Virus-like Particle Vaccines for Influenza

Ted M. Ross, Ph.D.
University of Pittsburgh
Center for Vaccine Research

Vaccine Renaissance IV
Providence, Rhode Island
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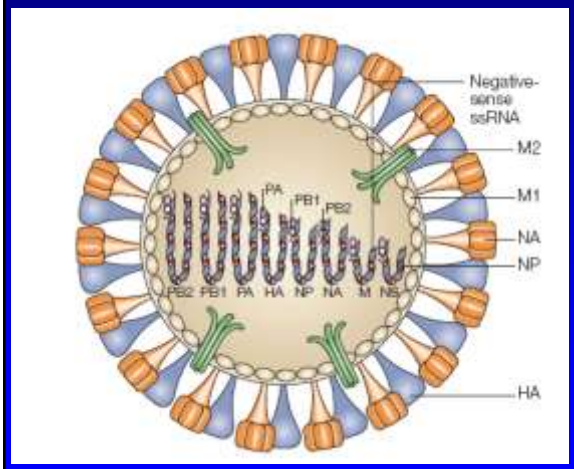
Influenza Viruses



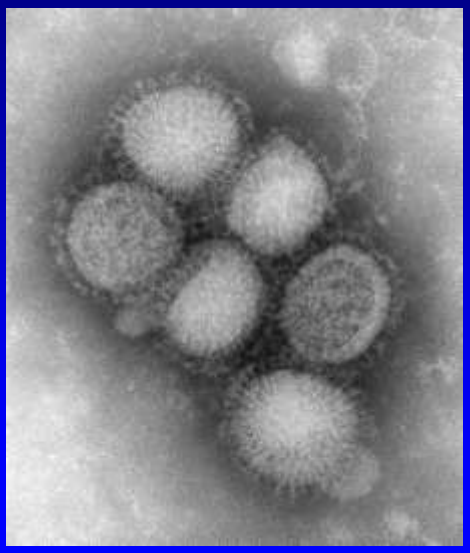
- Orthomyxoviridae
- SS negative-sense RNA virus
- Enveloped.
- Pleomorphic.
- Human pathogens: Influenza A and B
- 8 gene segments encoding at least 10 proteins

Nature Reviews Microbiology. 2005. 3:591.

Influenza Impact in U.S.

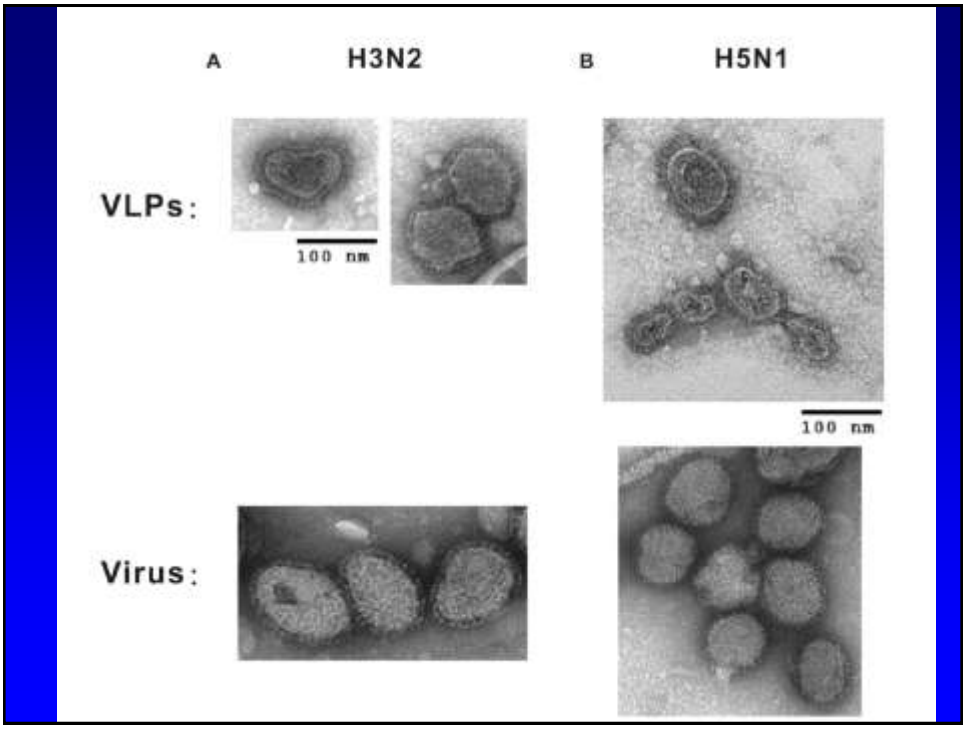
- Annual epidemics
  - 10% - 20% of US population infected
    - highest illness rates in children
    - highest complication rates in elderly
  - Annual average of 36,000 deaths
    - >90% among the elderly
- Occasional pandemics
  - > 500,000 deaths due to 1918 Spanish influenza
  - >100,000 deaths in 1957 and 1968

