Formaldehyde-Treated, Heat-Inactivated Virions with Increased HIV-1 env can be used to induce High-Titer Neutralizing Antibody responses

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Why do we need a vaccine?

- Human costs
- Economic costs

Human Costs

- 40 million living with HIV/AIDS
- 21.8 million cumulative deaths
- 11,000 new infections each day
- 4,000 of these in persons ages 15-49
- Almost 2 teenagers infected EVERY HOUR OF EVERY DAY in the US

Human costs

8,200 people die each day

Equals 20 747's crashing

Every day

Year in **Year out**

Economic Costs

- UN estimates that medical and human costs have reversed social and economic development in 16 countries
- HAART costs between $15,000-30,000/yr

Hlabisa, South Africa

- One of every three adults is HIV infected (40,000 people)
- With price reductions the most inexpensive combination therapy is approximately $400-1000 per year
- 11 can currently afford antiviral therapy

Government sponsored HIV preventative vaccine trials

- Approx. 45 vaccines in Phase 1 trials
- Only 3 vaccines in Phase 2 trials
- Only 1 vaccine in Phase 3 trials

VaxGen data

Vaccine: recombinant gp120

	TOTAL	INFECTED AT END OF TRIAL	PERCENTAGE WHO BECAME INFECTED	
All subjects				
	1,679	98	5.8 %	PLACEBO
	3,330	191	5.7	VACCINE

VaxGen, 2003

What happened?

- vaccine was recombinant gp120 protein
  - env exists as trimer
- used lab strain of HIV clade B
  - not representative of circulating HIV strains or other clades
- only targeting neutralizing Abs
- Pros:
  - can conduct Phase III HIV vaccine trial
  - learned that enrolling in trial did not increase risky behavior

Why is it so difficult to make effective HIV vaccine?

- Viral diversity
- Animal models are limited
- Natural, controlled infections do not protect against superinfections
- HIV pathogenesis is poorly understood
- HIV affects same cells a vaccine targets: CD4 T cells, macrophages, DCs

RV144

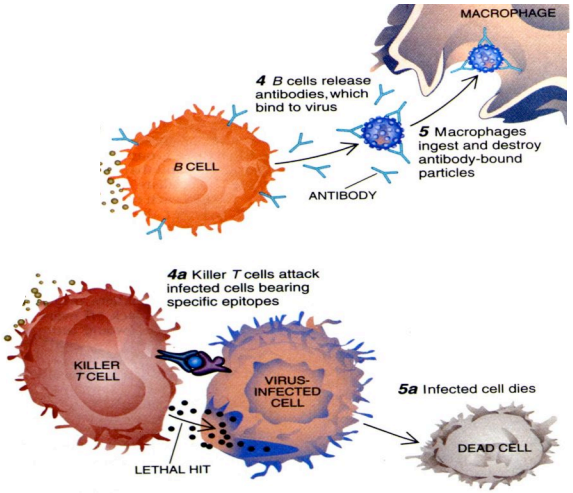
- vCP1521 Clade E Canarypox
- AIDSVAX B/E' gp120 from MN and A244
- 4 immunizations at 0, 1, 3, and 6 months
- 2 vCP1521 alone and 2 vCP1521 + AIDSVAX
- Estimated cost >$100 million

HVTN 502 and 503

- Merck Ad5 based vaccines
- Gag-Pol-Nef
- Clade B
- 502 began 2005—1500 volunteers needed
- 503 began 2007—3000 volunteers needed
- 503 tests a Clade B vaccine in a region where Clade C predominates!

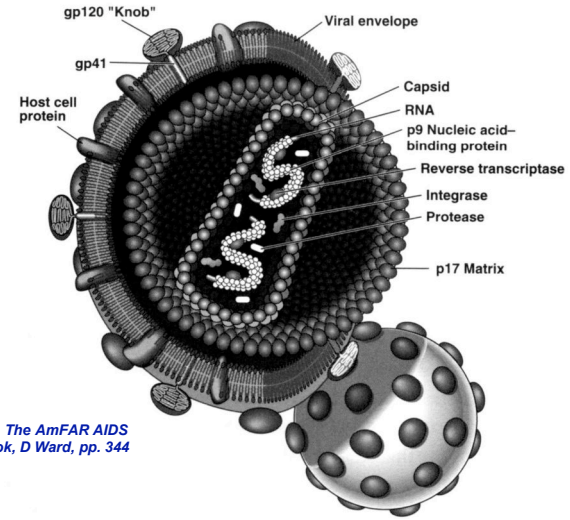
Your Immune System

- Antibody Responses:
  Prevent cells from becoming infected
- Cellular Responses
  Remove infected cells from circulation



