

Accelerated Viremia in Cats Vaccinated with Recombinant Vaccinia Virus Expressing Envelope Glycoprotein of Feline Immunodeficiency Virus

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THE SIMILARITIES BETWEEN FELINE immunodeficiency virus (FIV) and human immunodeficiency virus (HIV) and between the pathogenesis of the syndromes they cause have led us to use FIV infection of cats as a model with which to evaluate potential preventive vaccines for HIV infection in humans.¹⁻¹⁰ Protective immunity can be induced in cats against homologous and, to a lesser extent, heterologous FIV challenge, by vaccination with inactivated whole virus or FIV-infected cells,^{11,12} and the protection can be transferred to naive cats in plasma from vaccinated animals, indicating that antibodies may be the basis of this protection.¹³ Use of FIV vaccines based on recombinant envelope proteins would have clear advantages over inactivated or attenuated virus vaccines; however, so far, vaccination strategies using purified envelope glycoproteins of FIV or fractions of these proteins as immunogens have failed.^{14,15}

In the present study, we used recombinant vaccinia virus (rVV) or bacteria-expressed FIV envelope protein incorporated into immune-stimulating complexes or mixed with adjuvants in candidate FIV vaccines. The envelope glycoproteins of FIV AM19 were expressed by an rVV in baby hamster kidney cells, either in their native form (vGR657) or after deletion of the cleavage site between the surface (SU) and transmembrane (TM) proteins (vGR657 × 15) and incorporated into immunostimulating complexes (ISCOMs), resulting in two ISCOM preparations, vGR657 and vGR657 × 15, as previously described.¹⁶ A 1870-base pair (bp) fragment of the envelope gene was excised from pBluescript (Stratagene, La Jolla, CA) containing the whole envelope gene, by using the restriction enzymes *Bam*HI and *Bgl*III (nucleotide positions 350 and 2220, respectively) and subcloned into *Bam*HI-digested pEX vector (Stratagene, La Jolla, CA). This vector allows inducible expression of proteins such as the β -galactosidase fusion protein, which was partially purified as inclusion bodies, solubilized, and mixed with Quil A as an adjuvant (Gal-FIV Env plus Quil A). Simian immunodeficiency virus (SIV) envelope glycoprotein ISCOMs were prepared by E. Hulskotte (Institute of Virology, EUR, Rotterdam), by a method similar to that used for the cleavage site-deleted FIV envelope glycoprotein ISCOMs.¹⁷

Six groups of six cats each were vaccinated three times subcutaneously according to the following schedule: group 1, vGR657 ISCOMs; group 2, vGR657 × 15 ISCOMs; group 3, vGR657 × 15 plus Quil A; group 4, Gal-FIV Env plus Quil A; group 5, SIV Env ISCOMs; and group 6, phosphate-buffered saline. The cats were vaccinated with 10 μ g of protein at weeks 0, 4, and 10, and 2 weeks later were challenged with 20 times the dose required to infect 50% of cats (CID₅₀) of FIV AM19 intramuscularly. Peripheral blood mononuclear cells (PBMCs) and plasma samples were collected after challenge every 2 weeks for 8 weeks.

Plasma samples were tested for the presence of FIV-specific antibodies by enzyme-linked immunosorbent assay (ELISA) and the virus neutralization assay; PBMCs were used to determine the cell-associated viral load. Antibodies against Gag proteins p24 and p17 were detected with a commercially available kit, using recombinant p24 and p17 proteins (Cat. no. F1002-AB01; European Veterinary Laboratory BV, Woerden, The Netherlands). Antibodies against the envelope protein were detected by ELISA with synthetic peptides purchased from the European Veterinary Laboratory BV (Cat No. SU peptide [EVS-000-PE-003] and TM peptide [EVS-000-PE-004]). The first synthetic peptide contains the immunodominant virus-neutralizing epitope within variable region 3 (HIV-3) spanning amino acid residues 396-412 of the surface protein of the Petaluma strain (SU peptide).¹⁸ The second peptide contains a B cell epitope between amino acid positions 695 and 706 of the transmembrane protein of the same FIV strain (TM peptide).