Novel H1N1 Influenza Virus-2009



Challenges for the Development and Production of a Pandemic Vaccine

- Little time to identify pandemic vaccine strain
- Production of vaccine out of traditional seasonal timeframe
  - Shortage of embryonated eggs
- Vaccine needs to be immunogenic in naïve population
  - Two doses and/or higher antigen dose needed
- Limited supply of vaccine antigen
  - Global shortfall



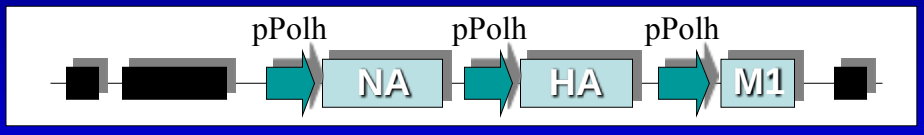
Goal

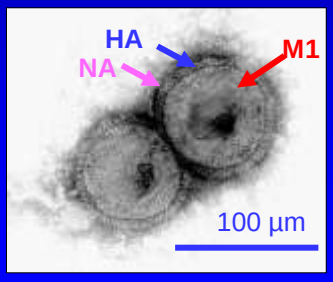
Design an effective non-replicating influenza virus-like particle vaccine

Hypothesis

An influenza virus-like particle vaccine will elicit broadly reactive immune responses to current and emerging influenza isolates

Recombinant Virus-like Particle Candidate Vaccine





- **Exact genetic match**
- **Correct 3D configuration of HA/NA proteins**
- **Efficient insect cell-based production**
- **No safety risks associated with live virus**

Seasonal Influenza VLPs

Influenza A

Influenza B

H1N1

A/New Caledonia/20/1999

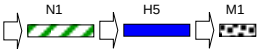
A/Solomon Is/03/2006

H3N2

A/Fujian/411/2002

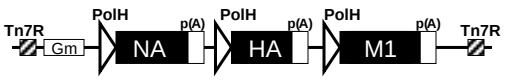
A/Wisconsin/67/2005

B/Shanghai/361/2002

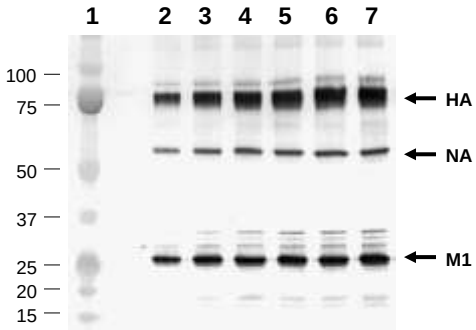


B/Malaysia//2006

A.

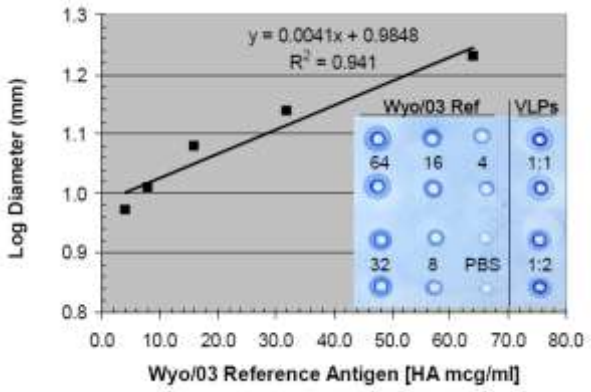


B.



Bright RA, Carter DM, Daniluk S, Toapanta FR… Vaccine. 2007. 25:3871-3878

Fig. 1C



Bright RA, Carter DM, Daniluk S, Toapanta FR… Vaccine. 2007. 25:3871-3878

Mice: BALB/c
Route: IM

Immunogen: VLP, rHA
VLP Dose: 3ug, 600ng, 120ng, 24ng
rHA Dose: 600ng

Vaccination: Week 0, 3

Vaccination Regimen

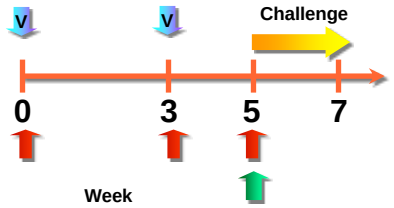


Table 1. Mouse responders to vaccination.

	Responders[a]				
Vaccine (Dose)	Wk 0	Wk 3	Wk 5	Wk 8	Wk 12
VLP (3 μg)	0/8	6/8	8/8	8/8	8/8
VLP (600 ng)	0/8	0/8	8/8	8/8	8/8
VLP (120 ng)	0/8	0/8	7/8	8/8	8/8
VLP (24 ng)	0/8	0/8	5/8	8/8	8/8
rHA (600 ng)	0/8	0/8	4/5	5/5	ND
WIV (600 ng)	0/8	2/8	8/8	8/8	8/8
Mock	0/8	0/8	0/8	0/8	0/8

[a]Number of mice exhibiting >4 fold rise in HAI titer (GMT >1:40) to A/Fujian/411/2002. Shaded values represent 100% responders.
[b]ug dose of HA in each vaccine.

Table 2. Hemagglutination-inhibition titer.

