Comparison of protection from homologous cell-free vs cell-associated SIV challenge afforded by inactivated whole SIV vaccines

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This study attempted to determine if SIV vaccines could protect against challenge with peripheral blood mononuclear cells (PBMCs) from an SIV infected rhesus monkey. Mature *Macaca mulatta* were vaccinated four times with formalin inactivated SIV_{mac32H} administered in MDP adjuvant (n = 8) or SIV_{mac32H} ISCOM vaccine (n = 8). Controls included animals vaccinated with measles virus in MDP adjuvant (n = 4) or ISCOM (n = 4) preparations. Of each group, half were challenged intravenously (IV) with ten MID_{50} of the cell-free SIV_{mac32H} (11–88) SIV stock and half were challenged with ten MID_{50} of PBMCs from the SIV_{mac32H} infected macaque 1XC. All SIV vaccinated animals challenged with the 11–88 cell free stock of SIV_{mac32H} were protected, whereas only half of the SIV vaccinated monkeys receiving the same infectious dose of the 1XC cell stock were protected.

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Introduction

The significance and importance of the SIV model in macaques for AIDS pathogenesis and vaccine development research has been well documented [3,4,6,15]. Whole inactivated SIV vaccines or SIV envelope-enriched vaccines have been demonstrated to protect macaques against low-dose intravenous challenge with homologous SIV strains propagated in culture on human T cells [2,5,6, 11,12,13]. Protection of macaques from SIV_{sm} or HIV-2 infection has been demonstrated by passive immunization [14] suggesting that protective immunity is humorally mediated. Considerable controversy has developed concerning the validity of SIV vaccine results since a study reported the problem of contaminating cell components from the same human T-cell line used to produce the vaccine as well as the virus challenge stock [16]. However, recent publication of SIV vaccine protection with recombinant antigens supports the premise that vaccine protection in this model is SIV antigen specific [8]. Realistic challenge of such vaccines by either cell-associated intravenous or mucosal challenge from virus produced from the host species are critical tests for true lentivirus development [15]. Evidence of protection from monkey propagated virus or virus infected macaque PBMCs may aid in clarifying this controversy. This study set out to determine if whole inactivated SIV vaccines capable of protecting from cell-free IV challenge could also protect against an infected cell challenge from an SIV-infected macaque with AIDS.

The importance of such vaccine efficacy studies with infected cells propagated in vivo in homologous species is clearly very relevant and of high priority for HIV vaccine development. This experiment revealed that the vaccinated group challenged intravenously with cells from an infected monkey with AIDS was protected from infection. These results are the first to demonstrate that vaccine protection against cell associated challenge is possible.

Materials and methods

Animals and vaccine challenge stocks

Mature *Macaca mulatta* were pre-bled for routine hematology and establishment of base-line values. All animals were SIV, STLV, and SRV free out-

bred macaques of northern India origin bred in captivity at TNO.

Vaccine Challenge Stocks consisted of the 11–88 stock of SIV_{mac32H} propagated by short term culture on the human T-cell line C8166 by M. Cranage and colleagues (Center for Applied Microbiological Research, Salisbury, U.K.), aliquoted and titrated in vivo as part of an EC collaborative effort. A dose of 10 MID_{50} of this stock was administered IV as a single challenge for group A. An intravenous dose of 50 MID_{50} of this stock was administered to a rhesus macaque (1XC) from which a large stock of PBMCs was prepared and titrated in vivo (H. Niphuis, ITRI-TNO, personal communication). A dose of 20,000 PBMCs was found to be equivalent to approximately one MID_{50}. Hence, the challenge dose of 2×10^5 1XC PBMCs was used to give an equivalent ten MID_{50} challenge to group B.

Vaccines

SIV_{mac32H} was derived from culture supernatant of infected C8166 cells and purified by ultracentrifugation and gel exclusion chromatography. Material was provided by programme EVA of the European Sponsored AIDS programme.