Serum antibody titers on the day of challenge against the SU and TM peptides are shown in Table 1. All cats in groups 1 and 2 (vGR657 and vGR657 × 15 ISCOMs) had high plasma antibody levels against both peptides, whereas the cats in group 3 (vGR657 × 15 with Quil A) had lower overall reactivities with these peptides. The cats in group 4 (fusion protein with Quil A) had also developed high titers of plasma antibodies against the TM peptide, but only one cat in this group had developed antibodies against the SU peptide.

All of the plasma samples collected on the day of challenge

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TABLE 1. VIRUS-NEUTRALIZING ANTIBODY TITER AT DAY OF CHALLENGE AND ANTIBODY RESPONSE AGAINST SURFACE AND TRANSMEMBRANE PEPTIDES IN VACCINATED CATS AT DIFFERENT TIMES AFTER CHALLENGE

Group	Cat	VN	Surface peptide (weeks after challenge) ^a					Transmembrane peptide (weeks after challenge) ^a				
			0	2	4	6	8	0	2	4	6	8
1	116	160	++	+++	+++	+++	+++	++	++	++++	++++	++++
	60	640	+++	++	+++	++	++	+	++	+++	++	++
	72	640	+++	+++	++++	+++	+++	+	+	+++	++	++
	84	640	++	++	+++	+++	++	+	+	+	+	+
	62	640	++	++	+++	+++	+++	+	+	+++	+++	+++
	105	320	++	++	+++	++	++	+	+	+	+	+
2	106	640	++	++	++++	++++	+++	++	++	++++	+++	+++
	64	320	++	+++	+++	+++	++	+	+++	+++	+++	+++
	73	80	+	+	+	++	+	+	++	++	++	++
	89	80	+	+	+	+	+	+	+	++	++	++
	92	40	+	+	+++	+++	++	++	++	++++	++++	++++
	117	80	+	+	++	++	++	+	+	+++	++	++
3	59	40	+	+	+++	+++	++			++	++	++
	70	40	+	+	+++	+++	+++	++	+	++++	++++	+++
	96	20	+	+	++	++	++	+	+	++	+++	+++
	115	40	+	+	++	++	++	+	+	++	++	++
	88	20			++	+	++	+	+	++	++	++
	13	10		+	+	++	+	+	+	++	++	++
4	63	<10				+	+	++	+	++	++++	+++
	82	<10				+	+	++	++	++++	++++	+++
	85	<10	+	+	+	+	+	+	+	++	++	++
	11	<10						++	++	++	++	+++
	112	<10						++	++	++++	+++	+++
	15	<10				+	+			+	+++	+++
5	43	<10				+	+			++	+++	+++
	83	<10	+	+	+	++	++	+	+	+	++	+++
	86	<10				+				++	+++	+++
	110	<10				+	+			++	+++	+++
	H333	<10				+	++			+	++	+++
	17	<10					+			+	++	++
6	42	<10				+	+				+++	+++
	68	<10				+	+			+	+++	+++
	104	<10			+	+	+			+	++	+++
	103	<10				+	+			++	++	+++
	25	<10									++	++
	27	<10				+	+				+++	+++

^aSurface peptide titers: blank, <200; +, 200–1000; ++, 1000–5000; +++, 5000–25,000; +++, >25,000. Transmembrane peptide titers: blank, <50; +, 50–300; ++, 300–3000; +++, 3000–10,000; +++, >10,000.