Rationale for inactivated whole virion vaccine

- A preventive HIV vaccine should elicit broad-based immune responses: cytotoxic T cell and neutralizing antibody responses
- Relevant neutralizing antibodies can offer protection from infection with HIV and SIV if they are present in sufficient concentrations at the time of infection
- The use of heat inactivated virions provides a complex antigen source with close to native envelope conformation



Source: The AmFAR AIDS Handbook, D Ward, pp. 344

Conditions for an Inactivated HIV Vaccine

- Eliminate infectivity
- Maintain or enhance antibody binding to envelope
- Generate protective immune responses

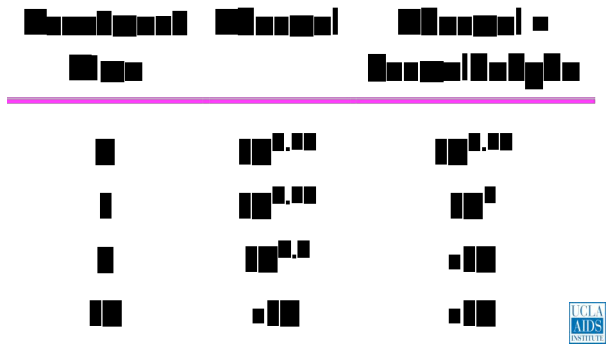
Methods of Inactivation

- Physical
  - Thermal
  - Irradiation
- Chemical
  - Formaldehyde
  - Gluteraldehyde
  - 2'2'dithiopyridine

Did we kill it?

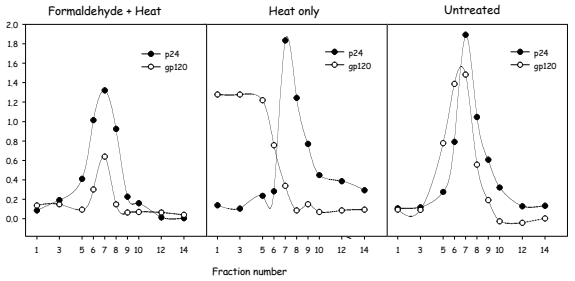
- Mix virus (treated or untreated) with target T-cells
- Limiting dilution: What is the highest dilution that you can still get an infection? Determine how much infectious virus exists.

Thermal and Chemical Treatment Reduces Infectivity



Why did we use both physical (heat) and chemical (formaldehyde) ?

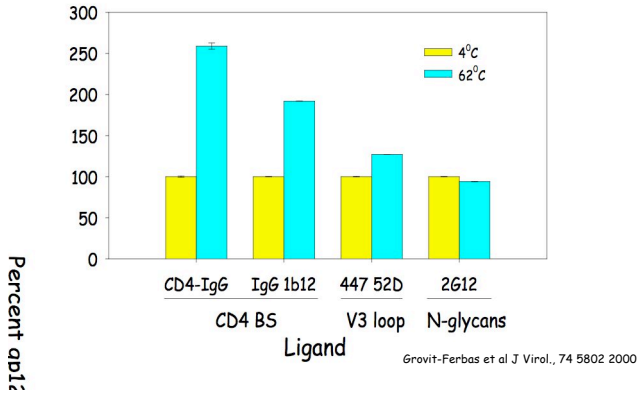
Treatment with formaldehyde decreases envelope shedding



Can the envelope still bind to neutralizing antibodies?

- gp120 capture ELISA:
- Mix virus with CD4-IgG or human anti-gp120 antibodies
- Add mixture to plate containing sheep anti-gp120 antibody
- Detect amount of human anti-gp120 ab bound to env on virus by adding labeled goat anti-human antibody

Binding of gp120 to envelope specific monoclonal antibodies



Grovit-Ferbas et al J Virol., 74 5802 2000

How can we improve our vaccine?

