IMMUNOGENICITY OF RECOMBINANT FELINE INFECTIOUS PERITONITIS VIRUS SPIKE PROTEIN IN MICE AND KITTENS

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SUMMARY

The gene encoding the fusogenic spike protein of the coronavirus causing feline infectious peritonitis (FIPV) was recombined into the genome of vaccinia virus, strain WR. The recombinant induced spike protein specific, in vitro neutralizing antibodies in mice. When kittens were immunized with the recombinant, low titers of neutralizing antibodies were obtained. After challenge with FIPV, these animals succumbed earlier than the vWR-immunized control group ("early death syndrome").

INTRODUCTION

Feline infectious peritonitis (FIP) is a progressive, debilitating, highly fatal disease in wild and domestic Felidae. In the pathogenesis of FIP the infection of cells of the monocyte/macrophage lineage appears to be of central importance (3, 4, 9, 10). The causative agent, FIPV, has been identified as a member of the family Coronaviridae (7). Thus far attempts to vaccinate against FIPV have failed. Protective immunity was obtained in some kittens after immunization with low-virulence FIPV strains or sublethal amounts of virulent FIPV; others, however, developed disease or became sensitized resulting in early death after challenge (1). Early death in experimental FIP has been demonstrated following passive transfer of anti-FIPV antiserum (11), suggesting that early death is caused by antibody dependent enhancement (ADE) of infectivity (6).

The viral proteins involved in protective immunity and early death have not been identified. The FIPV virion is composed of an RNA genome of about 30kb and three protein species: the 45kD nucleocapsid protein N, the 25-32kD membrane glycoprotein M and the 200kD spike glycoprotein S (7). The latter mediates attachment of the virus to the cell receptor, triggers membrane fusion and elicits virus neutralizing antibodies.

In this report we show that a challenge infection with FIPV of kittens previously immunized with a recombinant vaccinia virus expressing the major surface glycoprotein of FIPV results in early death.

Coronaviruses and Their Diseases
Edited by D. Cavanagh and T.D.K. Brown
Plenum Press, New York, 1990

METHODS AND RESULTS

Immunogenicity of recombinant FIPV spike protein in mice. The gene encoding the S protein of FIPV strain 79-1146 was inserted into the vaccinia virus genome by homologous recombination. Details of the isolation of the recombinant vaccinia virus, designated vFS, and demonstration of biological activity e.g. cell-cell fusion have been presented (see Vennema et al., this volume). After metabolic labelling in vitro with [^{35}S]-methionine a protein which comigrated with the FIPV spike protein was specifically immunoprecipitated from lysates of cells infected with vFS, using a polyvalent anti-FIPV antiserum (Fig. 1a).

