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# Early Death after Feline Infectious Peritonitis Virus Challenge due to Recombinant Vaccinia Virus Immunization

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**The gene encoding the fusogenic spike protein of the coronavirus causing feline infectious peritonitis was recombined into the genome of vaccinia virus. 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 feline infectious peritonitis virus, these animals succumbed earlier than did the control group immunized with wild-type vaccinia virus (early death syndrome).**

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 (10, 11). The causative agent, FIP virus (FIPV), has been identified as a member of the family *Coronaviridae* (6). Based on circumstantial evidence, FIPV immunity is thought to be largely cell mediated (2). Thus far, attempts to vaccinate against FIPV have failed (2). In some cases immunized kittens became sensitized, resulting in enhanced infection and reduced survival times upon challenge as compared with those of nonimmunized animals (3). This phenomenon, also known as early death, is thought to be caused by antibody-dependent enhancement (ADE) of infection.

ADE of viral infection in vitro has been described for a number of viruses (4). The mechanism of ADE involves binding of virus-antibody complexes to Fc or complement-receptor-bearing cells, i.e., monocytes and macrophages. Binding or opsonization of these complexes results in infection rather than neutralization of infectivity (4).

The role of ADE in viral pathogenesis has been difficult to establish, but it has been argued that ADE explains the development of dengue hemorrhagic fever in persons with pre-existing serum antibody to dengue viruses (1). FIPV infection of cats is one of the few homologous systems amenable to an experimental approach to study the involvement of ADE in viral infection in vivo. Passive transfer of anti-FIPV serum before experimental infection with FIPV induced early death, indicating that this phenomenon can be caused by ADE (12).

In the case of FIPV, 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 30 kilobases and three protein species: the 45-kilodalton nucleocapsid protein N, the 25- to 32-kilodalton membrane glycoprotein M, and the 200-kilodalton spike glycoprotein S (6). The latter mediates attachment of the virus to the cell receptor, triggers membrane fusion, and elicits virus-neutralizing antibodies. Recently, we have constructed a recombinant vaccinia virus with the S gene of FIPV. The recombinant, designated vFS, expressed a protein that resembles the authentic FIPV S protein in its mobility in polyacrylamide gels, expression at the cell surface, and biological activity (9). To study the role of the FIPV S protein in early death, we immunized kittens with this recombinant vaccinia virus. Subsequent challenge infection with FIPV resulted in markedly reduced survival times compared with those of control kittens.

**Immunogenicity of the recombinant S protein in mice.** To evaluate the immunogenicity of the recombinant vaccinia-virus-encoded FIPV S protein, vFS was used to immunize mice. Five male BALB/c mice were injected intraperitoneally with $5 \times 10^7$ PFU of recombinant vFS. Recombinant vaccinia virus expressing the infectious bronchitis virus S protein, designated vIS (9), was used for the immunization of five control mice. Three weeks later a second intraperitoneal immunization of $2 \times 10^8$ PFU was given. Sera taken on the days of the first and second immunization did not have detectable in vitro neutralizing activity of FIPV infectivity (Table 1). Pooled sera from mice immunized with vFS, collected 2 weeks after the second immunization, neutralized FIPV infectivity in vitro up to a 500-fold dilution, whereas sera from control mice did not (Table 1).

Sera were also tested in a radioimmunoprecipitation (RIP) assay with a lysate of metabolically labeled FIPV-infected fcwf-D cells, as described previously (9). All sera were used at a 100-fold dilution. The serum pool from vFS-immunized mice specifically immunoprecipitated the FIPV S protein; sera from control mice did not react in this assay (Fig. 1).

**Immunization of kittens with vFS; challenge of vaccinia-virus-immunized kittens.** The recombinant vFS was used to immunize kittens before challenge with FIPV. Five 13- to 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. Once daily, kittens were examined clinically, and rectal temperatures were measured. A second immunization with the same amount of the appropriate virus was given after 3 weeks. All kittens developed pox lesions at the site of primary inoculation; no lesions were observed after the

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TABLE 1. Recombinant vaccinia virus-induced FIPV-neutralizing antibodies in mice

| Pooled sera of mice immunized with: | Weeks after 2nd immunization | Titer[a] |
|---|---|---|
| vFS (5 mice) | 0 | <16 |
| | 2 | 513 |
| | 4 | 298 |
| | 8 | 355 |
| vIS (5 mice) | 2 | <16 |

[a] Titers are expressed as the reciprocal serum dilution that gave 50% reduction in cytopathic effect of 100 50% tissue culture infective doses FIPV on fcwf-D cells in a microtiter plate assay.

second immunization, suggesting an effective immune response to the primary vaccinia virus infection. A low level of in vitro FIPV-neutralizing antibodies was induced (see below).

