

## Mechanisms of protection induced by attenuated simian immunodeficiency virus. I. Protection cannot be transferred with immune serum

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**To evaluate its role in protection, immune serum was collected from four macaques which were chronically infected with live attenuated simian immunodeficiency virus (SIVmacC8) and had resisted challenge with wild-type SIVmacJ5. The immune serum was transferred to two naïve cynomolgus macaques by intraperitoneal injection (11 ml/kg). Four control macaques received an intraperitoneal injection of normal saline. One day later, all macaques were challenged with 10 MID<sub>50</sub> of the J5M challenge stock of SIV. After challenge, all macaques became infected as determined by virus co-culture and diagnostic PCR. Virus loads in PBMC at 2 weeks post-challenge were indistinguishable between the two groups of macaques. Thus, the failure of passive immunization to transfer protection indicates that serum components alone are not sufficient to mediate the potent protection obtained using live attenuated vaccines. This is the first time that serum has been transferred from animals known to be protected against superinfection.**

The experimental infection of macaques with simian immunodeficiency virus (SIV) is a powerful model in which the efficacy of AIDS vaccines may be evaluated (Stott & Almond, 1995). To date, the most potent protection has been obtained with live attenuated vaccines. The genetic disruption of the *nef* gene significantly restricts the replication of SIV in macaques and prevents the induction of disease (Kestler *et al.*, 1991; Rud *et al.*, 1994). Chronic infection with attenuated isolates of SIVmac can confer complete protection against superinfection with pathogenic SIVmac (Daniel *et al.*, 1992). Furthermore, chronically infected macaques resist challenge with virus-

infected cells as well as with cell-free virus (Almond *et al.*, 1995). However, safety issues preclude the widespread use of live retrovirus-based vaccines in naive populations (Baba *et al.*, 1995). In order to circumvent this problem, we have initiated a series of experiments aimed at elucidating the mechanism of protection conferred by live attenuated SIV. It may then be possible to reproduce the protective response by alternative, less hazardous methods. In this study, the role of components in immune serum was assessed by passive transfer into naive recipients.

Four cynomolgus macaques (L103–L106) were inoculated with 10<sup>4</sup> TCD<sub>50</sub> of the 9/90 pool of C8, prepared on C8166 cells (Rud *et al.*, 1994). All macaques became infected. At weeks 39 and 58 post-infection, these four macaques were challenged with 10 MID<sub>50</sub> of the wild-type SIV isolate J5M (Rud *et al.*, 1994) and 200 TCD<sub>50</sub> of the SIV/HIV-1 chimera SHIV-4 (Li *et al.*, 1992) respectively. No evidence for superinfection of these macaques was obtained by virus co-culture, diagnostic PCR or serological assays (Almond *et al.*, 1995; Stott *et al.*, 1994). At week 106, serum was collected from the four macaques, pooled and stored at –70 °C. This pool of serum was analysed for antibodies to SIV by ELISA, using methods described previously (Stott *et al.*, 1990). The log<sub>10</sub> titres of antibodies to envelope and Gag were 3·5 and 2·6 respectively.

The pool of unheated, pooled immune serum was transferred to two naive macaques (P203 and P204) by intraperitoneal injection at a dose of 11 ml/kg body weight. Four control macaques (P205–P208) received an intraperitoneal injection (11 ml/kg) of normal saline (0·85%, w/v, NaCl). Twenty-four hours later, titres of antibodies to envelope in P203 and P204 were log<sub>10</sub> 2·7 and 2·8 respectively. These results indicate observed dilution factors of log<sub>10</sub> 0·7–0·8 for the donated serum pool. Assuming a volume of 70 ml/kg, the infected serum at 11 ml/kg would be diluted 1:6·4. Hence, the antibody was absorbed efficiently into the plasma. At this time, all six macaques were challenged with 10 MID<sub>50</sub> of the J5M challenge stock (Rud *et al.*, 1994). On the day of challenge (day 1) and at days 13, 26 and 56 post-challenge, the presence of the challenge virus in 5 × 10<sup>6</sup> peripheral blood mononuclear cells (PBMC) was detected by co-cultivation with 10<sup>6</sup> C8166 cells