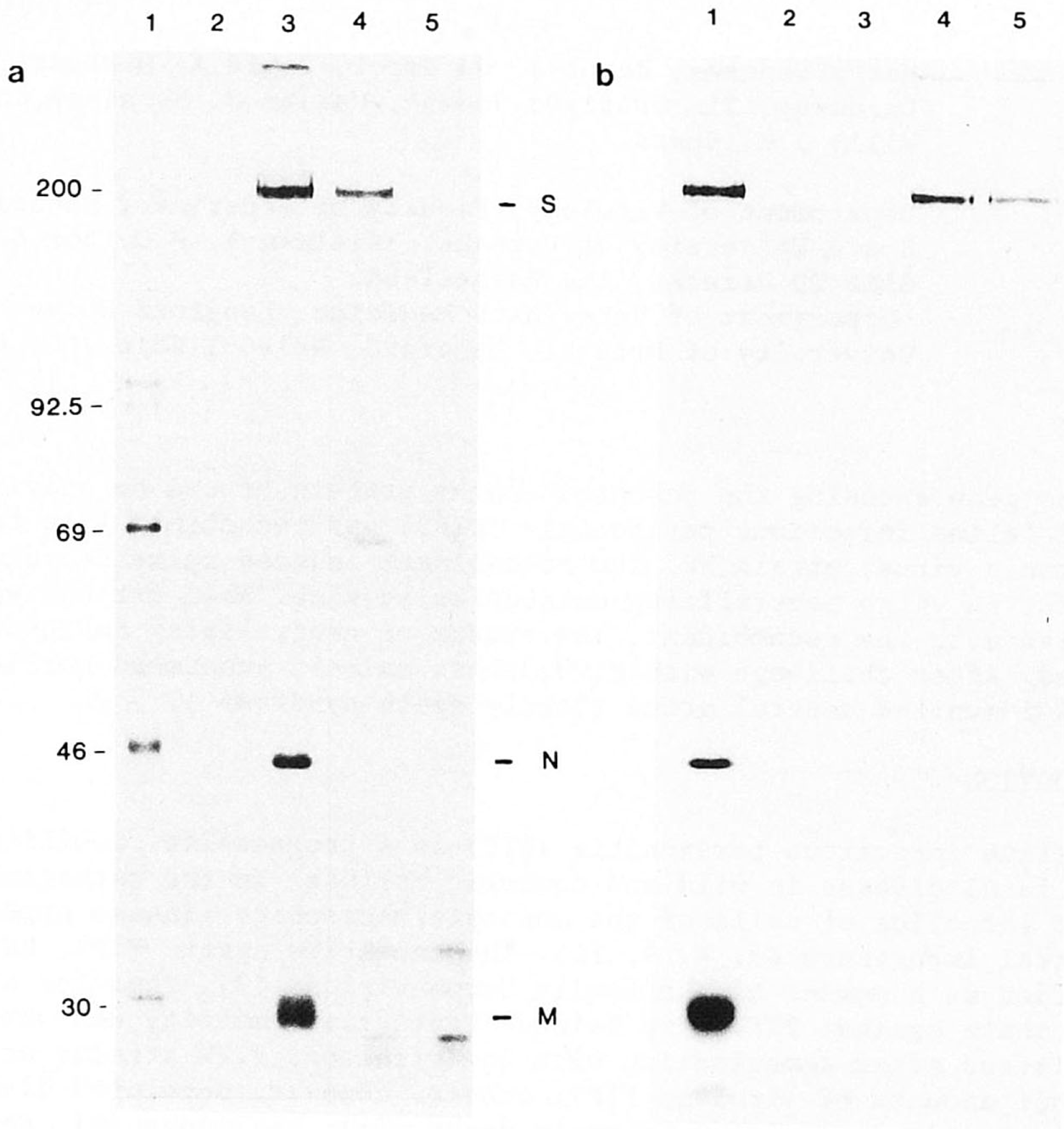

FIG. 1a. In vitro analysis of the recombinant spike protein of FIPV. Lysates of metabolically labelled mock (lane 2), FIPV (lane 3), vFS (lane 4) and vWR (lane 5) infected cells were used in a radio immunoprecipitation (RIP) assay with ascitic fluid (A36) of a field case of FIP.

FIG. 1b. Immunoprecipitation analysis of pooled sera of recombinant vaccinia virus immunized mice. Pooled sera of five mice immunized with vFS, collected on the day of booster (lane 3) and 2 (lane 4) or 4 (lane 5) weeks after the booster, were used in a RIP assay with labelled FIPV proteins. Pooled sera of five mice immunized with recombinant vaccinia virus vIS, collected 2 weeks after the booster served as negative control (lane 2). As a positive control RIP was carried out with A36 (lane 1). FIPV structural proteins are indicated.

To evaluate the immunogenicity of the recombinant S protein, vFS was used to immunize mice. Five male Balb/c mice were injected intraperitoneally (i.p.) with $5x10^7$ PFU of recombinant vFS. Recombinant vaccinia virus expressing the infectious bronchitis virus (IBV) S protein (see Vennema et al., this volume) was used for the immunization of five control mice. Three weeks later a second i.p. immunization of $2x10^8$ PFU was given. Sera were collected 2, 4 and 8 weeks after the second immunization. The sera from mice immunized with vFS neutralized FIPV infectivity _in vitro_ up to a 500-fold dilution in a neutralization assay and specifically immunoprecipitated the FIPV S protein (Fig. 1b). Sera obtained from control mice neither neutralized FIPV infectivity _in vitro_ nor immunoprecipitated FIPV S protein.

Immunization of kittens with vFS; early death syndrome. In order to study the role of the S protein in early death syndrome, the recombinant vFS was used to immunize kittens prior to challenge with FIPV. Five 13-14 week old specific pathogen-free kittens were injected subcutaneously with a total of 10^8 PFU of vFS; a second group of five kittens immunized with the same dose of wild type vaccinia virus strain WR (vWR) served as controls. A second immunization was given after three weeks. All kittens developed pox lesions at the site of primary inoculation; no lesions were observed after the second immunization, suggesting an effective immune response to the primary vaccinia virus infection. Two weeks after the second immunization all kittens were challenged orally with $5x10^5$ PFU of FIPV strain 79-1146. Once daily, kittens were examined clinically and rectal temperatures were measured. Euthanasia was carried out when the kittens became prostrate and a full post-mortem examination was performed.