The inactivated whole virus vaccine was prepared by the addition of 0.8% formaldehyde to SIV_{mac32H} and subsequent mixing with the adjuvant muramyl dipeptide (MDP, Sigma). The ISCOM vaccine consisted of a mixture of several components: (1) a whole SIV ISCOM preparation prepared from the same purified virus according to standard methods [10]; (2) lectin-purified gp130 incorporated into ISCOMs by the acidic method or covalently coupled to preformed ISCOM matrix; and (3) soluble p27, not incorporated during preparation of whole SIV ISCOM. The inactivated whole SIV vaccine and the SIV ISCOM vaccine were prepared in such a way that they contained approximately the same amounts of gp130 and p27. The inactivated whole MV and MV ISCOM preparations, which were used for immunizing the control monkeys have been described previously [10]. All monkeys were vaccinated intramuscularly four times at 0, 4, 10, and 16 weeks (group A) or 0, 4, 10, and 22 weeks (group B).

Serological analyses

Western blot analysis (WB) was performed by immunoblotting of SDS-PAGE separated SIV-infected cell proteins to nitrocellulose with antibody detection of antibodies to SIV using peroxidase conjugated antimonkey IgG as previously described [17]. SIV-specific monoclonal antibodies (KK5, 8, 11, and 33) were used to confirm the specificity of the antigens detected as previously described [9].

KK-5 inhibition enzyme linked immunosorbent assay (ELISA) was performed using detergent disrupted SIV_{mac32H}-infected cell lysate bound to Con A coated on polystyrene plates. Serum from vaccinated and challenged monkeys was competed for binding of KK-5, a monoclonal antibody that recognizes a conformation dependent virus neutralization epitope of SIV_{mac32H}.

Antigen detection in plasma

Serum and plasma samples were used to determine the presence or absence of viral antigen postchallenge by antigen capture analysis. Antigen capture was performed using the Coulter SIV core antigen detection kit according to the guidelines listed by the manufacturer (Coulter Corp., Hialeah, FL, U.S.A.).

Virus isolation

Ficol separated PBMCs were enumerated, and duplicate samples of 5×10^5 PBMCs in 0.5 ml (RPMI, 10% FCS) were added to 0.5 ml media contain 2.5×10^5 C8166 cells and incubated at 37°C for three weeks. Medium was refreshed two times per week and supernatant samples were retained for antigen capture analysis. Samples were confirmed negative or positive by reanalysis on cocultivated samples one week later. Samples were taken over a period of three weeks. Antigen capture values with an absorbance greater than or equal to the cut off value were considered positive for SIV antigen. All positive results were retested in duplicate for confirmation.

Results

Serological analyses

Western blots of group A (Fig. 1) and group B (Fig. 2) demonstrated a strong antibody response to p27 present in the vaccine preparations based on samples taken on the day of challenge. In both groups A and B, a stronger reactivity to gp130 was noted in animals vaccinated with ISCOMs than in animals vaccinated with formalin inactivated, MDP adjuvanted vaccine. ELISAs based on competive binding with the conformation dependent neutralizing antibody KK5 confirming the observation that SIV ISCOM induced higher antibody titers than the inactivated whole SIV preparation. SIV KK5 inhibition titers were not observed in measles vaccinated controls. Upon challenge all control animals developed SIV-specific antibody titers beginning

