AIDS vaccine developments

SIR — In assessing vaccine strategies for preventing AIDS, Stott *et al.* reported[1] that macaques immunized with uninfected C8166 cells[2] (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. 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 AID_{50} (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 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 AID_{50} of an homologous $SIV_{MAC}251$ strain, produced by co-cultivating spleen cells of an infected 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 antibody crossreactivity between cellular and lentivirus components, which Maddox suggests[5] 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 suggested[6]) 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.

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 determined[4] 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 $\log_{10}$.

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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 cells[7–9], 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 itself[1].

We have carried out a vaccination challenge experiment in the same SIV-macaque model as part of the European Communities' Concerted Action programme. 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 preparation[10] served as controls, each of which were inoculated into two separate groups of four rhesus monkeys. Two weeks after the fourth intramuscu-