Vaccine (Dose)	Wk 0	Wk 3	Wk 5	Wk 8	Wk 12
VLP (3 μg)	10[a]	48	1174	1050	760
VLP (600 ng)	10	12	905	875	440
VLP (120 ng)	10	10	84	275	220
VLP (24 ng)	10	10	48	128	170
rHA (600 ng)	10	10	121	160	ND
WIV (600 ng)	10	40	494	505	260
Mock	10	10	10	10	10

[a]Average HAI GMT titer from vaccinated mice against A/Fujian/411/2002.
[b]ug dose of HA in each vaccine.
ND=not done

Bright RA, Carter DM, Daniluk S, Toapanta FR… Vaccine. 2007. 25:3871-3878

Table 3. Hemagglutination-inhibition titer.

	H3N2[a]					H1N1
Vaccine (Dose)[b]	Wisc/05	NY/04	Wyo/03	Fuj/02	Pan/99	PR/8/34
VLP (3 µg)	40[c]	32	1024	1174	75	10
VLP (600 ng)	20	75	608	905	80	10
VLP (120 ng)	10	10	478	104	67	10
VLP (24 ng)	10	10	212	67	20	10
rHA (600 ng)	10	10	225	121	10	10
WIV (600 ng)	10	10	625	494	70	10
Mock	10	10	10	10	10	10

[a]Influenza viruses used in the HAI assay using sera collected at week 8.
[b]ug dose of HA in each vaccine.
[c]HAI GMT titer of responding mice.

Bright RA, Carter DM, Daniluk S, Toapanta FR… Vaccine. 2007. 25:3871-3878

Ferrets

Table 4. Ferret responders to vaccination.

	Responders[a]		
Vaccine (Dose)[b]	Wk 0	Wk 3	Wk 5
VLP (15 µg)	0/6	5/6	5/6
VLP (3 µg)	0/6	6/6	6/6
VLP (600 ng)	0/6	1/6	2/6
VLP (120 ng)	0/6	0/6	2/6
rHA (15 µg)	0/6	4/6	4/6
Mock	0/6	0/6	0/6

[a]Number of ferrets exhibiting >4 fold rise in HAI titer (GMT >1:40) to A/Fujian/411/2002. Shaded values represent 100% responders.
[b]ug dose of HA in each vaccine.

Table 5. Hemagglutination-inhibition titer.

	H3N2[a]						H1N1
Vaccine (Dose)[b]	NY/04	CA/04	Wyo/03	Fuj/02	Well/01	Pan/99	NC/99
VLP (15 µg)	28[c]	640	570	905	508	46	10
VLP (3 µg)	31	160	403	640	226	70	10
VLP (600 ng)	14	50	90	320	143	67	10
VLP (120 ng)	10	10	28	184	70	50	10
rHA (15 µg)	16	80	202	254	143	56	10
Mock	10	10	10	10	10	10	10

[a]Influenza viruses used in the HAI assay.
[b]ug dose of HA in each vaccine.
[c]HAI GMT titer of responding ferrets.

Bright RA, Mahmood K, Crevar CJ, Carter DM,…2008.

Avian Influenza in Asia

Pandemic Influenza

- Occur sporadically
- Criteria for pandemic strain
  - New influenza A subtype in humans
  - Little or no immunity in population
  - Easily transmitted
- Likely U.S. impact of another pandemic without effective intervention
  - 20-47 million illnesses
  - Up to 730,000 hospitalizations
  - > 200,000 deaths

Elicitation of Protective Immune Responses to Pandemic H5N1 Influenza Following Immunization with A/Indonesia/05/2005 Virus-like Particle Vaccines

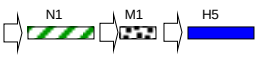
Bright RA, Carter DM, Crevar CJ, Toapanta FR… PLoS One. 2008. 3(1) e1501.

Pandemic Influenza H5N1 VLPs

H5N1

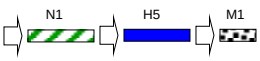
Clade 1

A/Viet Nam/1203/04



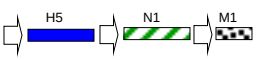
Clade 2

A/Indonesia/5/05



A/Bar headed goose/Qinghai/1A/05

A/Anhui/1/05



Bright RA, Carter DM, Crevar CJ, Toapanta FR… PLoS One. 2008. 3(1) e1501.

Recombinant VLP Vaccine Reduces Lead Time

CDC/WHO

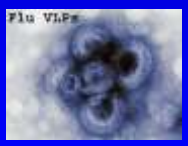
A/Indonesia/5/05 (H5N1)
HA, NA, and M1 baculovirus vector

rtPCR cloning
HA, NA, and M1 genes

Master and Working
Seed stocks

Day 1	Day 3	Day 7	Day 21	Day 30	Day 40	Day 45
rtPCR	Cloned	Bacmids	rBaculovirus	Master stock	Working stock	Seed stock

Wave Bioreactors
500 L working



HA/NA/M1 VLPs Secreted

Bulk VLPs

Days 47	Days 52	Day 60
cGMP Manufacturing	Purification	Formulation/Filling

Mice: BALB/c
Route: IM or IN

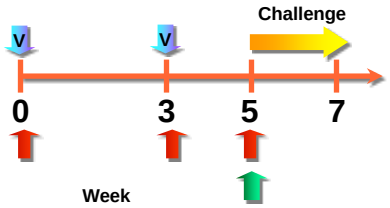
Immunogen: VLP, rHA
VLP Dose: 3ug, 600ng, 120ng
rHA Dose: 3ug