were also tested in two virus-neutralizing antibody assays. The feline lymphocyte assay was based on inhibition of infection of thymocytes with molecularly cloned FIV 19k1, as described previously.⁸ The CrFK virus-neutralizing assay was based on inhibition of infection of CrFK 1D10 cells with an FIV isolate (FIV-AM6c), which is adapted to replicate in these cells, as previously described, with minor modifications¹¹; the presence of FIV-specific antigen after 8 days was tested by ELISA.¹⁹ None of the samples had virus-neutralizing activity in the feline lymphocyte assay (data not shown); however, virus-neutralizing antibodies were demonstrated in cats in groups 1, 2, and 3 in the CrFK assay (Table 1). The virus-neutralizing titers in cats vaccinated with vGR657 ISCOMs (group 1) were not significantly different from those of cats vaccinated with vGR657 × 15 ISCOM (group 2), all ranging from 40 to 640. The virus-neutralizing plasma antibody titers of cats vaccinated with the

vGR657 × 15 protein with Quil A adjuvant varied from 10 to 40 and were significantly lower than those found for groups 1 and 2 (Student's *t* test; *p* < 0.05). No virus-neutralizing antibody response could be demonstrated on the day of challenge in cats vaccinated with the Gal-FIV-Env fusion protein (group 4) or in cats in the control groups (5 and 6).

Within 4 weeks after challenge infection, the plasma antibody titers against the SU and TM peptides of most of the cats immunized with the recombinant FIV envelope proteins (groups 1–4) had increased by 3- to 10-fold. Within 8 weeks, all of the cats, including the control animals, had developed anti-SU and anti-TM peptide plasma antibodies (Table 1). Eight weeks after challenge, the SU-specific peptide antibody titers in the cats vaccinated with rVV expressing FIV envelope glycoprotein were significantly higher than those of the nonvaccinated cats (Table 1; *p* < 0.05).

