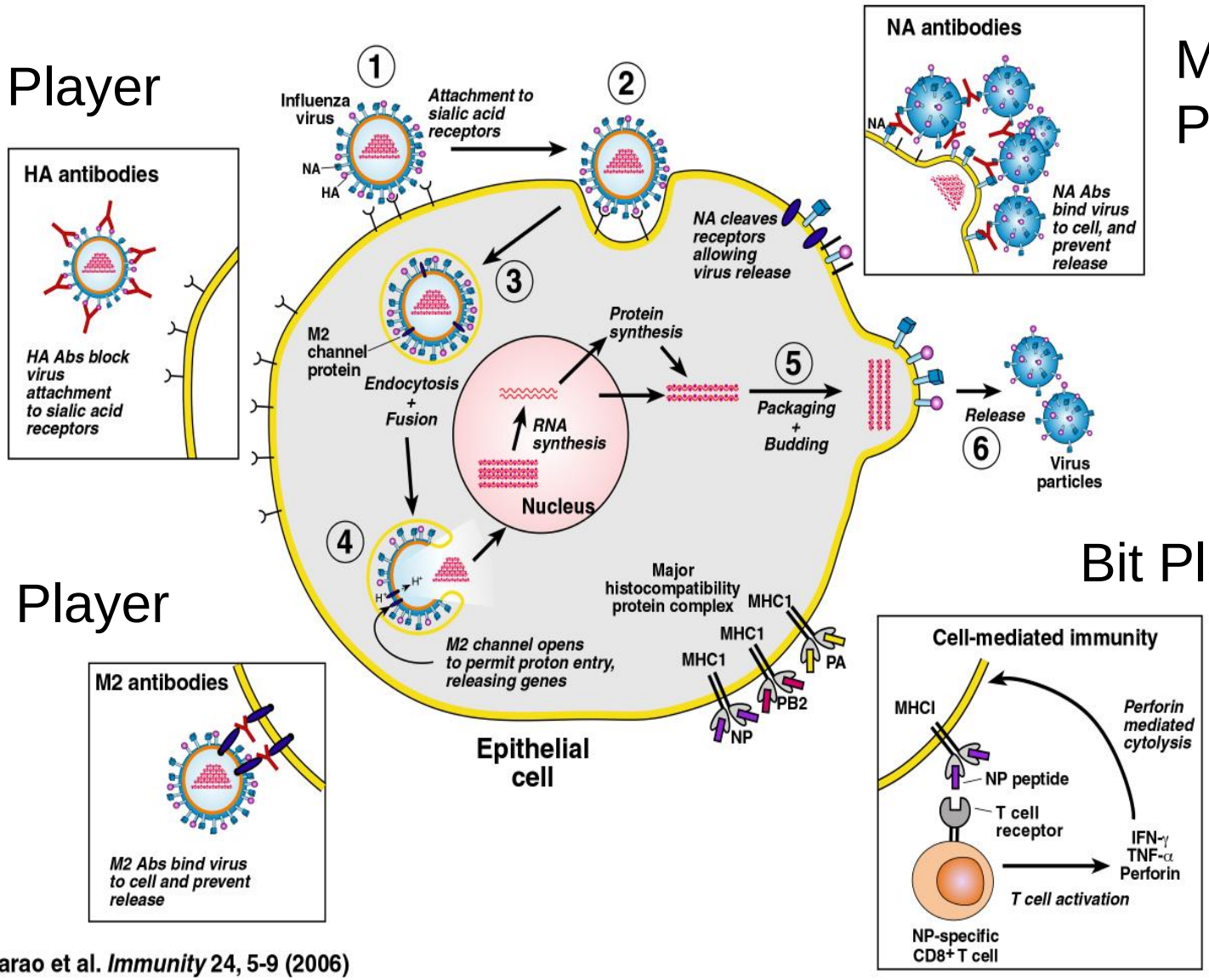
Adaptive Immune Response During Influenza Infection

Major Player

Moderate Player

Bit Player

Bit Player



Source: Subbarao et al. *Immunity* 24, 5-9 (2006)

Role for Serum Antibodies in Immunity to Influenza in Animals

Animals

a) Passive transfer of antibodies protect

Passively Administered Antibody Protects the LRT Better than the URT against Respiratory Syncytial Virus (RSV)