Vaccination: Week 0, 3

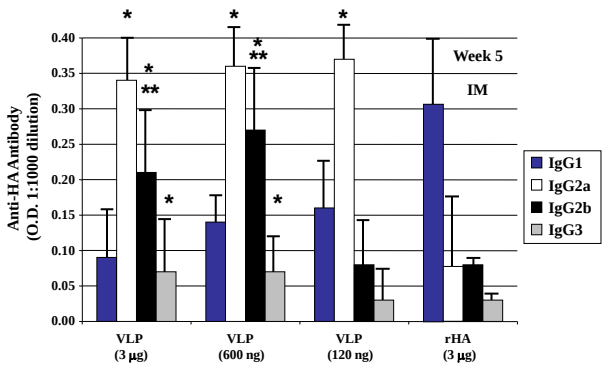
Serum: Weeks 0, 3, 5
Spleens/Lungs: Week 5

Challenge: 6:2 H5N1 reassortants- Clade 1 or 2

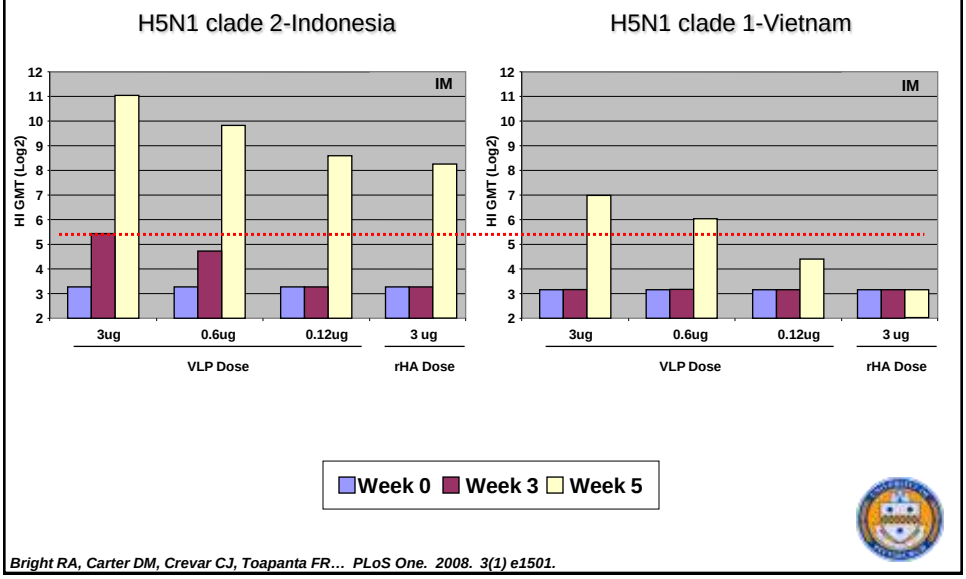
Vaccination Regimen



Serum Antibody Titers

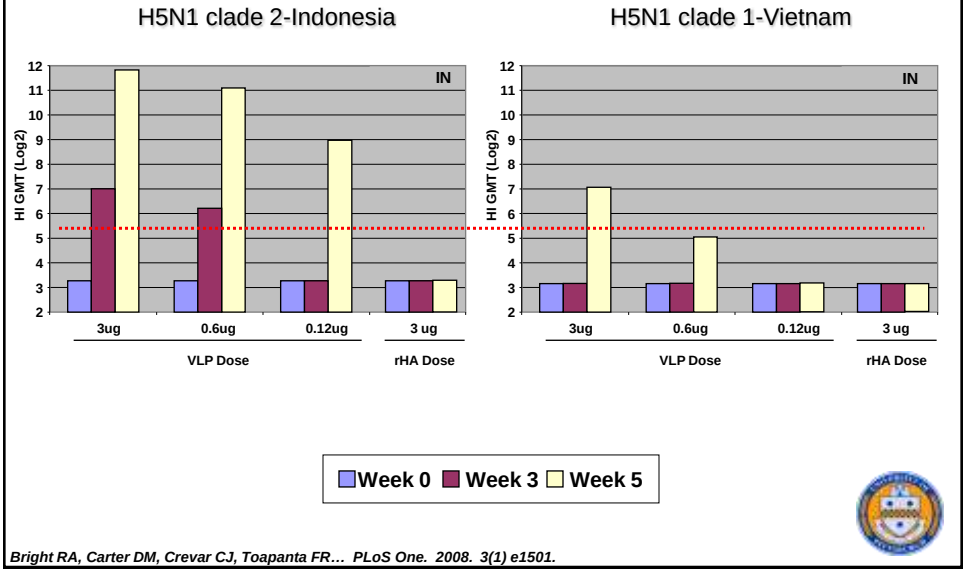


H5N1 clade 2 Dose Response



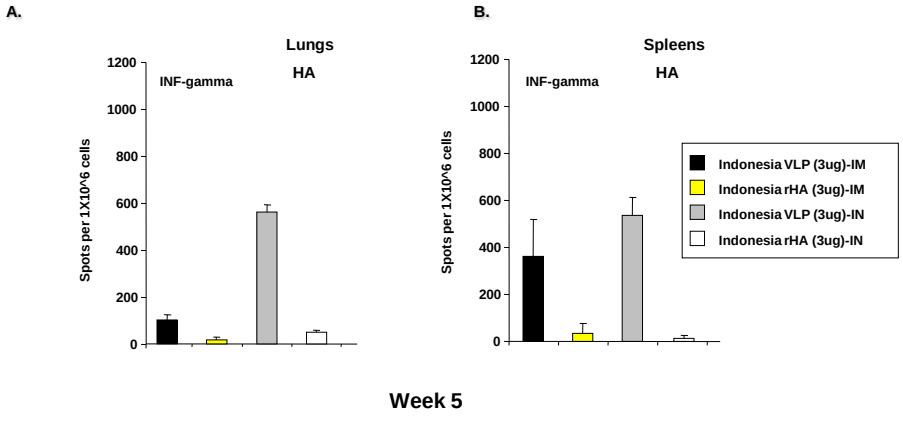
Bright RA, Carter DM, Crevar CJ, Toapanta FR… PLoS One. 2008. 3(1) e1501.

H5N1 clade 2 Dose Response



Bright RA, Carter DM, Crevar CJ, Toapanta FR… PLoS One. 2008. 3(1) e1501.

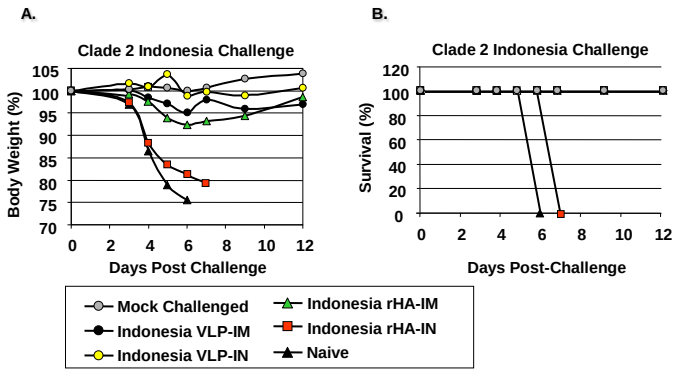
Cellular Responses



Week 5

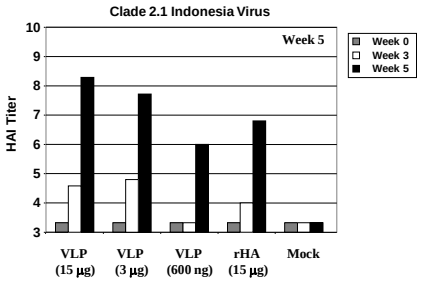
Schneider KS et al., 2008.

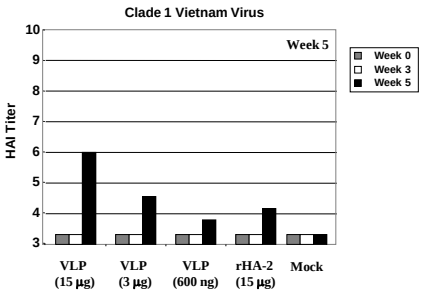
Bright RA, Carter DM, Crevar CJ, Toapanta FR… PLoS One. 2008. 3(1) e1501.



Bright RA, Carter DM, Crevar CJ, Toapanta FR… PLoS One. 2008. 3(1) e1501.

Ferrets





Bright RA, Crevar CJ, Carter DM, … 2008. in preparation

Summary

- **Virus-like Particles elicit a high titer and broadly reactive antibody responses against influenza compared to recombinant HA.**

- Immunogenic
  - **IM-low doses (*i.e.*, 0.12 µg HA) induce HAI titers ≥40**
  - **Mice and Ferrets**
- Protective
  - **All doses of clade 2 VLPs protect against clade 2 virus**
- Cross-Protective
  - **0.6 µg clade 2 VLPs protect against clade 1 virus in mice.**
  - **All doses of clade 2 VLPs in ferrets protect against clade 1 virus**

Two Types of Influenza Vaccines

- Inactivated split /subvirion trivalent vaccine
  - Intramuscular
  - Approved for persons >6 months of age
  - Preferred for those in close contact with immune-compromised persons
- Live-attenuated influenza vaccine (LAIV)
  - Intranasal
  - Approved for healthy persons 5 - 49 years olds

Inactivated (Killed or Subunit) Vaccines

- Requires suitable, high-yield culture system for growth of large quantities of virus in eggs
  - Reassortment of Influenza A viruses with high-growth donor virus A/PR/8/34
- Purification from embryonated eggs
- Chemical or physical inactivation of virus
- Most immunogenic in healthy, younger individuals
- May not stimulate all elements of comprehensive immune response
  - Humoral immunity but not cellular immunity

Influenza Vaccine Effectiveness

- Primary determinants
  - age and immune status of recipients
  - vaccine match with circulating strains