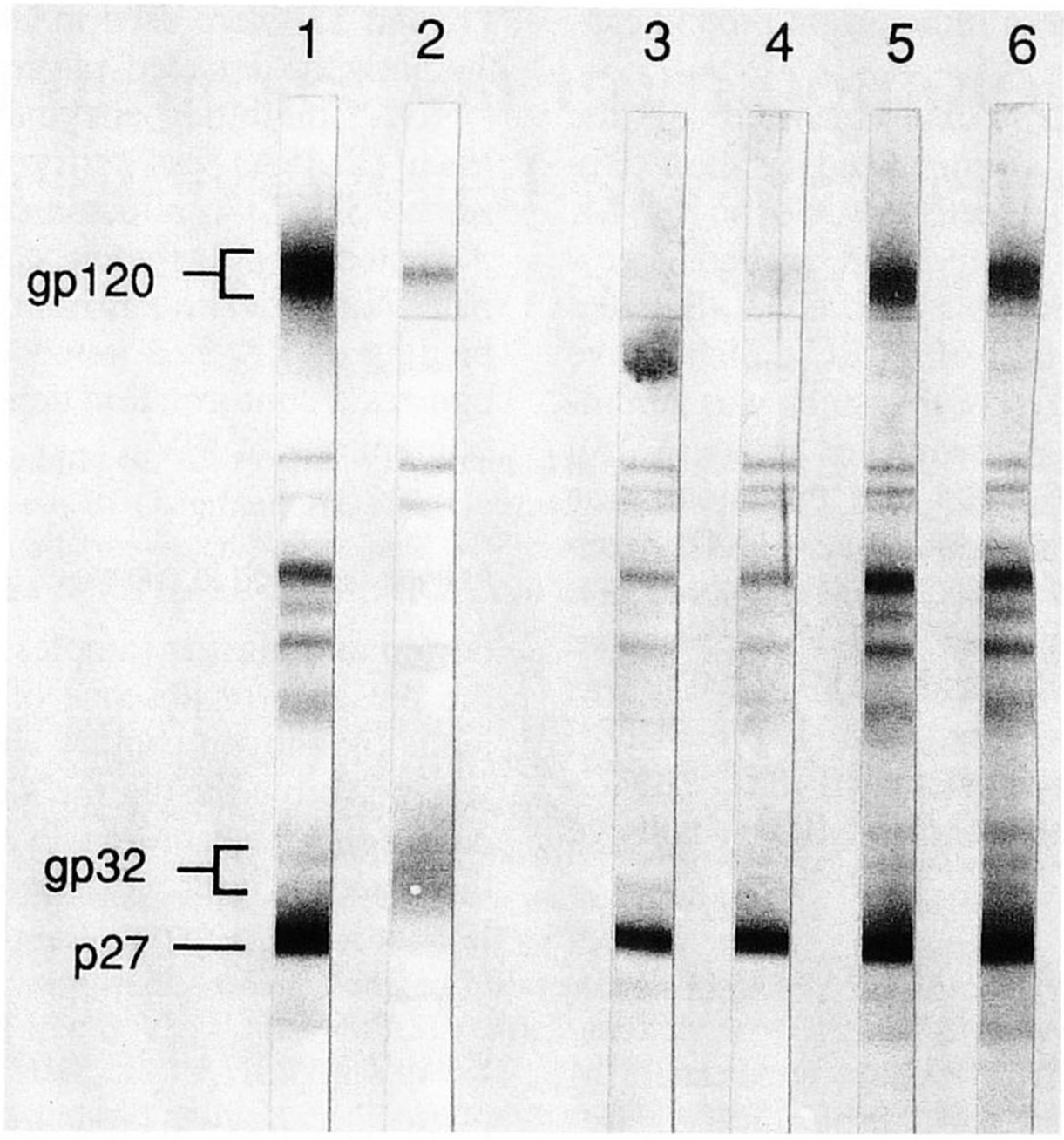

Fig. 1. Western blot analysis of four group A rhesus monkey plasma samples at day of challenge. The antigen used was a lysate of SIV_{mac32H} infected C8166 cells. Lane 1 shows the reactivity of monoclonal antibodies KK8 and KK33 with specificity for gp160/120 and p27, respectively. Lane 2 shows the reactivity of monoclonal antibody KK16, specific for gp160/32. Lanes 3 to 6 show antibody responses to monkey 4094, 1XD (both SIV-MDP vaccinated), 8656, and 1XB (both SIV ISCOM vaccinated).