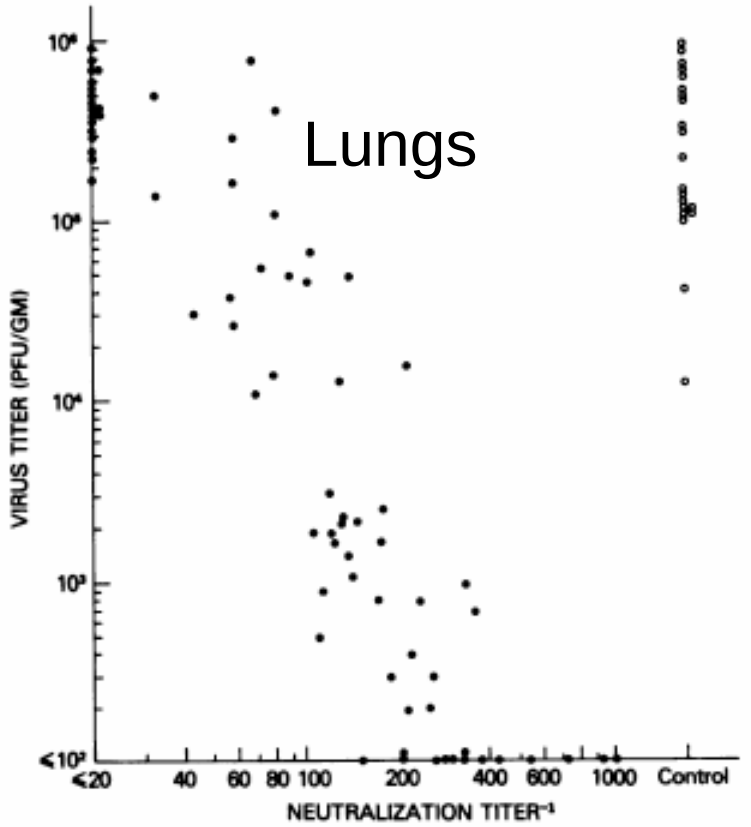


FIG. 1. Relationship of pulmonary virus titer and serum neutralizing antibody titer in animals receiving either immune cotton rat serum (●) or control cotton rat serum (○) intraperitoneally 24 h before intranasal challenge with 10^4 PFU of RSV. Animals were bled at the time of viral challenge; animals were sacrificed and the lungs were homogenized 4 days later.

Nasal Turbinates

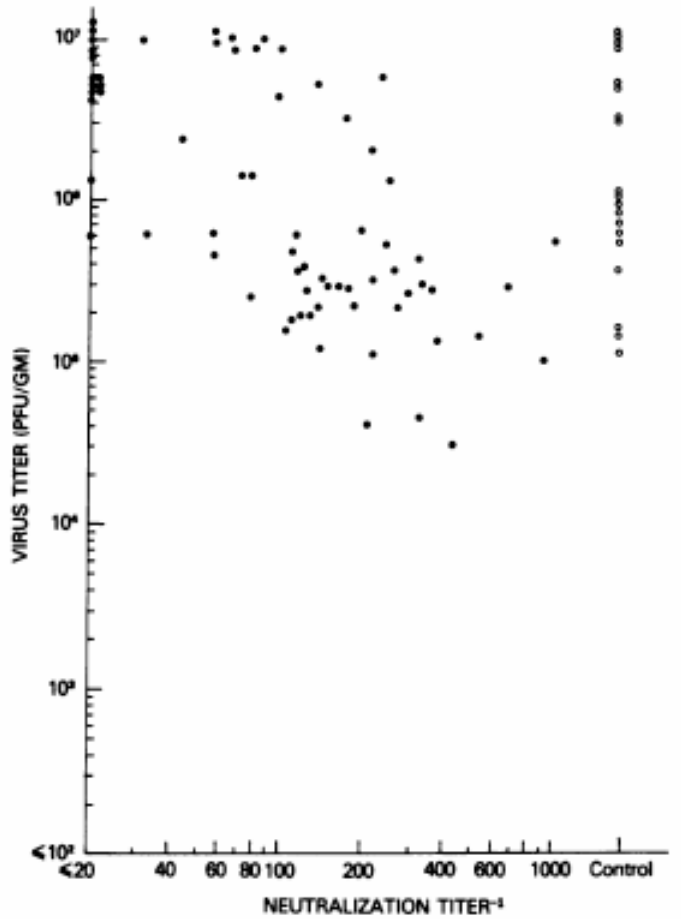


FIG. 2. Relationship of nasal virus titer and serum neutralizing antibody titer in animals receiving either immune cotton rat serum (●) or control cotton rat serum (○) intraperitoneally 24 h before intranasal challenge with 10^4 PFU of RSV. Animals were bled at the time of viral challenge; animals were sacrificed and the nasal tissues were homogenized 4 days later.

