Schedule + route

Chimp:

options:

1) multiple (non clinically relative) injectates
2) STD (0/4) + 6)

~~Reasons~~: Advantages

for 1 – want to guarantee success 1st exp.

for 2 – ~~use for clinical~~ save a chimp

Reasoning:

#1 want ~~make~~ add unless vaccine extremely low, unnecessarily.

Rec: use #2 evaluate at month 2 or 3 to see if AB present if none – add relevant dual

Chimp

Challenge timing

options: 1) T-3 mos
2) T-7 mos

Advantages
of 1: speed (turnaround)

of 2 maximize chance of success.

Reasoning:
for 1) 1) ~~Probably~~ adequate time for a good vaccine.
2) Gives close primacy for memory stimulation

Recc:
1) measure Ab at T-3. ~~If~~ if not present (>1:50). Challenge
2) ~~T~~. - if not - wait.

Chung

Challenge – dose & route

dose Virus 1×10^3 ARC-T11 (homologous)
rationale – best homologous follow
~~dose~~ x 6 mos. re challenge c Africa

route: IV
– rationale: 1) represent 17% of infections
2.) ~~applicable~~ natural lab/hosp workers
3.) not easily standardizable
3.) most expensive.

Mechanism

Antigen

~~mut~~

Gen:

live attenuated

whole

subunit

Ways:

Live or sub → grow ? in what

recombinant — subunit
vectored

what subunit — envelope

immunogenicity

Assay — do you have it?

small animals — ? neut?

chimpanzees → Challenge

Safety — humans

Immunogenicity — humans

Efficacy

Problems

- Govt → private sector ← Govt
- private sector

~~income~~

cost — benefit

development — return

liability

Govt role

Protocol – Bleedings + tests
at least parallel to fevers

ALT
WBC – T_4
– T_3
– $2T_4$
sed rate

11 APR 86

$1 \times 10^3 \times^3 = 1 \times 10^6$ ml

Larry:

GP – 5 + neut sn

50 ml @ 1:4 completely neut 10^2 HTLV-III/LS (in 200 ml)

Dose: 1 ml (1 mg)

3 ID (0.3 ml) – Back

.3 IM – thighs

.3 SQ – Arm

at 0, ~~1~~

~~Do~~ at 1, 2 – SQ + IM – 0.5 ml (Arm, Thigh)

Challenge: op #1 – wk 12 10^3 IV

Groopman –

Max human 1:100 – blood donor

Challenge: plateau

Get Jurkat pages.
Call Kelly re HTLV-III challenge
↳ any dp24 in killed virus challenge
check Purcell/Gallo data

Esther questions: use LAV/HTLV III as parallel data.

Rationale: ~10^3 – infected ~~100~~ 3/3 chimps
– concerned about challenge except in hospital + IV drug
IV – not natural, but controllable
– muted – later, if protective – mucosa

– Protective titer
ideally ≥ 1:100 neut
but any need be worth
challenging ±

– Timing of virus isolation
? passive – see Kelly
– not lymphocytes – focal

? need killed whole virus
IV as control for dp24
↳ will tell if virus req Ab+ + binding titer

– Spinal fluid
Ab + culture > 6 mos 12 mos

Title: Efficacy of Recombinant gp 120 Against Challenge with infectious AIDS Retrovirus.

Protocol No.: AID-86-001

Proposed Start Date: 1/1/86 Proposed First Dosing Date: 4/15/86

Proposed Completion Date:

Sponsor: Genentech, Inc.
460 Point San Bruno Blvd.
South San Francisco, CA 94080

Testing Director: Jorg Eichberg, D.V.M., Ph.D. _____
(Signature and Date)

Genentech Liaison Scientists: Phil Berman, Ph.D. _____
(Signature and Date)
and
Eric Patzer, Ph.D. _____
(Signature and Date)

Purpose:

To evaluate the capability of a subunit vaccine of gp120 produced by recombinant DNA technology to protect against AIDS retrovirus infection.

Declaration of Intent:

This study is intended to support research for a product regulated by the U.S. Food and Drug Administration.

Test and Control Articles:

A. Test:

Purified preparation of recombinant gp 120 of AIDS retrovirus expressed in mammalian cell culture and adsorbed onto aluminum gel.

B. Control and Diluent:
Aluminum hydroxide (0.5 mg/mL of Al^{+3}) in phosphate buffered saline (0.016M PO_4 pH 6.2, 0.15M NaCl, 0.004M KCl). One vial containing 2 mL of aqueous suspension.

C. Challenge Virus:
An isolate of HTLVIII from the laboratory of R. Gallo at NIH.

Dose:
A. Immunization:
1 mL of test or control preparations which contain 1.0 mg of gp 120 (test) and/or aluminum hydroxide (0.5 mg of Al^{+3} in test and control)injected per chimpanzee. Individual labeled syringes used for each animal.

B. Challenge:
1 mL of live HTLV III. Individual labeled syringes will be used for each animal.

Storage Conditions:
All solutions are to be stored at 2-8°C.