Envelope modifications

- Mutation in the endocytosis and sorting signal in gp41 of HIVSXSL9 (**Y706C**)
- Reported to result in better expression of Env at the surface of SIV infected cells
- increase the incorporation of SIV Env into virions

^{700}N R V R Q G **Y** S P L S F Q T

N R V R Q G **C** S P L S F Q T

Experimental protocol

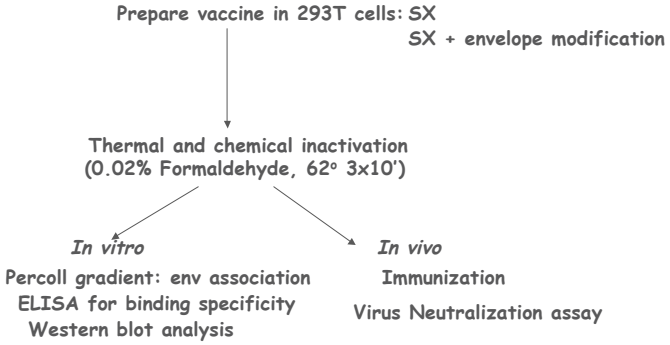


Table 1: Env content on virions and infectivity is enhanced by gp41 mutation (Y706C)

TCID50=amount of infectious virus/ml

CD4 IgG=amount of CD4 bound to virions

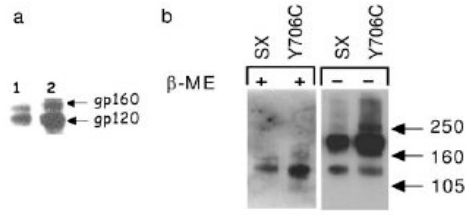
2G12=amount of anti-Env antibody bound to virions

Mutations	$TCID_{50}$/ml	CD4 IgG (ng/ml)	2G12 (ng/ml)
HIV_{SXSL9}	$10^{5.42}$	21	26
$HIV_{SXSL9,Y706C}$	**$10^{7.9}$**	**243**	**220**

Studying HIV envelope structure

- HIV envelope produced as gp160
- gp160 cleaved into gp120 and gp41
- One functional envelope spike exists as trimer (3 gp120/gp41 subunits)
- Measure amount of envelope on virions by Western blot analysis
- Use reducing agent (β-ME) to examine envelope trimer

Fig 1: Env modified virions retain oligomeric structures



What about immunogenicity in small animals?

Vaccination Schedule

Vaccinate with inactivated whole HIV virions +QS21
BALB/C mice: s.c 4 times
Rabbits: i.m. 6 times

↓

Draw sera 2 weeks post-final vaccination

↓

Remove xenogenic response by 3 adsorptions with 293T cells

↓

Neutralization assay

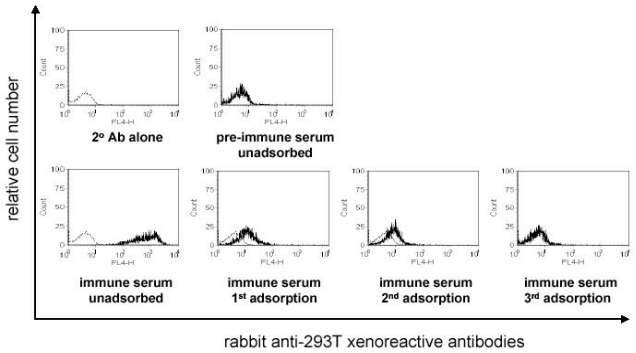
Serum Pre Absorption Conditions

- Goal: remove non-virus specific antibodies raised against proteins from virus producing cells
- 2 hours on 25 X 10^6 293T cells (remove high affinity abs)
- 1 hour on 25 x 10^6 293T cells (intermediate affinity)
- 30 minutes on 25 x 10^6 293T cells (low affinity)

Measuring removal of anticellular antibodies

- Take sera from vaccinated animals (rabbits)
- Mix with virus producing cells (293T cells)
- Add labeled second antibody that recognizes rabbit antibodies
- Detect amount of labeled antibody bound to cells (if high fluorescence, indicates presence of anticellular antibodies)