Prince et al, J Virol: 55:517, 1985

Role for Serum Antibodies in Immunity to Influenza in Humans

Humans

a) High titer of maternal antibodies to influenza results in increase in age of infant when flu illness occurs

b) Challenge studies that show that serum HA or NA antibodies independently contribute to restricted replication of virus

Protective Antigens - Human Studies

1- Anti-NA antibodies in serum associated with resistance

Relation Between Serum NA Antibody (anti-N2) Titer and Clinical Response of HA Antibody Negative Volunteers to Challenge with Wild Type H3N2 virus

Serum Anti-NA Titer†	Subjects not Ill	Subjects Ill: Afebrile & Febrile	Totals
<1:4	1*	10	11
>1:4	6	4	10

Note: Study conducted shortly after H3N2 virus arrived in 1968 - so HAI seronegative subjects available.

*$p < 0.05$, Fisher exact test (2-tailed).

†Hequi 1-N2(68) antigen used in neuraminidase inhibition assay - NA antibody induced by natural infection with H2N2 viruses which circulated from 1957 to 1968.

Murphy et al., NEJM;286:1329, 1972.

Relation between Serum NA Antibody Titer and Quantity of Virus Recovered from HA Antibody Negative Volunteers Infected with Wild Type H3N2 Influenza A Virus Challenge

Clinical Response	No. of Subjects	Serum Anti-NA Antibody with Use of Hequi 1-N2(68) Enzyme (Reciprocal Geometric Mean)	Titer of Virus in Nasopharyngeal Wash (Geometric Mean) ($log_{10}TCID_{50}$/ML)
Not ill	6	6.7*	1.5 †
Afebrile illness	6	3.8	1.7**
Febrile illness	6	2.0*	4.9 †**

* Statistically significant difference - student t-test $p < 0.05$

†,** Statistically significant difference - student t-test $p < 0.005$. Note: all volunteers infected.

Murphy et al., NEJM;286:1329, 1972.

Independent Contribution of NA Antibodies in Serum and Nasal Wash to Decreased Replication of WT Influenza Challenge Virus

Protective antigen	Decreased replication of virus associated with antibody in:	
	Serum	Nasal Wash
	IgG	IgA or IgG
HA	+	+
NA	+	Not Tested

Level of antibody versus level of virus replication in 163 volunteers challenged with wild type H1N1 or H3N2 virus. * Presumed to be IgG

Clements, et al. J. Clin. Micro. 24: 157, 1986

Conclusions on the Role of NA Antibodies in Resistance to Replication of Wild Type Virus

1- NA antibodies clearly associated with resistance

2- Prevent disease, not infection

3- Moderate strength

4- Prevent disease by restricting replication of virus
- magnitude and duration of virus replication reduced

5- Antibodies in serum associated with resistance - mucosal NA antibodies not measured

Protective Antigens - Human Studies

1- HA antibodies associated with resistance

2- Contribution of serum and mucosal HA antibodies to resistance

Relation between Pre-inoculation Nasal Wash or Serum ELISA HA Antibody Titer and Resistance to Infection with Influenza A/Alaska/6/77 *ca* Vaccine Virus.

Response to vaccine[a]	No. of volunteers tested	Mean pre-inoculation HA antibody titer (reciprocal)[b]		
		Serum		Nasal wash IgA
		IgG	IgA	
Not Infected	8	8.7±0.6	6.4±0.6	4.0±0.5[c]
Infected	29	8.1±0.3	6.6±0.3	1.5±0.5

[a] Virus recovery or antibody response or both signified infection.
[b] Log_2 titer plus or minus the standard error.
[C] $P < 0.005$ by the Wilcoxon rank sum test (two tail).

Clements et al., Infect and Immun;40:1044, 1983

Antibody to HA (neut Ab) in Nasal Wash can Mediate Resistance to H3N2 Wild-type Virus Challenge

Group (no. of volunteers)	Antibody titer before challenge*			No. of men with indicated response to wild-type influenza A challenge virus†		
	Neutralizing antibody (anti-HA)		Antibody to NA	Febrile illness	Shed virus	Immunologic response
	Nasal wash	Serum	Serum			
H3N2 *Ts*-1[E] vaccinees						
A (5)	33	6.3	<2	0	0	2
B (7)	34	49	10	0	0	5
Seronegative controls (7)	<4	<4	2.4	6	7	7

*Reciprocal geometric mean titers; samples of sera from vaccinees and controls taken seven day and four days, respectively, before challenge with wild-type virus.