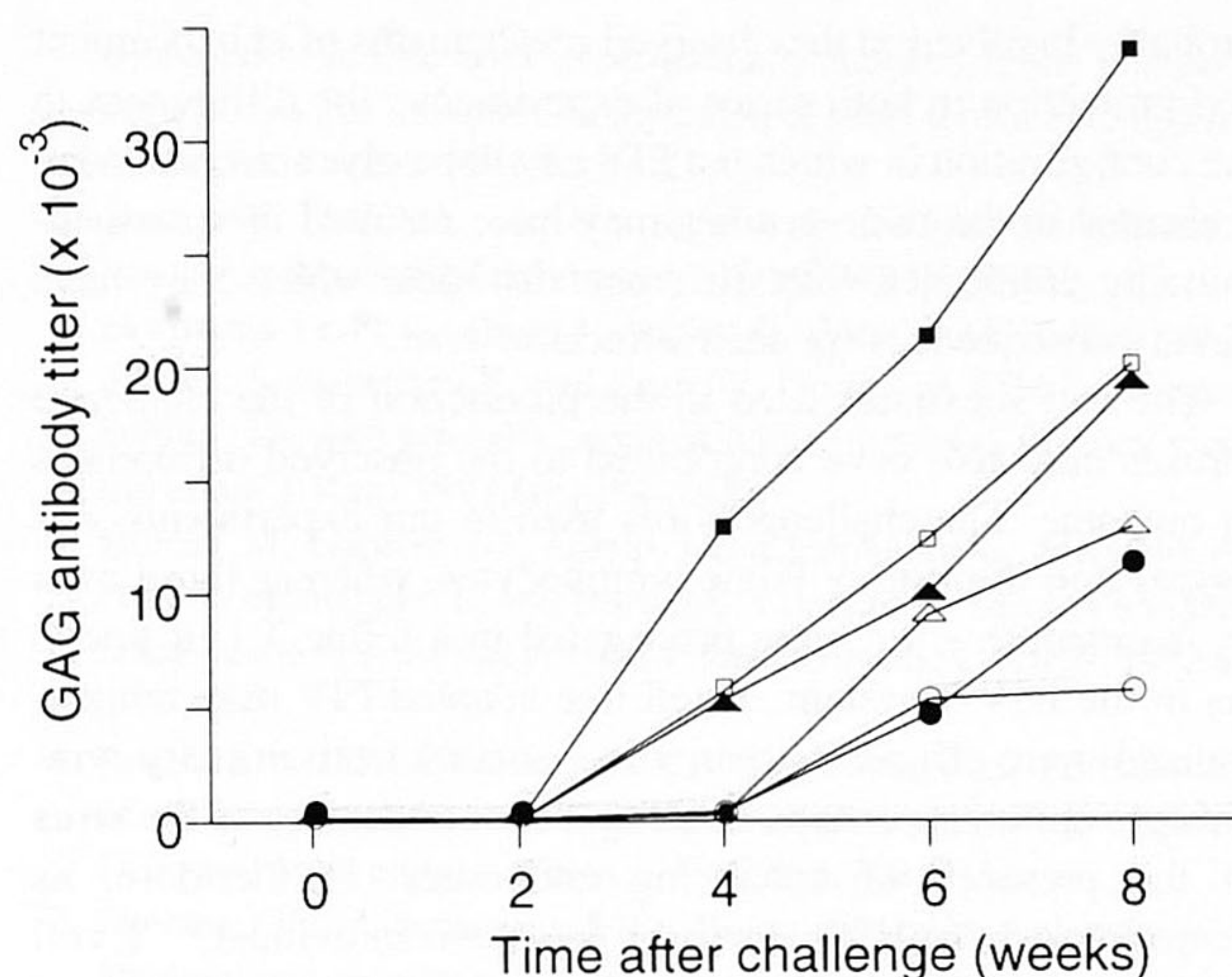


FIG. 1. Kinetics of plasma antibody development against Gag protein in cats in respective vaccination groups. Mean anti-Gag titers at different times after challenge: open squares, group 1; filled triangles, group 2; filled squares, group 3; filled circles, group 4; open circles, group 5; open triangles, group 6.

Plasma antibodies to the FIV Gag protein were detected 4 weeks after challenge in all of the cats vaccinated with rVV expressing FIV envelope glycoproteins (groups 1, 2, and 3) (Fig. 1) and about 2 weeks later in the cats vaccinated with the Gal-FIV envelope fusion protein (group 4) and in those in the two control groups. Eight weeks after challenge, the plasma titers of FIV Gag-specific antibodies were higher in cats in groups 1–3 than in the other groups (Fig. 1).

The cell-associated viral load was measured 2 and 4 weeks after challenge by cocultivating serially diluted PBMCs (10^3 , 3×10^3 , and 10^4 cells) eightfold with 10^5 PBMCs stimulated with concanavalin A and interleukin 2 from a specific pathogen-free cat. After 3 weeks, the culture supernatants were tested for the presence of FIV antigen by ELISA. The number of infected PBMCs *in vivo* was calculated from the results obtained *in vitro*. Two weeks after infection, FIV-infected PBMCs were found in all of the cats in groups 1 and 2 and in four of six cats in group 3 (Fig. 2) but in none of the other three groups. Two weeks later, however, FIV-infected PBMCs were seen in all cats in all groups. Although a lower average FIV load was observed in cats of group 4 than in the other groups, the differences between the groups were not significant.

The more rapid development of PBMC-associated viremia and Gag-specific plasma antibodies shows that immunization with rVV expressing FIV glycoproteins, resulting in virus-neutralizing plasma antibodies, leads to accelerated FIV infection on challenge. To determine whether this acceleration is plasma mediated, naive cats were inoculated intramuscularly with plasma collected from cats immunized with these candidate vaccines. Pools of plasma collected on the day of challenge from cats in groups 1 and 2 (pool A) and in group 6 (pool B) were prepared, and the virus-neutralizing titers were determined in the CrFK assay as 320 and <10 , respectively. No virus-neutralizing antibodies could be detected 6 hr after the transfer in groups of four specific pathogen-free kittens, weighing 800–1200 g, inoculated intravenously with 7 ml of pool A or B plasma.