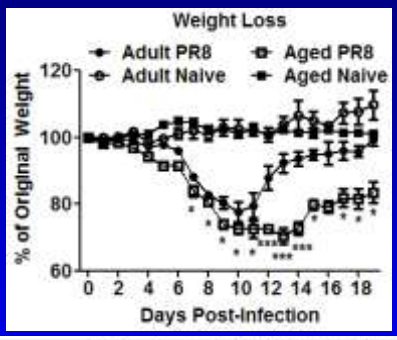
- Effectiveness by age and status
  - 70-90% effective in preventing influenza in healthy persons < 65 years
  - 30-70% effective in preventing influenza community-dwelling persons > 65 years

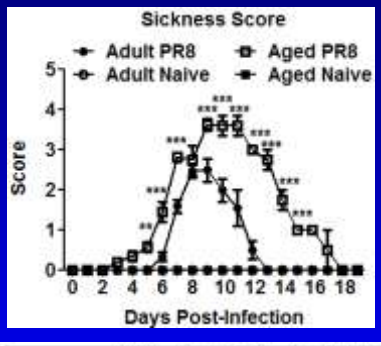
  - In persons > 65 years residing in nursing homes
    - 30-40% effective in preventing influenza
    - 50-60% effective in preventing hospitalization
    - 80% effective in preventing death

Impairments of the Aged Immune System to Influenza Infection

Using Adjuvants to enhance
VLP vaccines in the elderly

Infection of Aged Mice with PR8 Virus



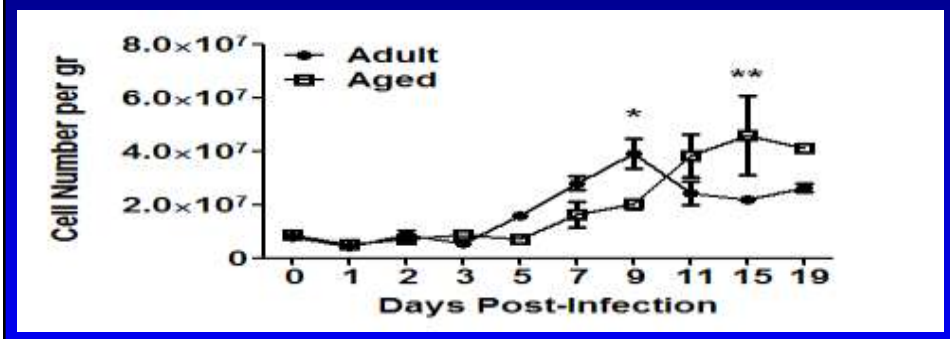


	DPI	0	2	4	6	8	10	12	14	16	18
Adult Naive	N	6	6	6	6	6	6	6	6	6	6
Aged Naive	N	6	6	6	6	6	6	6	6	6	6
Adult Challenge	N	60	48	42	36	30	24	18	18	12	12
Aged Challenge	N	60	48	42	36	30	24	18	18	10	10

	DPI	0	2	4	6	8	10	12	14	16	18
Adult Naive	N	6	6	6	6	6	6	6	6	6	6
Aged Naive	N	6	6	6	6	6	6	6	6	6	6
Adult Challenge	N	60	48	42	36	30	24	18	18	12	12
Aged Challenge	N	60	48	42	36	30	24	18	18	10	10

* P<0.05; ** P<0.01; *** P<0.001

Toapanta and Ross. *Respiratory Research* 2009, 10:112



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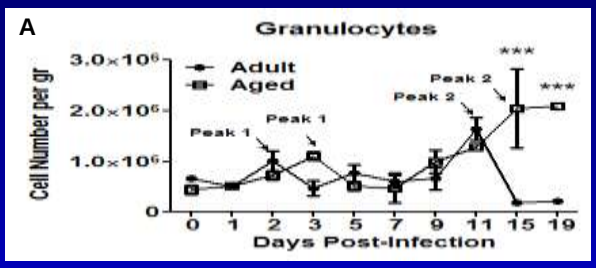

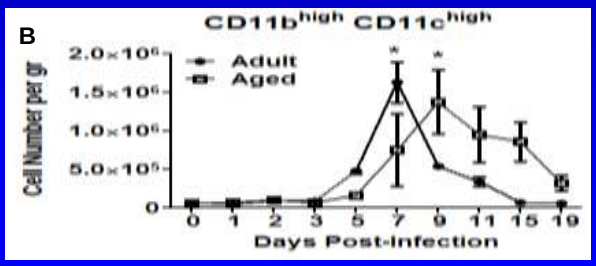

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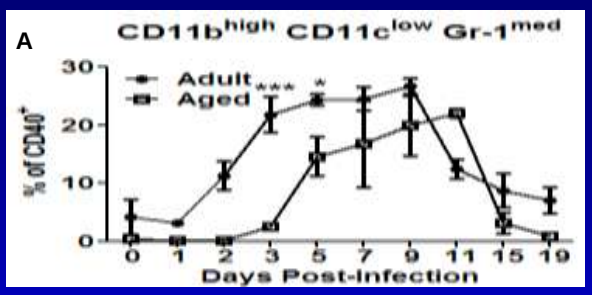