Preparation of Test and Control Articles:
To be injected as supplied.

Dosage Calculation:
A. Test:
1 mL/animal at: 1.0 mg/mL gp 120 = 1.0 mg/animal
0.5 mg/mL aluminum in the form of aluminum hydroxide = 0.5 mg/animal

B. Control:
1 mL/animal at: 0.5 mg/mL aluminum in the form of aluminum hydroxide = 0.5 mg/animal

C. Challenge:
1 mL/animal of HTLV III.

Route of Administration and Rationale:

A. Immunization:
Test and control preparations will be administered i.m. and subcutaneously in two sites, since this route has been shown to be efficacious with other vaccines. The preferred sites of injection are the thigh and the arm. If not accessible, alternative sites will be selected and noted.

B. Challenge:
Infectious HTLV III will be administered intravenously since this has been shown to result in infection of unvaccinated chimpanzees. The preferred site of injection is the saphenous vein. If not accessible, an alternate site will be selected and noted.

Test species:
Chimpanzees (Pan troglodytes) free of any serologic or histopathologic evidence of present or prior hepatitis B or AIDS infection.

Identification of subjects:
Tattoo (arm), unique number.

Rationale for choice of species:
Chimpanzees are the only non-human species which are susceptible to infection by human AIDS retrovirus; therefore, they are the only species in which a challenge study can be done.

Basis of animal selection:
Healthy animals which exhibit no serologic or histopathologic evidence of present or prior hepatitis B virus or AIDS infection.

Diet:
The animals will be fed commercial monkey chow supplemented with fruits and vegetables.

Records:
1. Routine clinical records (include intercurrent illness, treatment, change in behavior).
2. Anesthesia, injection, bleeding schedule.
3. Full clinical and experimental history on file at the study center.
4. Clinical and hematological records.
5. Weight records.
6. Physical exam including special attention to lymphadenopathy and splenomegaly.

Anesthesia:
Ketamine hydrochloride, i.m. Minimal dosage to immobilize.

Experimental Design:

Group (Animal numbers)	Treatment (per animal)	Virus Challenge
1 (1,2)	1.0 mg recombinant gp 120 in aluminum hydroxide adjuvant	HTLV III
2 (3,4)	aluminum hydroxide adjuvant alone	HTLV III

All injections, venipuncture and biopsies will be performed using a squeeze cage and ketamine hydrochloride i.m. to anesthetize the animals.

Quarantine:
Animals will be observed for a baseline period of 16 weeks. During this period serum will be collected every 2 weeks and assayed for the activities described under "venipuncture". If any animal exhibits signs of a HBV or AIDS infection prior to the virus challenge, it will be removed from the study.

Immunizations:
Animals will receive a primary injection on day 0 and subsequent injections at 4 and 8 weeks. The injections will be administered in a total volume of 1.0 mL. 0.25 mL will be injected in each of four separate sites. Two subcutaneous and two intramuscular injections will be administered in shaved and alcohol swabbed sites (back of the arm and the opposite thigh to the anesthetic injection). The injection sites will be inspected for any adverse reactions at 1, 2 and 7 days post-immunization, and the results of the inspection will be recorded.

Venipuncture:
30 mL of blood will be drawn from the femoral vein on day 0 and every other week thereafter until week 64. If not accessible, an alternate site will be chosen and noted. 10 mL of blood will be used directly for cell based assays. The remaining blood will be allowed to clot at room temperature for 2 hours and then incubated at 4°C overnight in a tube with a tight fitting cap to permit clot retraction. The clot will be removed from the tube with a sterile forceps and the serum fraction will be centrifuged at 1300 xg for 20 minutes at 4°C. The clarified serum (supernatant) will be transferred to individually labeled sterile tubes (log number, date, animal ID number) and heated to 56°C for 2 h in a regulated water bath. This heat treatment will inactivate the AIDS retrovirus as well as serum complement. 5 mL of serum will be frozen at -20°C and sent on dry ice to Genentech via air express carrier to the attention of Phil Berman. The remainder will be used for assays at the testing facility and subsequently frozen and stored at -20°C at the testing facility. When venipuncture and immunization are coincident, blood will be drawn first.

Lymph Node Biopsy:
Biopsies will be taken if an enlargement, i.e., lymphadenopathy develops in any of the animals. The tissue will be fixed for subsequent histopathological examination at Southwest Foundation.

Virus Challenge:
Animals will be injected with 1mL of infectious HTLV III in the saphenous vein (opposite leg from venipuncture) after venipuncture has been taken. The challenge will be performed on week 12 provided that a protective anti-gp 120 titer is observed in all of the immunized animals. If deemed necessary, fourth and fifth booster injections will be given on weeks 12 and 16 to increase the anti-gp 120 titer.

Elimination of bias:
Animal groups will be balanced as closely as possible according to susceptibility to AIDS infection.

Other considerations:
Chimpanzees will be caged separately.

Environmental considerations:

Lights: On 0700 and off 1900 hours.

Ambient temperature: 80±10°F.