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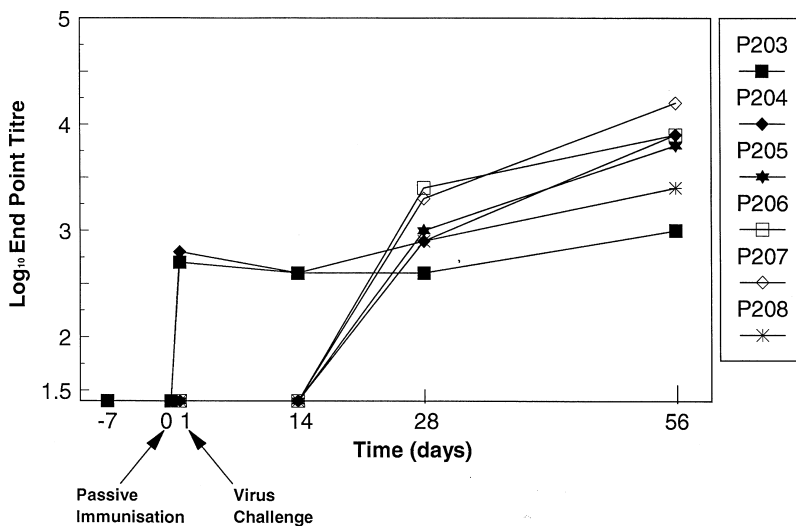
**Table 1. Recovery of virus from macaques following challenge with J5M**

VI, virus isolation: co-culture of PBMC with indicator C8166 cells (\* virus titration: number of SIV-infected cells per 10<sup>6</sup> PBMC co-cultured with C8166 cells).

PCR, diagnostic PCR which differentiates challenge virus J5 from live attenuated vaccine virus C8.

ND, Assay not performed.

Macaque	Injection day 0	Virus detection							
		Day 1		Day 14		Day 28		Day 56	
		VI	PCR	VI*	PCR	VI	PCR	VI	PCR
P203	Immune serum	—	—	1 000	J5	+	ND	—	J5
P204		—	—	3 000	J5	+	ND	+	J5
P205	Normal saline	—	—	10 000	J5	+	ND	+	J5
P206		—	—	1 000	J5	+	ND	—	J5
P207		—	—	30	J5	+	ND	—	J5
P208		—	—	30	J5	+	ND	+	J5



**Fig. 1.** Titres of antibodies with specificity for SIV rgp140 detected in macaque plasma by ELISA. End-point titres were determined for each plasma sample taken on days -7 to 56 from macaques P203 and P204, which received intraperitoneal injections of 11 ml/kg of unheated immune serum pool on day 0, and macaques P205, P206, P207 and P208, which received 11 ml/kg normal saline on day 0. On day 1, all macaques were challenged with 10 MID<sub>50</sub> of SIVmac J5M.

and by a diagnostic PCR. In some instances, the virus load was assessed by co-cultivating 10-fold dilutions of PBMC between 10<sup>2</sup> and 10<sup>6</sup> cells with C8166 cells in 24-well plates. The diagnostic PCR is capable of differentiating C8 virus which, theoretically, may have been present in the donated unheated plasma pool, from the challenge virus, J5M, by amplification of the *nef* gene (Rose *et al.*, 1995). The results of these assays are summarized in Table 1. In addition, antibodies to SIV core protein (p27) and envelope (gp140) were assessed before and after virus challenge, by ELISA as described previously (Stott *et al.*, 1990; Fig. 1).

The intraperitoneal injection of the immune serum resulted in the successful transfer of anti-SIV antibodies, detectable in the blood of macaques P203 and P204 on the day of challenge (Fig. 1). Following challenge, the titres of anti-envelope

antibodies in macaques P203 and P204 remained steady through to day 26 before increasing. In contrast, control macaques seroconverted between days 13 and 26 post-challenge and continued to rise until day 56.

Upon challenge, all six macaques became infected with SIV, as judged by virus co-cultivation and diagnostic PCR (Table 1). At day 13 post-challenge the frequency of SIV-infected PBMC was determined by titration. The virus load detected in the blood of the two macaques which received immune serum was indistinguishable from the loads in the infected controls. Proviral DNA was detected in all the samples taken from the six macaques following challenge. The size of the DNA fragments obtained following restriction endonuclease digestion of the PCR products with the enzyme *RsaI* indicated that the template DNA was derived from virus J5 and not C8.

In addition, the virus isolates in positive co-cultures were confirmed to be J5, by immunostaining with KK70, a MAb specific for SIV Nef which is capable of differentiating between J5 and C8.

There was no evidence from this study that the potent protection obtained by chronic infection with live attenuated SIV could be conferred through serum components alone. Intraperitoneal injection successfully transferred the immune serum, since anti-SIV antibodies were detected in the serum of recipient animals on the day of virus challenge. Nevertheless, both recipients of immune serum became infected when challenged with  $10 \text{ MID}_{50}$  of J5M, a virus challenge which the donors of the immune serum had resisted previously (Almond *et al.*, 1995). Furthermore, there was no evidence from the virus loads measured at 2 weeks post-challenge that the immune serum had conferred upon the recipient any capacity to restrict virus replication.