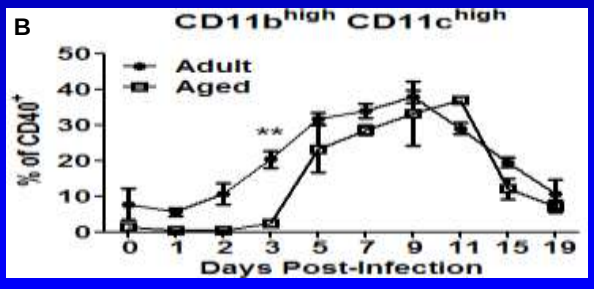

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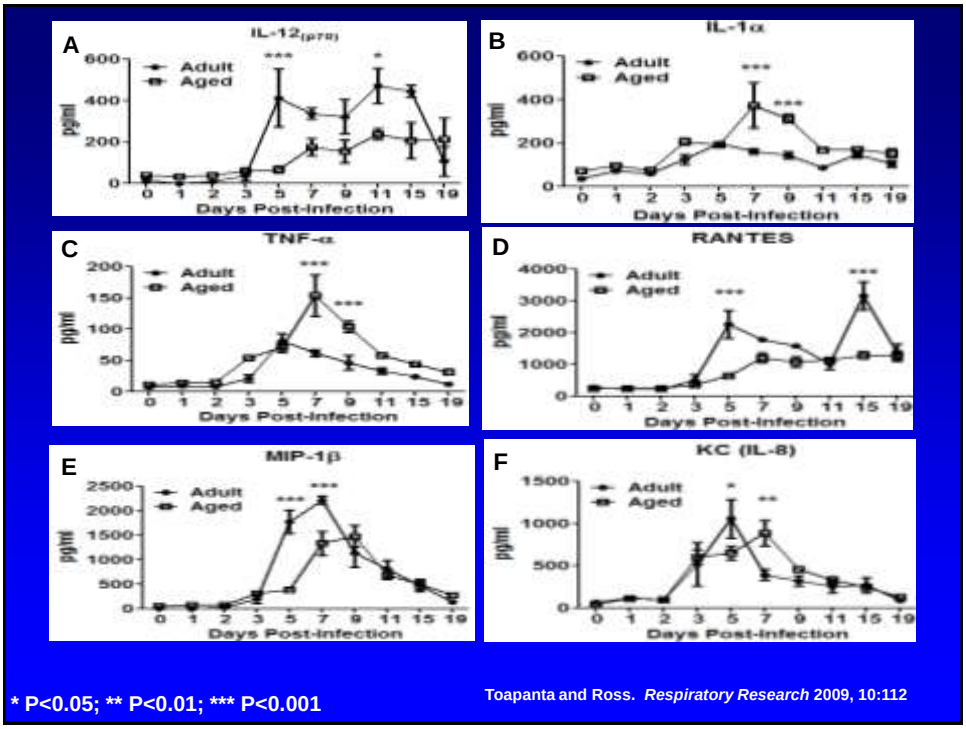

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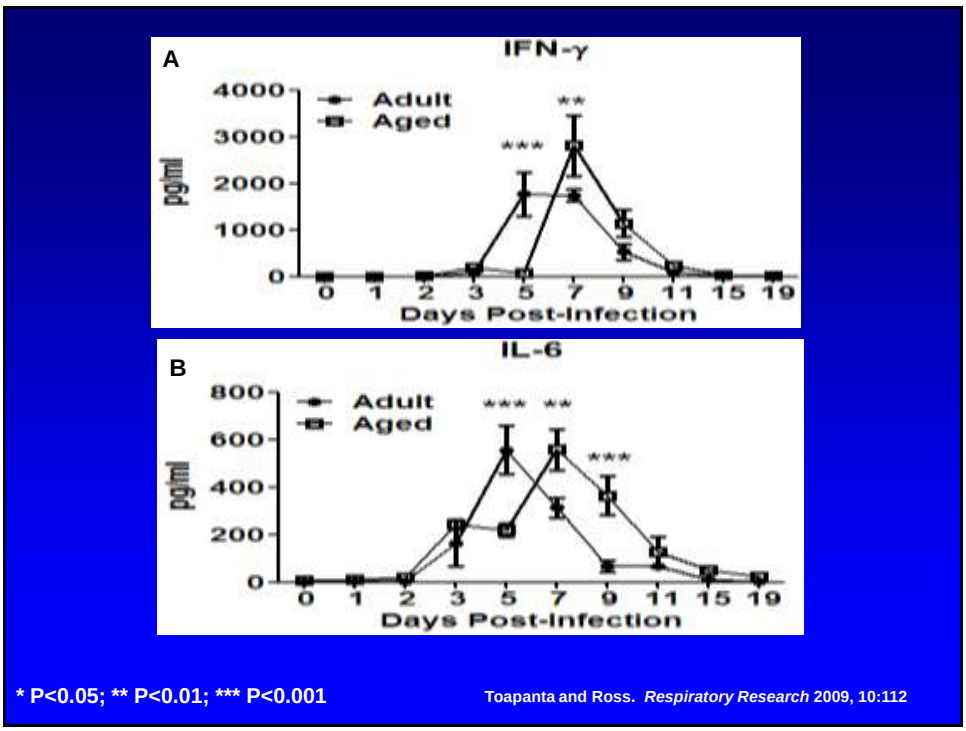