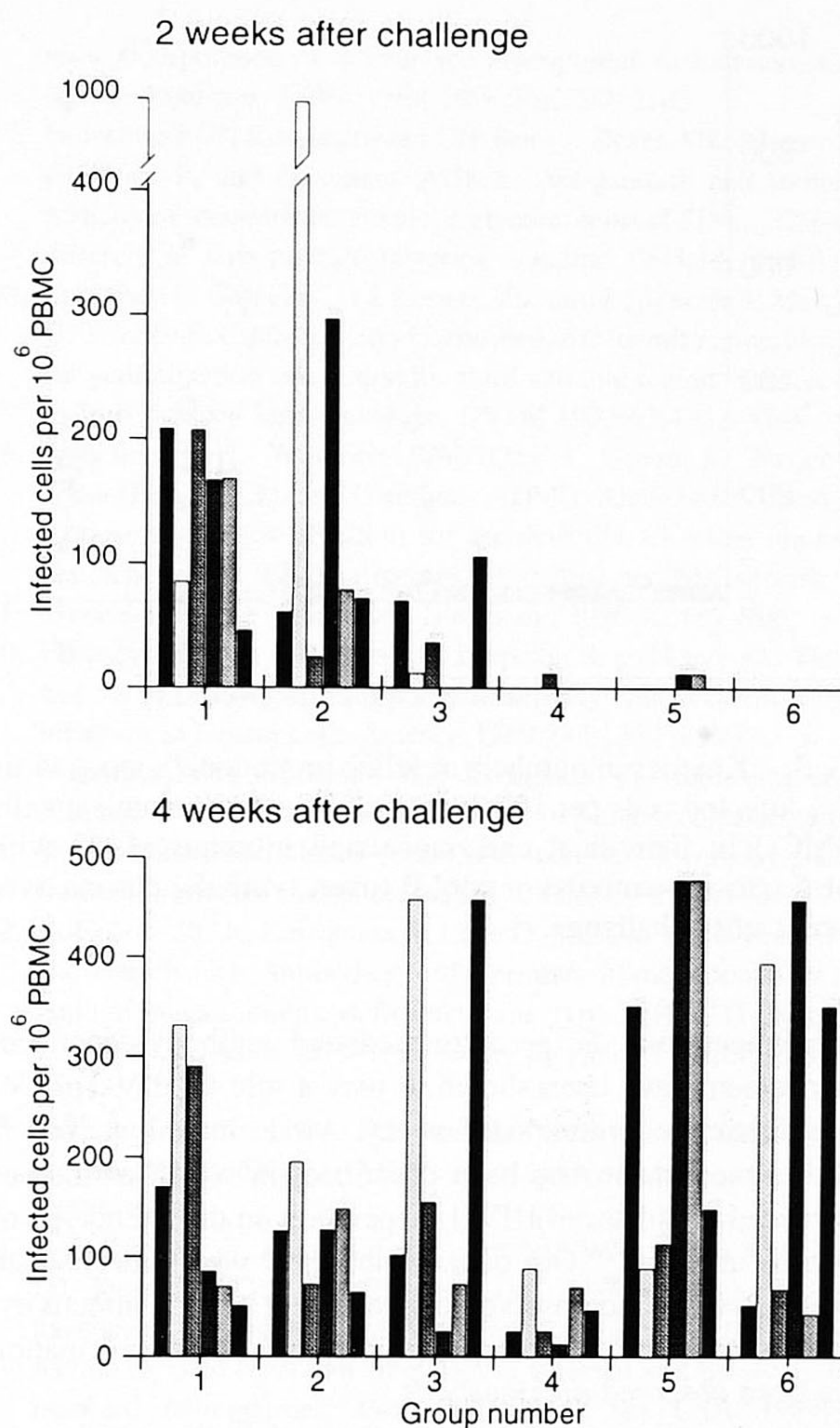


FIG. 2. Numbers of feline immunodeficiency virus (FIV)-infected cells per 10^6 peripheral blood mononuclear cells (PBMCs) in individual cats in respective vaccination groups 2 and 4 weeks after challenge.