Animal Care and Health:
Animals will be cared for as described by existing SOP's at the testing facility except as provided herein.

Should an animal become ill during the course of the study, it will be treated at the discretion of the veterinarian and the data recorded. The experimental protocol will be continued if possible.

Should an animal die during the course of the study, a gross autopsy will be conducted under the direction of the veterinary pathologist and representative tissue samples will be saved for microscopic examination.

Nonstandard Safety Routines:
None.

Proposed Assays for Safety and Efficacy in Chimpanzees of Recombinant gp120 AIDS Retrovirus Vaccine

Assay	Interval	Location
I. Immunological assays		
A. Cellular Assays		
1. OKT4/OKT8 ratios	biweekly	Southwest Foundation
2. CD4 positive cells	biweekly	Southwest Foundation
3. p24 in adherent cells	biweekly	Southwest Foundation
4. p24 in non-adherent cells	biweekly	Southwest Foundation
5. Mitogen induced proliferation in PBLs	biweekly	Southwest Foundation
6. gP120 induced proliferation in PBLs	biweekly	Southwest Foundation
7. Cytokine production	biweekly	Genentech, Inc.
B. Serological assays		
1. Western blots (high and low percentage gels) of virus proteins	biweekly	Genentech, Inc.
2. Radioimmunoprecipitation of 35-S methionine labeled virus	biweekly	Genentech, Inc.
3. Titer to gp120	biweekly	Genentech, Inc.
4. Reactivity in commercial HTLV III assay kit	biweekly	Southwest Foundation
5. In vitro neutralization (fluorescence and reverse transcriptase)	biweekly	J. Groopman, Boston
6. In vitro neutralization (VSV pseudotypes and inhibition of syncytia formation)	biweekly	P. Clapham, London
7. In vitro neutralization (antibody dependent cytotoxicity)	biweekly	C. Lane, Bethesda
8. Immunoblot		
9. Local Abs incl IgA		
C. Miscellaneous assays		
~~1. Skin testing (Merieux panel)~~	monthly	Southwest Foundation
2. gp 120	monthly	Southwest Foundation
II. Clinical Chemistry		
A. CBC platelets and differential	biweekly	Southwest Foundation
B. SMA panel	biweekly	Southwest Foundation
~~C. Coagulation: PT, PTT~~	biweekly	Commercial laboratory

III. Virological Assays			
#1 A.	Co-cultivation of PBLs with HTLVIII permissive host cell	biweekly	Southwest Foundation
IV.	Physical Exam	biweekly	Southwest Foundation

Salk
Chimp

Don Francis

HIV Immunization of Chimps

Summary of Meeting at Southwest Foundation on May 15, 1987

PRESENT: Drs. Salk, Gardner, Eichberg

An agreement was made to carry out the following studies:

1. Immunization of chimps with HIV. Two HIV-uninfected chimps will be actively immunized with gamma irradiated HIV + IFA. One uninfected chimp will receive IFA alone.
   a. The HIV antigen will be gamma irradiated (~3 megarads, 90 hrs), whole, unconcentrated HIV (HB-2 strain). The immunogen will then be concentrated, and given in a single dose of 100 ug IM, consisting of 50% aqueous antigen mixed with 50% oil [9 parts oil, 1 part arlacel A (mannide monooleate). Administration of booster doses of HIV will depend on dynamics of the immune response. The challenge HIV is a strain (from Jerry Groopman), which has been characterized at Southwest including titration in vitro and infectivity in chimps. It will be given IV at a time to be decided later depending on quantitative and qualitative aspects (e.g. HIV neut. titer) of the immune response to the primary vaccination.
2. The same HIV immunogen will be used to boost 1 already HIV-infected chimp (after obtaining permission from Dr. Harvey Alter in whose HIV pathogenesis study this chimp belongs). Another of Alter's HIV infected chimps will be an adjuvant (IFA) control. A third HIV-infected chimp for immunotherapy will be the adjuvant (IFA) control from (1) above.

3. Assays to be done on the chimp sera include:

Southwest	UCD	UCSD	UCLA
Clinical status (WT, temp., swelling, CBC, SMAC)	Characterization of HIV immunogen	In situ hybridization	RTI
	ELISA		
T cell subsets	WB		
Mitogen stimulation	Neuts (neut index)		
CMI: ADCC			
CMI: Blastogenesis			
Virus isolation			
Western blot			
RIPAs			

4. Agreement re: authorship

Immunoprevention: SoWest, 1st authorship

Immunotherapy: UCD, 1st authorship

5. Budget

Per diem $19/day x 3 chimps

Lab tech 25%

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30 K/yr = $2500/mo: from MacArthur fund

6. Timing:
   a. Present protocol to SoWest Animal Care Committee (Eichberg) May 26

   b. Inoculate chimps (Eichberg ± Salk ± Gardner) Early June

cc: Dr. Salk
Dr. Eichberg
Dr. Carlson
Dr. Jennings
Dr. Francis
Anita Moore
Betsy Bencken