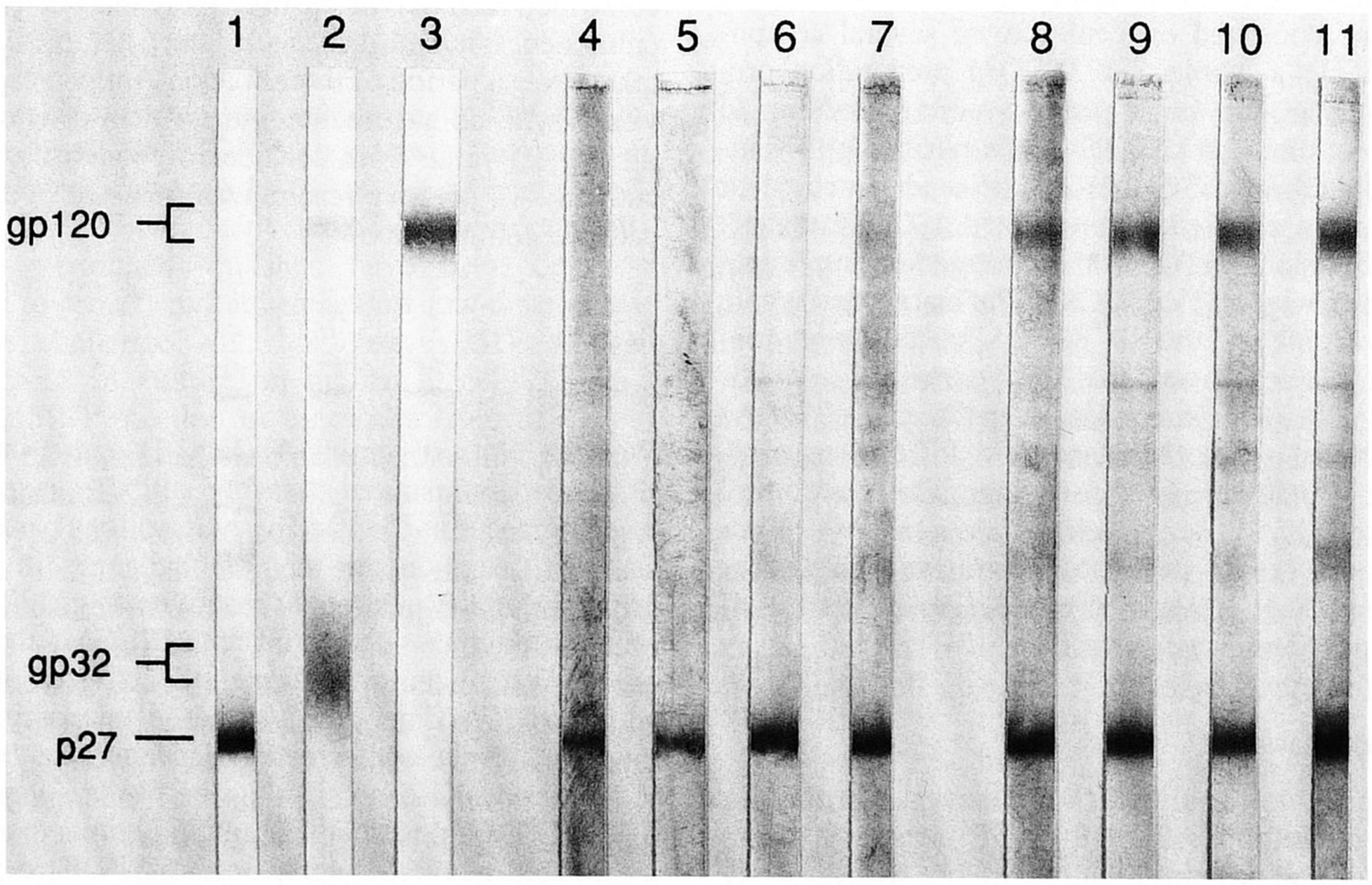

Fig. 2. Western blot analysis of eight group B rhesus monkey plasmas at day of challenge. Lanes 1 to 3 show the reactivity of monoclonal antibodies KK33, KK16, and KK8, respectively. Lanes 4 to 7 show antibody responses of monkey 8645, KP, 8649, 1IM (all four SIV-MDP vaccinated), 8730, 4097, 8653, and 8666 (all four SIV ISCOM vaccinated).

Table I. Vaccination/challenge schedule and postchallenge status

Vaccine	Monkey	Vaccine schedule (months)	Challenge[d] 10 MID_{50}	Weeks positive		Status
				Pl.Ag[a]	V.I.[b]	
MV ISCOM	8789	0, 1, 2, 4	cell-free	2,8	4,9	infected
	8791	0, 1, 2, 4	cell-free	2	4,9	infected
	8672	0, 1, 2, 5	cell-associated	2,4	4,9	infected
	8679	0, 1, 2, 5	cell-associated	2	4,9	infected
MV MDP	4060	0, 1, 2, 4	cell-free	2	4,9	infected
	1YH	0, 1, 2, 4	cell-free	2	4,9	infected
	2CA	0, 1, 2, 5	cell-associated	2	4,9	infected
	1JH	0, 1, 2, 5	cell-associated	2	4,9	infected
SIV ISCOM	4053	0, 1, 2, 4	cell-free	neg.	neg.	protected
	1XV	0, 1, 2, 4	cell-free	neg.	neg.	protected
	1JB	0, 1, 2, 4	cell-free	neg.	neg.	protected
	8656[c]	0, 1, 2, 4	cell-free			
	4097	0, 1, 2, 5	cell-associated	neg.	4,9	infected
	8653	0, 1, 2, 5	cell-associated	neg.	4,9	infected
	8668	0, 1, 2, 5	cell-associated	neg.	neg.	protected
	8730	0, 1, 2, 5	cell-associated	neg.	neg.	protected
SIV-MDP	4083	0, 1, 2, 4	cell-free	neg.	neg.	protected
	4094	0, 1, 2, 4	cell-free	neg.	neg.	protected
	1XD	0, 1, 2, 4	cell-free	neg.	neg.	protected
	3B	0, 1, 2, 4	cell-free	neg.	neg.	protected
	KP	0, 1, 2, 5	cell-associated	neg.	4,9	infected
	1IM	0, 1, 2, 5	cell-associated	neg.	4,9	infected
	8645	0, 1, 2, 5	cell-associated	neg.	neg.	protected
	8649	0, 1, 2, 5	cell-associated	neg.	neg.	protected

