

AIDS vaccine developments

SIR — In assessing vaccine strategies for preventing AIDS, Stott *et al.* reported¹ that macaques immunized with uninfected C8166 cells² (a T-cell line of human origin) were protected against subsequent intravenous infection by simian immunodeficiency virus (SIV), which induces an AIDS-like disease in macaques. Further investigation of the humoral response of vaccinated monkeys with SIV-infected and inactivated cells or with purified inactivated virus, revealed that all animals have antibodies directed against cellular components.

the strongest humoral immune response to both SIV and C8166 antigens. We also detected high ELISA titres against fresh human PBLs, whereas these animals raised weak antibody responses against rhesus macaque PBLs.

Eighteen weeks after the first challenge experiments, the protected animals of the high-dose vaccinated group were reboosted with the same vaccine preparation and challenged intravenously 2 weeks later with 10 AID₅₀ of an homologous SIV_{MAC}251 strain, produced by co-cultivating spleen cells of an infected

antibody crossreactivity between cellular and lentivirus components, which Maddox suggests⁵ could contribute to the understanding of AIDS pathogenesis. Nevertheless, our results show that protection of macaques cannot be related to an immune crossreactivity between cellular components and SIV antigens. Rather, we suggest that the strong anti-human cell immunity developed by our vaccinated macaques efficiently participates in protection (as previously suggested⁶) through cellular antigens on the envelope of the human PBL-grown viruses used for the first challenge. The results we report here demonstrate the absolute necessity of designing vaccine experiments where viruses used for challenge infection are grown in PBL compatible with the host species. In addition, an alternative strategy to the investigation of the anti-lentiviral specific immune responses can be provided by highly purified antigens whatever their origins: inactivated viruses, recombinant or synthetic subunits.

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ELISA TITRES AGAINST SIV AND CELLULAR ANTIGENS AT THE DAY OF CHALLENGE									
Antigens detected	2 mg vaccinated				0.4 mg vaccinated			Control	
	8,715	8,738	8,744	8,770	8,758	8,762	8,778	8,771	8,783
SIV	5	5	5	5	5	4	4	Neg	Neg
C8166 cells	4	4	5	4	4	3	3	Neg	Neg
Human PBL	3	3	4	3	ND	ND	ND	Neg	Neg
Rhesus PBL	1	2	2	1	1	1	1	Neg	1

Neg, negative result. Regarding the high sequence homology of HIV-2 and SIV, the specific anti-SIV serum reactivity was determined⁴ using an HIV-2 antigen detection assay. We used the ELAVIA II kit (Diagnostics Pasteur) using as second antibody a peroxidase-labelled anti-monkey immunoglobulin G. Titres were determined as the last serum dilution, giving a significant positive optical density (OD) at 490 nm when compared to measures of 10 preimmune sera at the same serum dilution (cut off = OD average of preimmune sera + 2.26 s.d.). ND, not determined. ELISA titres expressed as log₁₀.

Moreover, protection of these macaques against an homologous SIV challenge correlates with the ELISA titres against C8166 cells. Both vaccine preparations and the challenge virus stock were obtained from the same culture system: SIV-infected C8166 human cells.

As part of the European Communities' AIDS Concerted Action programme, we immunized rhesus macaques with the same column-purified vaccine preparation (formalin-inactivated SIV_{MAC} strain 251, 32H isolate, grown on human C8166 cells, provided by M. Cranage and P. Greenaway) in alum adjuvant. Two groups of animals received either 2 or 0.4 mg immunogen (days 0, 30, 60 and 135) and were challenged, 2 weeks after the final boost, with 10 AID₅₀ (animal infectious dose 50%) of an heterologous SIV_{SM} virus stock (provided by P. Putkonen) produced on fresh human peripheral blood lymphocytes (PBL)^{3,4}. All four macaques in the high-dose immunized group, and two of three animals in the low-dose group, were protected more than 4 months after intravenous challenge.

We analysed the antibody responses of these monkeys. All animals exhibited high ELISA titres to uninfected C8166 cells as well as lentiviral-specific antigens. The high-dose vaccinated animals developed the highest humoral response to lentiviral and cellular antigens (see table). Surprisingly, ELISA titres were 10 times lower in the low-dose vaccinated group except for the unprotected monkey (No. 8,758), which developed

monkey with fresh macaque PBLs: this virus has therefore never been exposed to a human cell. All monkeys developed clinical and biological evidence of infection (virus isolation by coculture, PCR, anamnestic response) 15 days after the second challenge experiment.