An increased FIV-Gag-specific antibody response and accelerated cell-associated viremia were found in cats of group A in comparison with those of group B during the first weeks after challenge with 20 times the CID_{50} of FIV AM19 (Figs. 3 and 4). Although not formally proven, this indicates that the enhancement was mediated by FIV envelope-specific antibodies. It is not clear from these studies why the presence of virus-neutralizing antibodies on the day of challenge is not correlated with protective immunity but rather with enhanced susceptibility to FIV infection. It should be noted, however, that the virus-neutralizing activity was demonstrable only in the CrFK assay and not in the feline lymphocyte assay. The latter assay is probably more relevant in terms of protective immunity against FIV infection *in vivo*. In the transfer experiment, it was shown that the acceleration phenomenon could be transferred to naive cats with the plasma of cats in groups 1 and 2. The mechanism of enhancement proved to be operational at relatively high dilutions: after plasma transfer, no virus-neutralizing antibody activity could be demonstrated in the plasma of the kittens that subsequently showed enhanced susceptibility to FIV infection.

Various mechanisms of antibody-dependent enhancement have been described in relation to lentivirus infections.

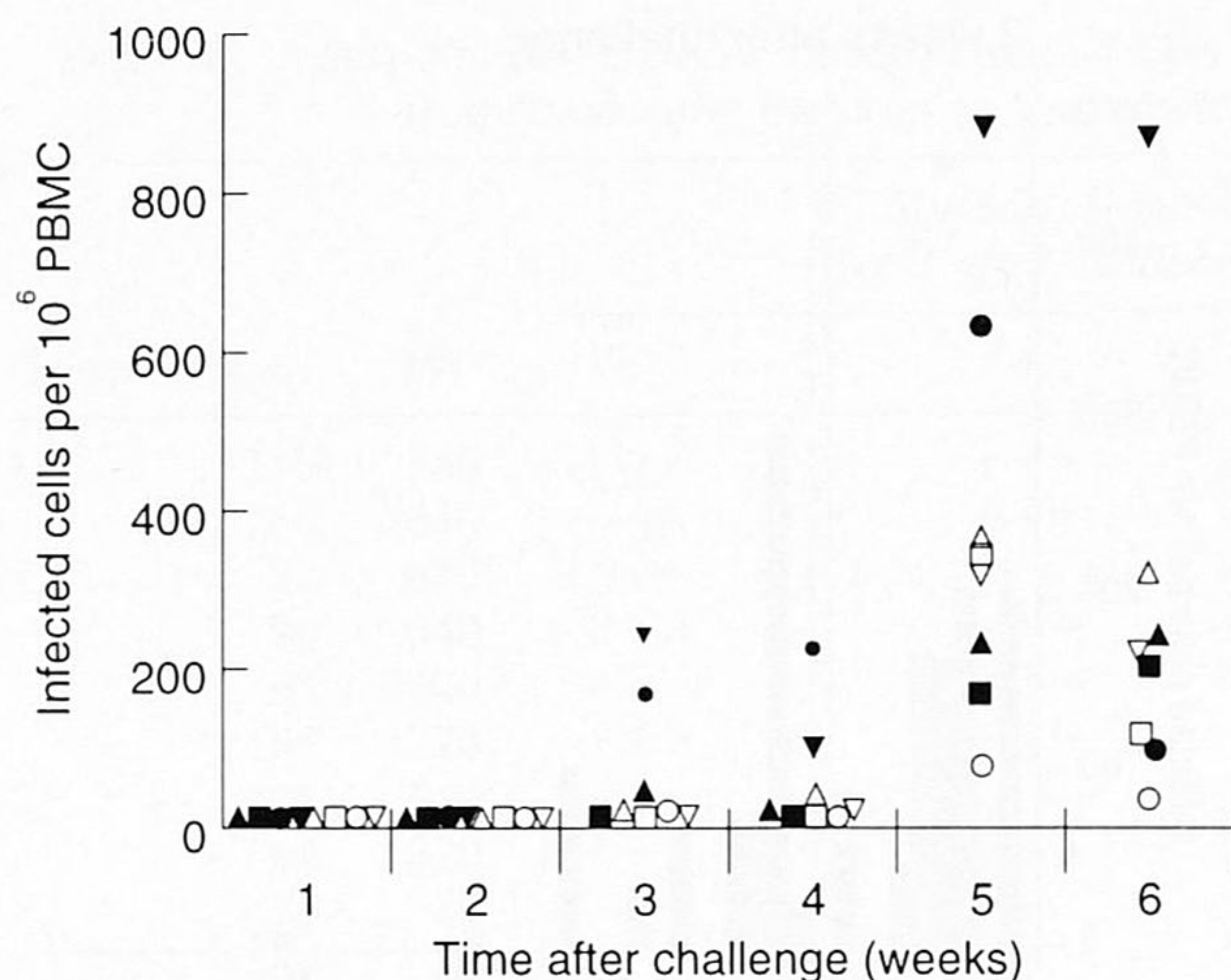


FIG. 3. Kinetics of numbers of feline immunodeficiency virus (FIV)-infected cells per 10^6 peripheral blood mononuclear cells (PBMCs) in individual cats inoculated intramuscularly with pool A (closed symbols) or pool B (open symbols) plasma over 6 weeks after challenge.

Complement- and Fc receptor-mediated antibody-dependent enhancement have been shown to play a role in HIV-1, HIV-2, and simian immunodeficiency viral infections.^{13,20-24} Another mechanism has been described, in which antibodies neutralized or enhanced HIV-1, depending on the phenotype of the virus involved.²⁵ Our data do not show which mechanism was involved in the observed enhancement of FIV infectivity, but indications of enhanced infectivity after FIV vaccination have been observed previously.²⁶

In the experiments of Yamamoto *et al.*,¹² the presence of virus-neutralizing antibodies, demonstrated in an FeT1 cell (feline lymphoid cell line) assay, was correlated with protective immunity rather than with enhancement of infectivity. The main difference from our vaccination approach is that Yamamoto *et al.* used inactivated whole virus or virus-infected cells as immunogens whereas we used recombinant envelope proteins. Since transfer experiments²⁷ show that plasma antibodies are