[a] Plasma antigen, weeks positive.
[b] Virus isolation, weeks positive.
[c] Died during recovery from anaesthesia at day of challenge (not included).
[d] Group A = cell-free, group B = cell-associated challenge.

one month postchallenge when challenged with either cell-free SIV (group A) or cell associated SIV (group B) challenge (Table I).

Virus isolation

Macaques vaccinated with either formalin inactivated SIV in MDP adjuvant or SIV ISCOMs challenged with ten MID_{50} of cell-free C8166 propagated virus stock (group A) were all protected (seven out of seven) based on repeated negative virus isolation and negative plasma antigen on day 14 (one of the original eight animals died incidentally on the day of challenge). Measles vaccinated control monkeys became positive for SIV antigen at day 14, and virus isolation was possible from PBMCs at all subsequent time points (see Table I).

SIV vaccinated macaques challenged with ten MID_{50} (200,000 cells) PBMCs from an SIV_{mac32H} infected macaque were not uniformly protected. Measles virus vaccinated controls became plasma SIV antigen positive at day 14 and positive for SIV by virus isolation at subsequent time points. SIV vaccinated animals were negative for plasma antigen on day 14, but in both formalin inactivated/MDP and ISCOM SIV vaccine groups (two out of four), animals in each group were virus isolation positive (Table I). To date four out of eight SIV vaccinated animals challenged with SIV infected macaque PBMCs remain protected based on repeated negative virus isolation attempts. In addition, protected animals were noted to have decreasing titers, while infected animals developed rising SIV antibody titers postchallenge.

Discussion

Vaccine protection (active) of nonhuman primates from lentivirus infection has been achieved in chimpanzees vaccinated with recombinant HIV-1 env gp120 [1] or with a combination based on boosts with V3 specific peptides [7], and in macaques with both whole virus vaccines [5,11] and enriched virus glycoprotein [12,13]. Protection in the macaque model with recombinant vaccinia priming ($SIV_{mne\,gp160}$) followed by recombinant baculovirus expressed gp160 resulted in protection of pig-tailed macaques from biologically cloned, human T-cell propagated SIV_{mne} challenge [8]. However, to date these results have not been reproducible in the SIV_{mac} system (A. Schultz, NIH, personal communication).

Whole virus vaccines of $SIV_{DeltaB670}$ or $SIV_{mac251(32H)}$ inactivated with formalin have been demonstrated to give protection against homologous and heterologous challenge. These results have been achieved using cell-free SIV of various strains propagated on human T-cell lines. This study set out to determine if protection could be achieved by the same dose of infected PBMCs har-

vested from a monkey (1XC) 11 months after infection with the same SIV strain used to produce the whole virus vaccines.

Our results demonstrate that two different whole vaccine preparations that are able to protect against cell-free challenge are also able to elicit protection in 50% of animals challenged intravenously with PBMCs from a macaque infected with homologous virus 11 months earlier. The reasons for achieving only partial protection may be explained by at least two possibilities. First, cell associated virus may result in an intracellular persistent infection resulting in infection from donor cells to host cells without sufficient opportunity for extracellular neutralization. The second possibility may be due to the genetic diversity of the challenge stock obtained from an animal 11 months postinfection with AIDS. The in vivo period of propagation of SIV_{mac32H} may have permitted variant virus to evolve that is capable of escaping neutralizing immunity induced by the vaccine strain.

These results demonstrate the feasibility of developing a practical efficacious vaccine for HIV protection in man. The mechanisms by which cell-associated protection or failure are achieved must be elicited for the development of vaccine strategies capable of providing complete protection against cell associated challenge.

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