Controversial results from vaccine experiments highlight the difficulty in interpreting such experiments: cautious conclusions should include detailed discussions about controls. Our experiments do not exclude the possibility of

SIR — Several groups have independently demonstrated that macaques may be protected from infection with simian immunodeficiency virus (SIV) by immunization with inactivated vaccines, based on either whole inactivated SIV or fixed SIV-infected cells⁷⁻⁹, raising expectations that the development of a vaccine for the protection from human immunodeficiency virus (HIV) infection in man may be successful. But it has become apparent that non-virus-specific cellular antigens present in SIV vaccine preparations used in those experiments may have played a role in the protection observed. The SIV virus stock used for challenge of vaccinated monkeys had been prepared from SIV-infected human T-cell lines, the same or similar to those used for production of the vaccine preparation itself¹.

We have carried out a vaccination challenge experiment in the same SIV-macaque model as part of the European Communities' Concerted Action prog-

ramme. Our results indicate that at least part of the protection induced by inactivated SIV preparations is not due to immunization with non-virus-specific human T-cell antigens. To this end we have compared the efficacy of two SIV whole virus vaccine preparations, administered to two groups of seven and eight rhesus monkeys (*Macaca mulatta*), respectively. The first vaccine was an inactivated whole SIV preparation adjuvanted with muramyl dipeptide (MDP) previously demonstrated to elicit protection; the second was an SIV-iscom preparation containing both the Gag and Env proteins of SIV. These vaccines had been prepared from the SIV_{MAC} strain 251 (32H), propagated on the human T-cell line C8166 as described by Stott *et al.*¹. Inactivated measles virus adjuvanted with MDP (MV-MDP) and an MV-iscom preparation¹⁰ served as controls, each of which were inoculated into two separate groups of four rhesus monkeys. Two weeks after the fourth intramuscu-

PROTECTION INDUCED BY SIV VACCINES IN RHESUS MACAQUES

Group	Type of vaccine	Type of i.v. SIV challenge (10 MID ₅₀)	Monkeys protected from viraemia*/ monkeys per group	Serum antibody titres measured in ELISA (log ₁₀ ± s.d.) at day of challenge†		
				Antigen		
A	SIV-MDP	cell-free	4/4	SIV-env	2.4±0.4	C8166 2.0±0.2 RhPBMC 1.9±0.1
	SIV-iscom‡	cell-free	3/3		3.4±0.2	2.7±0.2 2.7±0.2
	MV-MDP	cell-free	0/2		≤0.8	2.1±0.1 1.8±0.2
	MV-iscom	cell-free	0/2		≤0.8	1.9±0.1 1.9±0.3
B	SIV-MDP	infected PBMC	2/4		3.3±0.4	2.1±0.1 1.9±0.1
	SIV-iscom	infected PBMC	2/4		3.6±0.3	2.8±0.2 2.7±0.2
	MV-MDP	infected PBMC	0/2		≤0.8	≤1.5 1.9±0.3
	MV-iscom	infected PBMC	0/2		≤0.8	≤1.5 2.1±0.1

* Viraemia was demonstrated by co-cultivation of PBMC with C8166 cells and subsequent demonstration of SIV antigen in the medium by P27-antigen capture assay. Results were confirmed by showing serum antibody induction (MV-vaccinated monkeys) or booster reaction, in an SIV env-specific ELISA and Western blot assay.

† SIV-Env: inhibition of reactivity of labelled SIV-neutralizing mouse monoclonal antibody (KK5) by serial dilutions of monkey sera. KK5 kindly provided by Dr K. Kent (through MRC-ADP).

‡ One animal not included (died during recovery at day of challenge).

C8166/RhPBMC, reactivity of serial dilutions of monkey sera with solubilized membrane protein fraction (about 10 µg ml⁻¹) of respective cells attached to ConA-coated wells.