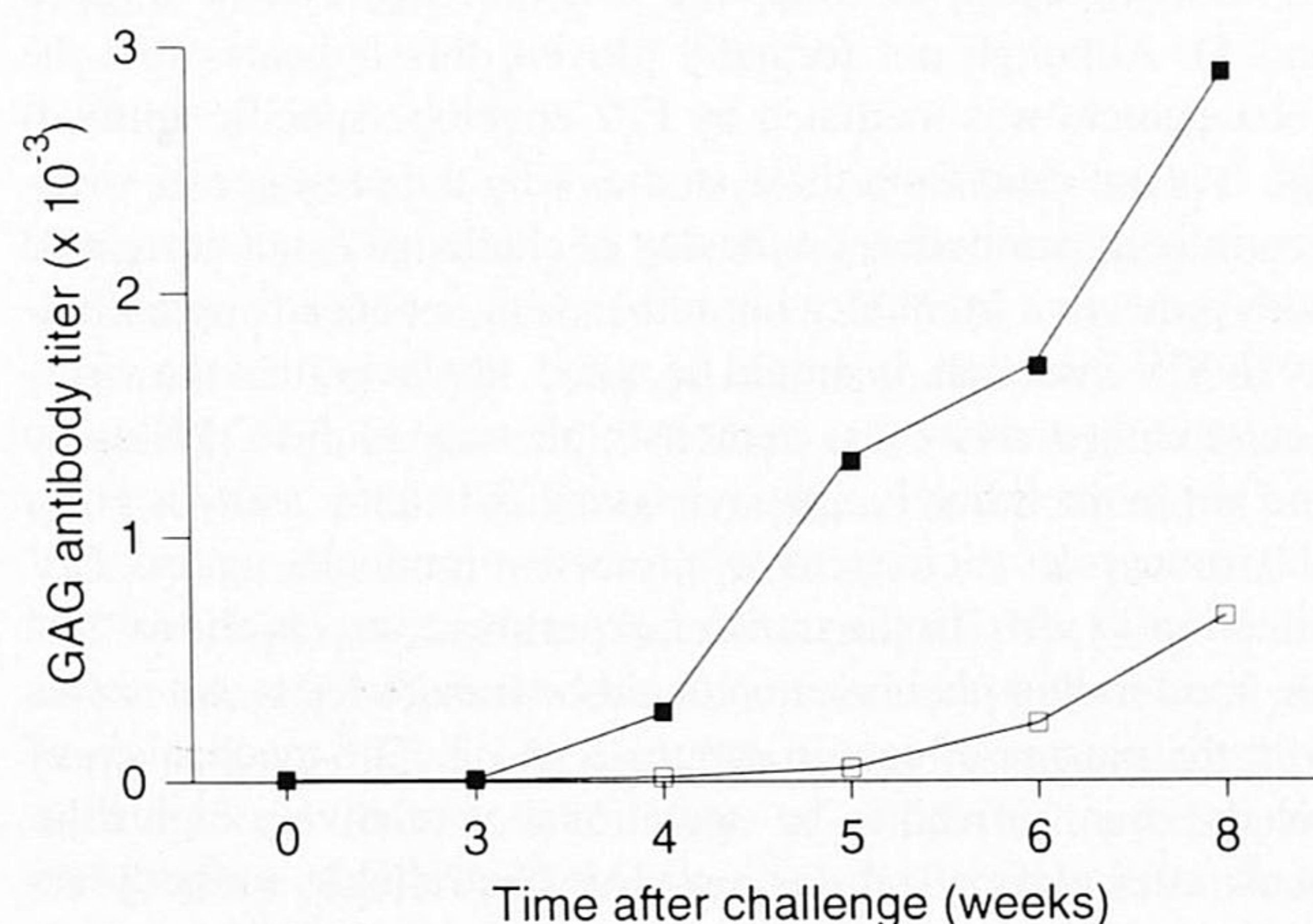


FIG. 4. Kinetics of mean plasma antibody development against Gag protein in cats inoculated intramuscularly with pool A (closed symbols) or pool B (open symbols) plasma.

probably involved in the observed mechanisms of enhancement and protection in both series of experiments, the differences in the configuration in which the FIV envelope glycoproteins were presented in the two vaccines may have resulted in virus-neutralizing antibodies with different affinities, which may have direct consequences for their effects *in vivo*.

The cell substrates used in the production of the challenge viruses may also have contributed to the observed differences in outcome. The challenge virus used in our experiments was propagated in primary feline lymphocytes, whereas those used by Yamamoto *et al.* were propagated in a feline T cell line.¹¹ As in the HIV-1 system, T cell line-adapted FIV may be neutralized more efficiently than virus isolated from primary lymphocyte cultures, perhaps resulting in neutralization of the virus in the presence of enhancing antibodies. Furthermore, as demonstrated for HIV-1 isolates from one individual,²⁵ T cell line-adapted FIV may be less susceptible to antibody-dependent enhancement.