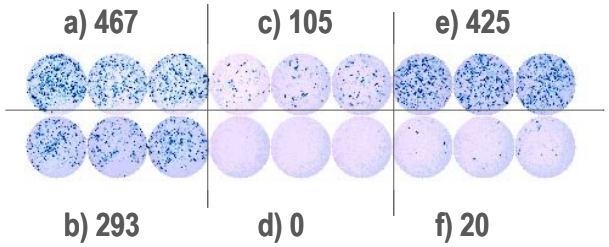
Preabsorption of immune sera removes xenoreactive Abs



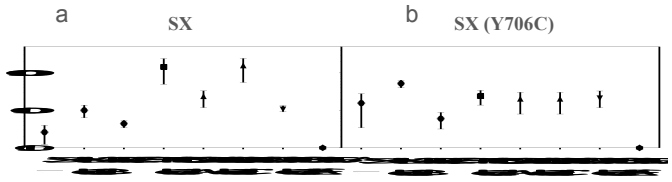
Neutralization Assay

- Magi cells: express CD4, CXCR4 and CCR5, HIV LTR-Bgal
- Mix virus with sera at various dilutions
- Add to Magi cells- if infected, will turn blue
- Count blue cells
- Neutralizing antibodies will reduce number of blue cells (high GMT indicate more neutralizing antibodies)

Quantitation of MAGI cell assay



Modifications in viral envelope can increase neutralizing antibody titers in vaccinated mice



Why do we care about cross-clade neutralizing antibodies?

Table 1 Proposed Reference Sequences of HIV-1 Genetic Subtypes

Subtype	*env*	*gag*	*pol*	Main geographical area
A	U455 IBNG DJ258 SF1703	U455 IBNG DJ258 VI32	U455 IBNG	Central Africa
B	LAI JRFL OYI RF	LAI JRFL OYI RF	LAI JRFL OYI RF	Europe North and South America Australia Asia
C	UG268 ZAM18 ZAM20 DJ256	UG268 ZAM18 ZAM20 DJ259		East and South Africa
D	NDK JY1 UG274 SE365	NDK VI203 UG274 UG270	NDK ZZZ6[1] ELI[1]	Central Africa
E	TN235 TN239 TN242 CM240X			East Asia
F	BZ163A BZ126A 93BR029.2 93BR020.17	BZ162 VI69 VI174		South America East Europe
G	LBV217[1] 92UG975.10 92RU131.9	LBV217[1] VI191 SE6165[2]	SE6165[2]	Central Africa
H	VI557[2] CA13[2]	VI557 VI525		Central Africa
J	SE702[2] SE7887[2]	SE7022[2] SE7887[2]		Central Africa (Europe)

[1] These sequences have been found to be partly recombinant in *env* or *gag*.
[2] Full length gene sequences do not yet exist.

Are these preparations immunogenic in rabbits?

Neutralization of HIV by sera from $SXSL9_{Y706C}$ immunized rabbits

Rabbit Number	HIV $_{SXSL9}$	TK1135 (Clade A)	93IN109 (Clade C)	93TH305 (Clade A/E)	SIV
3065	40	80	20	640	<20
3249	160	80	20	320	<20
3058	80	160	20	40	<20
GMT	**93**	**106**	**20**	**333**	**<20**
Human polyclonal sera	80	20	40	40	

Conclusions from our studies

- Inactivation protocol ↓ infectivity of HIV-1
- Inactivation protocol ↑ antigenicity of HIV-1
- Cross clade neutralizing Abs can be raised in mice and rabbits
- Improved neutralizing Ab titers by increasing the amount of env on virions

How can we improve safety and immunogenicity?

Developing Pseu

- Safety mutations to impact key steps in virus replication: packaging, reverse transcription, and integration

Developing Pseu

- Safety mutations to impact packaging, reverse transcription, and integration
- Env specific mutations to remove specific N glycans and to increase Env content

Preliminary data: Pseu is immunogenic

Immunogen	Mean ND_{50} HIV_{SXSL9}	Mean ND_{50} HIV_{NL4-3}	Mean ND_{50} HIV_{TK1135}	Mean ND_{50} $HIV_{93IN101}$	Mean ND_{50} $HIV_{93TH305}$
Pseu	60 (20-80)	900 (540-1620)	106 (80-160)	47 (20-80)	80(80)
Pseu $_{Y706C,N141Q}$	**47 (20-80)**	**2220 (180-4860)**	**480 (160-640)**	**86 (20-160)**	**186 (80-320)**

Collaborators

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