For vaccination all the monkeys were challenged intravenously with either 10 monkey infectious doses (MID₅₀) SIV_{MAC}251 (32H) propagated in C8166 cells (group A), or with SIV-infected peripheral blood mononuclear cells (PBMC) obtained from an SIV-infected rhesus macaque (1×C) (group B). These PBMC had been prepared from heparinized blood of the monkey, 11 months after experimental infection with the same cell-free challenge stock of SIV_{MAC}251 (32H), frozen in 5×10⁵ cell aliquots and subsequently titrated *in vivo* in rhesus macaques. The equivalent of 10 MID₅₀ of these cells was used as challenge dose for group B.

The results are shown in the table. All the monkeys of group A vaccinated with SIV-MDP or SIV-iscom were protected from developing SIV viraemia for the 12-week observation period after intravenous cell-free SIV_{MAC}251 (32H) challenge, whereas all the MV-MDP and MV-iscom vaccinated monkeys developed SIV viraemia within 4 weeks after receiving the same cell-free challenge. Also all the MV-MDP and MV-iscom vaccinated monkeys of group B developed SIV viraemia within 2 weeks after intravenous challenge with SIV-infected PBMC. Two out of four SIV-MDP vaccinated monkeys and two out of four SIV-iscom vaccinated monkeys of group B were protected from SIV viraemia for the 9-week observation period after intravenous challenge with the SIV-infected PBMC.

These data were confirmed by demonstrating that all the monkeys that became SIV-viraemic also showed booster responses in their serum antibody titres measured in an SIV env-specific enzyme-

linked immunosorbent assay (data not shown). At the day of challenge, all SIV-vaccinated animals had developed serum antibody titres in this assay. In addition, antibodies directed against C8166 cells and rhesus PBMC present at that day were measured (see table). The protection found in group B among SIV-vaccinated monkeys did not correlate with the levels of serum antibody titres to C8166 cells or PBMC in these animals.

This is the first demonstration in the SIV-macaque model that vaccination can protect against challenge with cell-associated SIV, and is the first report that vaccinated, previously unchallenged non-human primates can be protected from infected PBMC from another infected animal. As the SIV challenge material used in group B was directly prepared from infected PBMC of the homologous species, the partial protection observed against this severe intravenous challenge should be attributed to immunization with SIV-specific antigens. Consequently the suggestion by Stott *et al.*¹, that virus- as well as cell-specific components can still be involved in protection after vaccination with inactivated SIV preparations, is strongly supported by our observations.

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SIR — Stott *et al.*¹ have presented evidence that the protection elicited with an SIV vaccine⁹ containing fixed SIV-infected cells of human origin (C8166) may, at least in part, be mediated by anti-cell responses resulting from xenoinmunization. This surprising observation raises important questions about the nature of the protective responses elicited by other SIV vaccines.

As part of an AIDS vaccine development project, within the MRC AIDS Directed Programme and the European Communities' Concerted Action programme, we have immunized 10 rhesus macaques with a partially purified, inactivated virus vaccine based on the 32H isolate of SIV_{MAC} strain 251 grown in C8166 cells (manuscript in preparation). Four animals (group A) each received four 500-µg doses of vaccine given in the Syntex adjuvant formulation 1 (saf-1). Another two groups of three animals each received the same vaccine given in alum, either as four 500-µg doses (group B) or as four 100-µg doses (group C). All animals were challenged intravenously 2 weeks after the final vaccine boost with 10 median monkey infectious doses (MID₅₀) of cell-free virus. Group A were challenged with SIV_{SM}B670, a related but antigenically distinct strain of SIV, grown on human peripheral blood mononuclear cells (PBMC)⁸. Animals in groups B and C were challenged with homologous virus, that is the 32H isolate of SIV_{MAC}251 grown on C8166 cells.

Although unvaccinated control animals all became infected, all of the vaccinated animals were protected from infection as determined by the inability to recover virus from PBMC, inability to detect proviral DNA in PBMC and lack of an anamnestic antibody response. Animals were subsequently boosted further with vaccine, formulated as previously, at 16 months (group A) and 6.5 months (groups B and C) after initial challenge. At 2 weeks after the additional boost, all 10 animals plus 4 unvaccinated controls were challenged with 10 MID₅₀ of cell-free SIV_{MAC}251 grown in monkey PBMC. Virus was recovered from all of these animals as early as 2 weeks after challenge. Thus no protection from challenge was elicited despite the fact that the additional vaccination boosted the SIV-specific antibody response. In this experiment we have not

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