Apart from minor pyrexia shortly after the primary vaccinia virus immunization, temperatures were normal during the period before challenge. The mean rectal temperature curves after challenge were similar to those presented previously (11). Briefly, temperatures in both groups rose to a peak on PCD 3. This peak was slightly higher in the vFS immunized group than in the control kittens. In the vFS immunized kittens body temperatures remained elevated. In the control group temperatures dropped and reached normal values on PCD 7 and remained normal until PCD 14. Thereafter, the temperature rose again in 4 out of 5 control kittens and remained high for an extended period. Only 1 of the 4 recovered from this second fever.

Survival times after challenge (Table 1) were reduced significantly ($P<0.05$) in the group immunized with vFS, as tested by the Mann-Whitney procedure. Mean survival times ±SEM after challenge were 8.2 ±1.1 days and 29.0 ±1.7 days for vFS and vWR-immunized kittens respectively. Two kittens of the control group survived challenge.

Histological studies on tissues taken at post-mortem examination showed that the vFS-immunized kittens had suffered from a form of FIP much more severe than naturally occurring FIP, even though no gross changes were observed. The vFS-immunized kittens had multiple lesions in liver, spleen and brain. Mesenteric lymph nodes and Peyer's Patches of the small intestine showed a histiocytic response. The lesions in the vFS-immunized kittens largely represent histiocytic infiltration and proliferation. The presence of multiple small lesions in many organs is atypical of naturally occurring FIP. The diseased kittens of the control group had gross lesions and peritoneal exudate characteristic of effusive FIP. In one cat the brain had focal lymphoid cell infiltrates, in another the liver showed multiple foci of necrosis and the third cat had focal pyogranulomatous lesions in spleen, small intestinal serosa and mesenteric lymph nodes.

Humoral immune response to FIPV spike protein. Serum samples were taken on the days of primary and secondary immunization, on the day of challenge and on PCD 3, 9, 17, 24 and 31 or on the day when euthanasia in extremis was performed. Sera were tested for FIPV neutralizing activity _in vitro_ by

Table 1. Survival times after challenge and neutralizing antibody titers of vaccinia virus immunized, FIPV challenged kittens.

immunized with vaccinia virus:	kitten	survival time in days	titer[a] PCD 0	titer[b] PCD 9(*=7)	titer[b] PCD 17
vFS	G62	7	32	2455*	
	G67	9	4	725	
	G72	7	10	727*	
	G76	9	4	305	
	118	9	10	610	
vWR	G63	31	<4	77	8192
	G68	>400	<4	75	258
	G73	28	<4	299	2907
	119	>400	<4	150	867
	123	28	<4	<10	514

[a] reciprocal of the serum dilution that gave 50% plaque reduction of 250 PFU of FIPV on fcwf cells.
[b] reciprocal of the serum dilution that gave 50% CPE reduction of 100 $TCID_{50}$ on fcwf cells in a standard microtitre assay.

reduction of plaque formation or by inhibition of viral CPE (see footnotes Table 1). On the day of challenge vFS-immunized kittens had low titers of neutralizing antibodies, detectable only in a plaque reduction assay (Table 1). No neutralizing antibodies were detectable in sera of the control kittens (Table 1). Titers remained unchanged on PCD 3 but by PCD 7 or 9 all sera had increased neutralizing activity (Table 1). Except for kitten G76 all kittens immunized with vFS had significantly higher titers than control kittens.

The sera were then used in a RIP assay with labelled FIPV proteins. Figure 2 shows the results for one representative kitten of both groups. No response was detected in this assay with sera from PCD 3 and before. Serum from vFS-immunized kitten G76 of PCD 9 precipitated the S protein but none of the other structural proteins. Using the standard RIP assay which detects only IgG antibodies, the PCD 9 serum from control kitten G73 did not show an FIPV specific response. Hence, the antibodies in this serum detected in the neutralization assay, were probably of IgM isotype, as would be expected in a primary response. Immunoprecipitation of all structural proteins was obtained with sera from the control kitten from PCD 17 onwards. Similar patterns were found for all kittens (manuscript submitted).

DISCUSSION

The data presented here show that immunization of kittens with a recombinant vaccinia virus, expressing the FIPV spike protein resulted in early death after challenge with FIPV. From the analysis of the humoral immune response we conclude that immunization with vFS led to an S protein specific priming and a low level of <u>in vitro</u> neutralizing antibodies. The S antigen produced in the initial rounds of replication boosted the primed S response to

a relatively high level of IgG antibodies early in infection. The early death syndrome was probably caused by a combination of these factors, through a mechanism consistent with ADE. The demonstration of early death in experimental FIP following passive transfer of anti-FIPV antiserum (11) supports the hypothesis that early death can be caused by ADE. Furthermore, ADE of FIPV infectivity in vitro in cultured feline macrophages has recently been reported (8). The data presented here show that an immune response against the viral spike protein alone can trigger early death syndrome after challenge with FIPV.