Previous attempts to confer passive protection against SIV with immune serum or immunoglobulins have given conflicting results. In contrast to the results obtained with SIVmne, SIVsm and HIV-2 (Lewis *et al.*, 1993; Putkonen *et al.*, 1991), where protection was successfully transferred, experiments with SIVmac have been uniformly unsuccessful (Gardner *et al.*, 1995; Kent *et al.*, 1994). In the first study, neither immune serum pools prepared from early acute (12 weeks) or chronically (> 2 years) infected macaques nor a mixture of four neutralizing anti-SIV MAbs detectably altered the course of infection in naive macaques, following challenge with a genetically closely related virus prepared on simian PBMC (Kent *et al.*, 1994). Similarly, Gardner *et al.* (1995) reported that neither plasma nor purified immunoglobulin prepared from SIV-infected macaques were able to confer passive protection against challenge with SIVmac251. In contrast, if the challenge stock of SIVmac has been prepared on xenogeneic human T cells, then passive protection against SIVmac can be transferred with immune serum which contains antibodies against host-cell components (Gardner *et al.*, 1995; Stott *et al.*, 1994). This is possible despite the dilution of protective serum components.

In these previous reports, where serum pools from SIVmac-infected macaques were used, a formal demonstration that the donors were protected against superinfection with SIVmac had not been performed. In the study reported here, the immune serum pool was prepared from macaques which had been infected with C8 and had resisted a subsequent challenge with J5M (Almond *et al.*, 1995). Nevertheless, there was no evidence that passive transfer of the immune serum transferred any protective immunity against the same virus challenge.

Live attenuated vaccines confer potent protection against pathogenic SIV challenge. However, considerable safety issues would preclude the application of this approach for a prophylactic vaccine against human AIDS. This study was designed to elucidate the role of serum components in conferring protection. Wyand *et al.* (1996) observed that

macaques which had been immunized with live attenuated SIV and protected against superinfection exhibited high titres of neutralizing antibody against primary isolates of SIV. However, these observations have not been reproduced by others (Norley *et al.*, 1996; Stahl-Hennig *et al.*, 1996). Furthermore, live attenuated vaccines seldom elicit antibody titres (total or neutralizing) which compare with those obtained using recombinant envelope vaccines, which fail to confer protection against infection. Clements *et al.* (1995) also proposed that the protection obtained with an attenuated macrophage-tropic SIV isolate correlated with a broad cross-neutralizing response. Furthermore, they demonstrated passive protection against SIVsmB670 in two of four macaques which received heat-inactivated serum from donor macaques chronically infected with isolate 17E-Cl (Clements *et al.*, 1995). Thus, whilst it may be possible to transfer protection to some recipients with serum, our study indicates that it need not be an essential component.

Recently it has been reported that the  $\beta$ -chemokines MIP-1 $\alpha$ , MIP-1 $\beta$  and RANTES can inhibit infection by HIV-1 and SIV, possibly by competitive blocking of the secondary receptor needed for virus entry into T-cells (Cocchi *et al.*, 1995; Feng *et al.*, 1996). The serum pool used for the transfer in this study was not heat-inactivated or fractionated, so that all soluble serum components could be assessed, in addition to the immunoglobulin fraction. The serum pool transferred contained 7500, < 47 and < 31 pg/ml of RANTES, MIP-1 $\alpha$  and MIP-1 $\beta$  respectively. However, analyses of the plasma of each macaque before and after transfer of immune serum or saline did not identify a significant change in RANTES concentration (data not shown).

In this study, virus loads were assessed at 2 weeks following infection. Comparison of the virus loads in macaques which received immune serum and normal saline failed to demonstrate any evidence that the immune serum conferred even partial protection. In contrast, a recent study by Haigwood *et al.* (1996) found that injection of high-titre purified immunoglobulin into macaques 1 and 14 days after challenge with SIVsm had profound effects upon the infection and subsequent course of disease. Macaques that received immune globulin from an SIV-infected macaque exhibited suppressed virus loads throughout the course of the primary viraemia and delayed progression to disease. It is intriguing whether this distinct result reflects the relative timing of challenge and passive transfer or differences in the SIVsm/macaque and SIVmac/macaque models.

Thus, this study did not identify serum components that are, alone, capable of conferring the protective mechanism of live attenuated SIV vaccines. Further studies are required to define the role of cellular immunity in this protection. For example, it has been reported that infection with C8 virus results in a persistent proliferative T-cell response in macaques, in contrast to infection with pathogenic SIV (Stahl-Hennig *et al.*, 1996). However, macaques are outbred and this presents a

technical challenge for many of these studies. Nevertheless, these problems must be overcome if we are to understand the protective mechanism of live attenuated viruses and so develop an effective, safe AIDS vaccine.

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