In summary, immunization of cats with rVV expressing FIV envelope glycoprotein or with an FIV envelope bacterial fusion protein, using different adjuvant systems, resulted in the development of FIV-specific serum antibodies in all cats. The former group also developed FIV-neutralizing antibodies, as demonstrated in an assay using cell line-adapted FIV, but not in an assay using homologous FIV and feline lymphocytes. After three immunizations, the cats were challenged with homologous FIV. Cats vaccinated with rVV expressing glycoprotein developed Gag-specific serum antibodies and cell-associated viremia more rapidly than those vaccinated with the bacterial fusion protein. This accelerated response could be transferred to naive cats with plasma collected on the day of challenge. The mechanisms of FIV enhancement after vaccination and passive transfer are not yet fully understood. For the development of an effective FIV vaccine, elucidation of the underlying mechanisms may be crucial and may also lead to a more rational strategy for the development of HIV-1 vaccines.

ACKNOWLEDGMENTS

We thank Geert van Amerongen and Nico Schmidt for biotechnical assistance and Ellen Hulskotte for the SIV envelope ISCOMs. We also thank Rob van Herwijnen of the European Veterinary Laboratory for the SU and TM peptide ELISA and Conny Kruyssen for help in preparing the manuscript.

This work was supported by grants from Mallinckrodt Veterinary Ltd., Harefield, United Kingdom, and the Dutch Advisory Council on Health Research (RGO Grant No. 88-90/89028).

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