†Volunteers received $10^{4.5}$ $TCID_{50}$ of influenza A/Bethesda/68 (H_3N_2) HEK-2 intranasally 35 days after vaccination.

Murphy, et al. J. Infect. Dis. 128:479, 1973

Independent Contribution of HA Antibodies in Serum and Nasal Wash to Decreased Replication of WT Influenza Challenge Virus

Protective antigen	Decreased replication of virus associated with antibody in:	
	Serum	Nasal Wash
	IgG	IgA or IgG
HA	+	+
NA	+	Not Tested

Level of antibody versus level of virus replication in 163 volunteers challenged with wild type H1N1 or H3N2 virus. * Presumed to be IgG

Clements, et al. J. Clin. Micro. 24: 157, 1986

Conclusions on the Role of HA Antibodies on Resistance to Replication of Wild Type Virus

1- Anti-HA antibodies clearly associated with resistance

2- Prevent both disease and infection

3- Strongest antibody

4- Prevent disease by preventing/restricting replication of virus
- magnitude and duration of virus replication reduced

5- Serum and mucosal antibodies independently contribute to resistance

Heterosubtypic immunity -
Evidence suggests that it is weak in humans

Effects of Heterosubtypic Immunity to Influenza A Virus in Children who Received Live Attenuated Influenza A Virus Vaccine [A]

Vaccine virus	Heterotypic immunity induced by:	No. of vaccinees	% Infected	Vaccine shedding		Antibody response to vaccine virus		
				%	Score	% with Sero-conversion	Mean ELISA antibody titer[B]	
							Pre-vaccination	Post-vaccination
H3N2*ca*	Wild type H1N1 virus							
	HAI ≥ 1:8 (6.2[B])	21	81	33	2.7	76	7.5	9.6
	HAI < 1.8 (2.0 [B])	27	71	46	3.9	74	6.7	9.5
H1N1*ah*	Live H3N2 [C] vaccine							
	Infected	17	88	59	2.0	82	8.5	11.8
	Not infected	22	91	41	1.9	91	7.6	10.9

[A] The differences in means and proportions between vacinees with and without serotypic immunity do not reach statistical significance.

[B] Mean of reciprocal $\log_2$ antibody titers.

[C] Infected subjects are those who demonstrated H3N2 vaccine virus shedding or seroconversion among children who participated in H3N2 vaccine studies; uninfected subjects were not infected in H3N2 vaccine studies and had H3N2 HA* titers of <1:8 (2.0)

Steinhoff et al., JCM, 31:836, 1993

Effects of Heterosubtypic Immunity to Influenza A Virus in Children who Received Live Attenuated Influenza A Virus Vaccine [A]

Vaccine virus	Heterotypic immunity induced by:	No. of vaccinees	% Infected	Vaccine shedding		Antibody response to vaccine virus		
				%	Score	% with Sero-conversion	Mean ELISA antibody titer[B]	
							Pre-vaccination	Post-vaccination
H3N2	Wild type H1N1 virus							
IMMUNE →	HAI ≥ 1:8 (6.2[B])	21	81	33	2.7	76	7.5	9.6
NON-IMMUNE →	HAI < 1.8 (2.0 [B])	27	71	46	3.9	74	6.7	9.5
H1N1	Live H3N2 [C] vaccine							
IMMUNE →	Infected	17	88	59	2.0	82	8.5	11.8
NON-IMMUNE →	Not infected	22	91	41	1.9	91	7.6	10.9

[A] The differences in means and proportions between vacinees with and without serotypic immunity do not reach statistical significance.

[B] Mean of reciprocal $\log_2$ antibody titers.