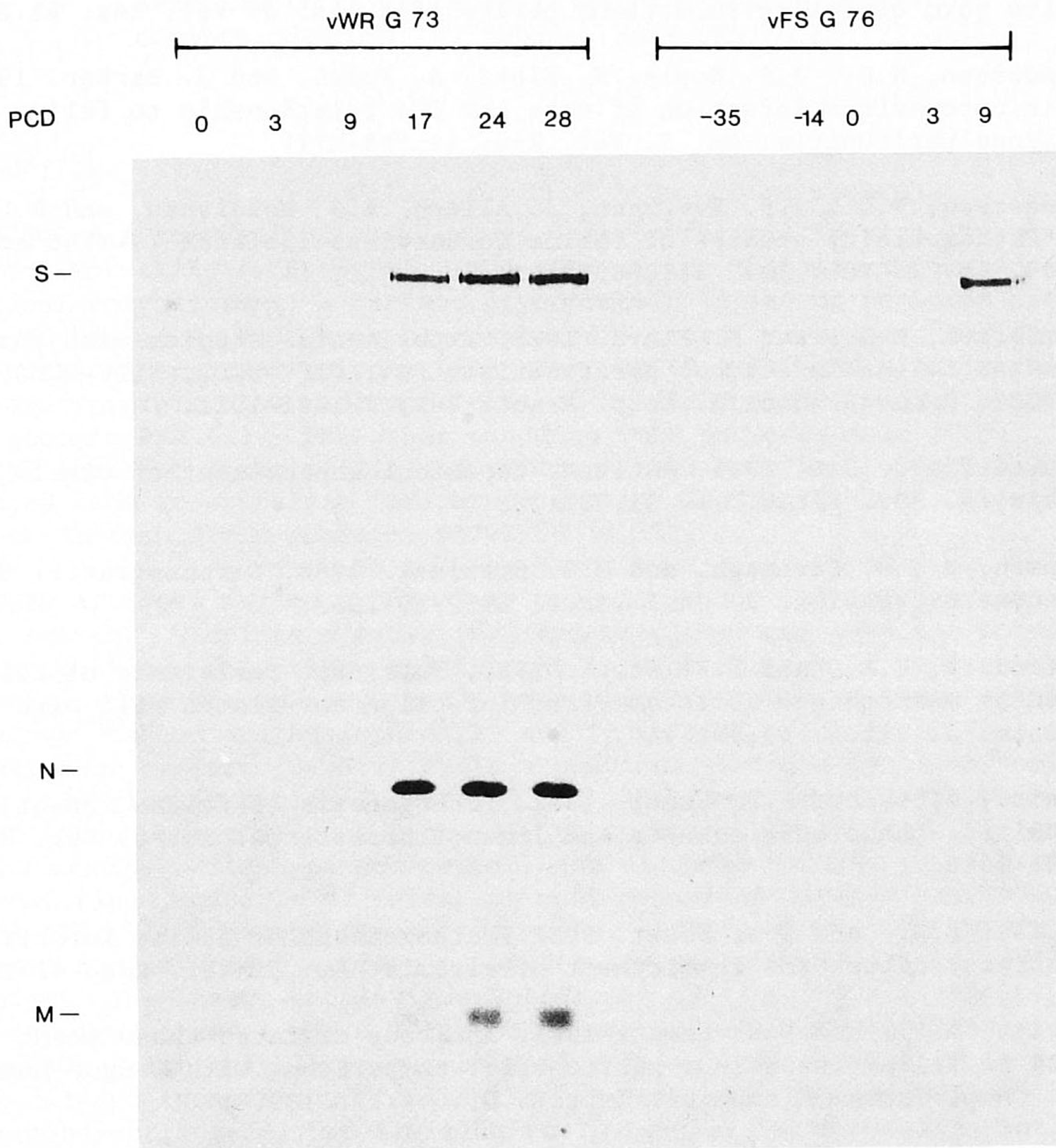

FIG. 2. Immunoprecipitation analysis of sera of experimentally infected kittens. Sera of vaccinia virus immunized kittens were tested in a RIP assay with labelled FIPV proteins. The vaccinia virus used for immunization, vFS or vWR, and the code number for the kitten are indicated at the top of the panels. Above each lane the post challenge day on which the serum was taken has been indicated.

ACKNOWLEDGEMENTS

We thank P.J. Brown and G.R. Pearson for the post-mortem examination, A. Boots for immunizations of mice, H. Egberink and E.D. Chirnside for reviewing the manuscript and B. Moss for vaccinia virus insertion vector pGS20. Supported by a grant from Duphar BV, Weesp, the Netherlands.

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