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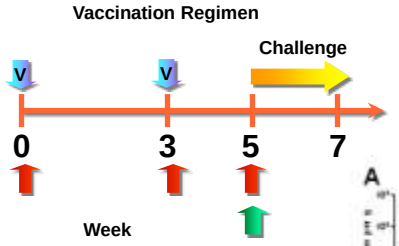
Toapanta and Ross. *Respiratory Research* 2009, 10:112

VLP Vaccination plus TLR3 activation by Poly I:C



Mice: BALB/c
Aged: 24 month
Adult: 6 month

Route: IN

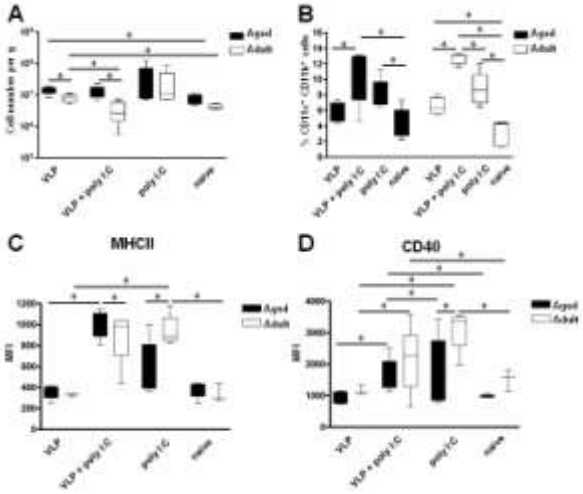
Immunogen: VLP
VLP Dose: 3ug

Adjuvant: Poly I:C

Vaccination: Week 0, 3

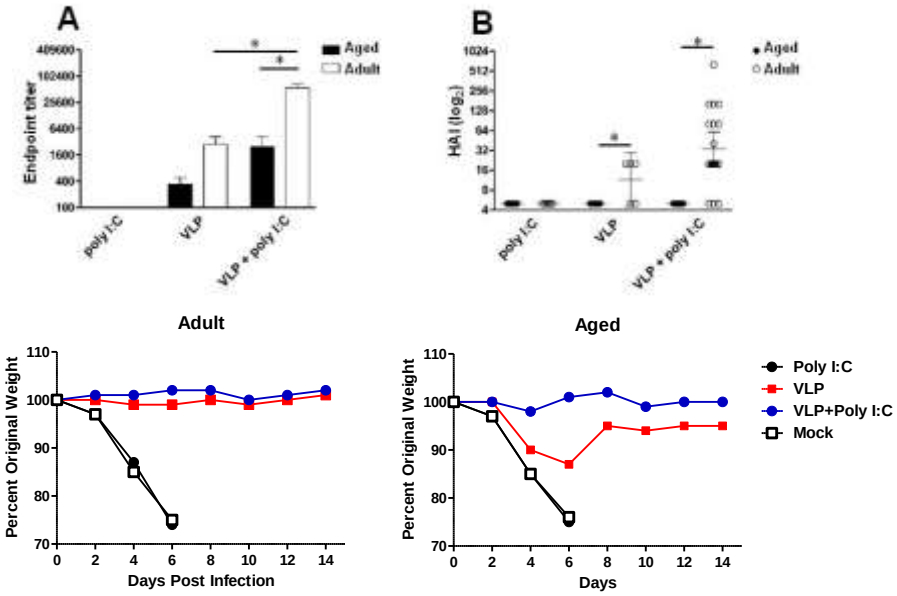
Serum: Weeks 0, 3, 5
Lungs: Post-Challenge

Challenge: A/PR/8/34
$10LD_{50}$



Schneider-Orhum and Ross. 2010.

VLP Vaccination plus Poly I:C



Schneider-Orhum and Ross. 2010.

E

	Poly I:C	VLP	VLP + Poly I:C	Naive
Aged	0/7 (0%)	2/6 (33%)	7/7 (100%)	0/5 (0%)
Adult	0/5 (0%)	5/5 (100%)	5/5 (100%)	0/5 (0%)

Schneider-Orhum and Ross. 2010.

Summary

- Aged Mice have an impairment in the innate immune responses to influenza infection.
- VLP vaccines elicit HAI antibodies in adult mice, which are enhanced by TLR3 agonist, Poly I:C.
- However, adjuvants are needed to elicit protective immunity to influenza challenge in aged mice, but this protective immunity is not related to HAI titers.

Overall Conclusions

- Virus-like particles elicit antibody and cell-mediated immune responses to both seasonal and pandemic influenza responses.
- Virus-like Particles are effective immunogens, delivering multiple antigens to the host.
- Resulting in stimulation of innate immune responses and eliciting high titer antibodies in adult immune systems.
- VLPs are effective in both adult and aged models, however, the use of innate stimulating adjuvants enhances the adaptive immunity induced by VLPs in the aged.

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Questions?