[C] Infected subjects are those who demonstrated H3N2 vaccine virus shedding or seroconversion among children who participated in H3N2 vaccine studies; uninfected subjects were not infected in H3N2 vaccine studies and had H3N2 HA* titers of <1:8 (2.0)

Steinhoff et al., JCM, 31:836, 1993

Relative **strength** of the immune mediators of resistance to reinfection with influenza virus and illness upon reinfection

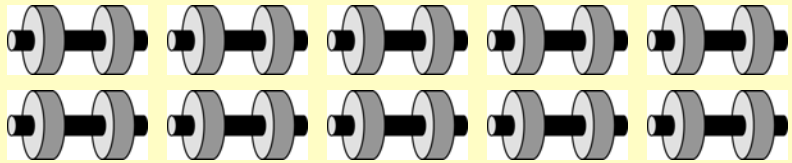
1- Anti-HA antibodies
   a) IgG (Serum)
   b) IgA/IgM (NW)

2- Anti-NA antibodies
   a) IgG (Serum)
   b) IgA/IgM* (NW)

3- All other: anti-M2;
   $CD8^+$ and $CD4^+$
   T-cells

Relative **strength** of the immune mediators of resistance to reinfection with influenza virus and illness upon reinfection

The immune strength scoring index - dumbbell units



1- Anti-HA antibodies
   a) IgG
   b) IgA/IgM

2- Anti-NA antibodies
   a) IgG
   b) IgA/IgM*

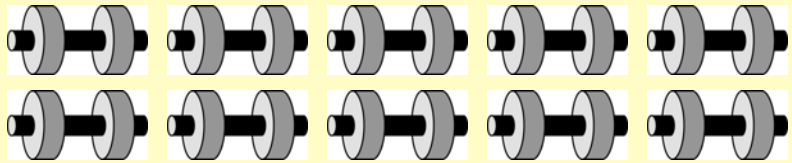
3- All other: anti-M2;
   $CD8^+$ and $CD4^+$
   T-cells

Relative **strength** of the immune mediators of resistance to reinfection with influenza virus and illness upon reinfection

1- Anti-HA antibodies

a) IgG

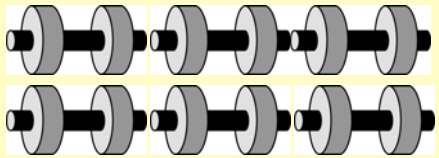
b) IgA/IgM



2- Anti-NA antibodies

a) IgG

b) IgA/IgM*



*

3- All other: anti-M2;

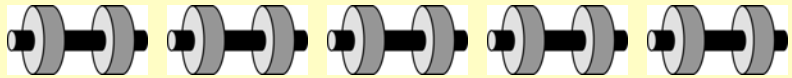
$CD8^+$ and $CD4^+$

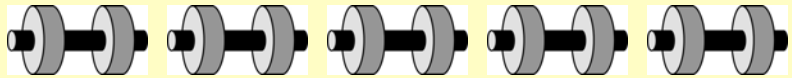
T-cells

* Educated guess - not aware of data to support this

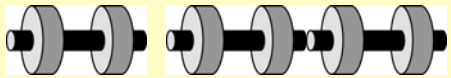
Relative **strength** of the immune mediators of resistance to reinfection with influenza virus and illness upon reinfection

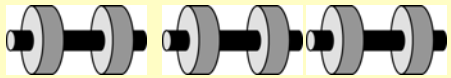
1- Anti-HA antibodies

a) IgG 

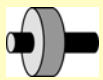
b) IgA/IgM 

2- Anti-NA antibodies

a) IgG 

b) IgA/IgM* 

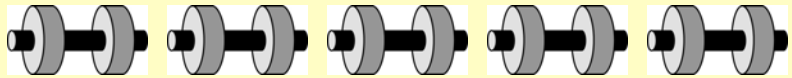
3- All other: anti-M2;

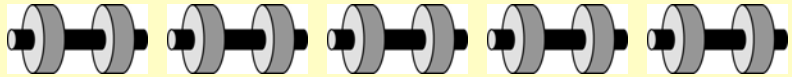
$CD8^+$ and $CD4^+$ 

T-cells

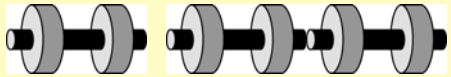
Conclusion

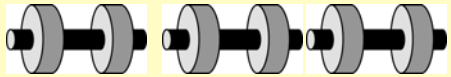
1- Anti-HA antibodies

a) IgG  1

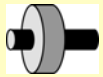
b) IgA/IgM  2

2- Anti-NA antibodies

a) IgG  3

b) IgA/IgM  4

3- All other: anti-M2;

$CD8^+$ and $CD4^+$ 

T-cells

Immunity = 1 + 2 + 3 + 4

No single correlate or surrogate of immunity