Two weeks after the second immunization, all kittens were challenged orally with $5 \times 10^5$ PFU of FIPV strain 79-1146. Euthanasia was carried out when the kittens became prostrate, and a full postmortem examination was performed. Apart from minor pyrexia shortly after the primary vaccinia virus immunization, rectal temperatures were normal during the period before challenge. The mean rectal temperature curves after challenge were similar to those presented by Weiss and Scott (12). Temperatures in both groups rose to a peak on postchallenge day (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 on PCD 4 and reached normal values on PCD 7 and remained normal until PCD 14. Thereafter, the temperature rose again in four (G63, G73, 119, and 123) out

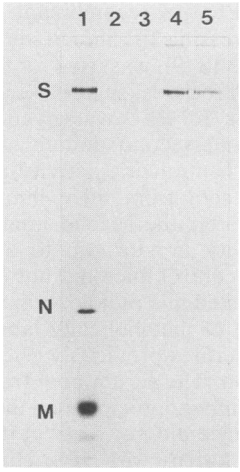


FIG. 1. Immunoprecipitation analysis of pooled sera of recombinant vaccinia virus immunized mice. Pooled sera of five mice immunized with vFS, collected on the day of secondary immunization (lane 3) and 2 (lane 4) or 4 (lane 5) weeks after the second immunization, were used in an RIP assay with labeled FIPV proteins. Pooled sera of five mice immunized with recombinant vaccinia virus vIS, collected 2 weeks after the second immunization, served as a negative control (lane 2). As a positive control RIP was carried out with ascitic fluid of a field case of FIP (lane 1). FIPV structural proteins are indicated. Pooled sera of mice of 2 and 4 weeks after a second immunization with vFS immunoprecipitated the FIPV spike protein.

TABLE 2. Survival times after challenge and neutralizing antibody titers of vaccinia virus-immunized, FIPV-challenged kittens

| Immunized with vaccinia virus: | Kitten | Survival time (days) | Titer[a] on PCD: | | |
|---|---|---|---|---|---|
| | | | PCD 0 | PCD 9 | PCD 17 |
| vFS | G62 | 7 | 32 | 2,455[b] | |
| | G67 | 9 | 4 | 725 | |
| | G72 | 7 | 10 | 727[b] | |
| | G76 | 9 | 4 | 305 | |
| | 118 | 9 | 10 | 610 | |
| vWR | G63 | 31 | <4 | 77 | 8,192 |
| | G68 | >400 | <4 | 75 | 258 |
| | G73 | 28 | <4 | 299 | 2,907 |
| | 119 | >400 | <4 | 150 | 867 |
| | 123 | 28 | <4 | <10 | 514 |

[a] Titers on PCD 0 are expressed as the reciprocal serum dilution that gave 50% plaque reduction of 250 PFU of FIPV on fcwf-D cells. Titers on PCD 7, 9, and 17 are expressed as the reciprocal serum dilution that gave 50% reduction in cytopathic effect of 100 50% tissue culture infective doses on fcwf-D cells in a microtiter plate assay.
[b] Titer on PCD 7.

of five control kittens and remained high for an extended period. One (kitten 119) of the four recovered from this second fever.

Survival times after challenge (Table 2) were reduced significantly in the group immunized with vFS, as tested by the Mann-Whitney procedure ($P < 0.05$). Mean survival times ± standard errors of the mean after challenge were 8.2 ± 1.1 days and 29.0 ± 1.7 days for vFS- and vWR-immunized kittens, respectively. Two kittens (G68 and 119) of the control group survived challenge.

**Postmortem examination and histological characteristics of diseased kittens.** Histological studies on tissues taken at postmortem examination showed that 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 represented histiocytic infiltration and proliferation. The diseased kittens of the control group had gross lesions and peritoneal exudate characteristic of effusive FIP. In one cat (G73) the brain had focal lymphoid cell infiltrates, in another (G63) the liver showed multiple foci of necrosis, and a third cat (123) had focal pyogranulomatous lesions in spleen, small intestinal serosa, and mesenteric lymph nodes.

The postmortem 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 presence of multiple lesions in several organs is atypical of naturally occurring FIP and indicates a rapid dissemination. The relatively small size of the lesions is probably due to the short incubation period.

**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. Neutralizing antibody titers were determined in a neutralization assay or in a plaque reduction assay (Table 2). None of the kittens had FIPV-specific antibodies on the days of primary and secondary immunization. Two weeks after the second immunization (PCD 0), vFS-immunized kittens had low titers of neutralizing antibodies, detectable only in a plaque reduction assay (Table 2). No neutralizing antibodies

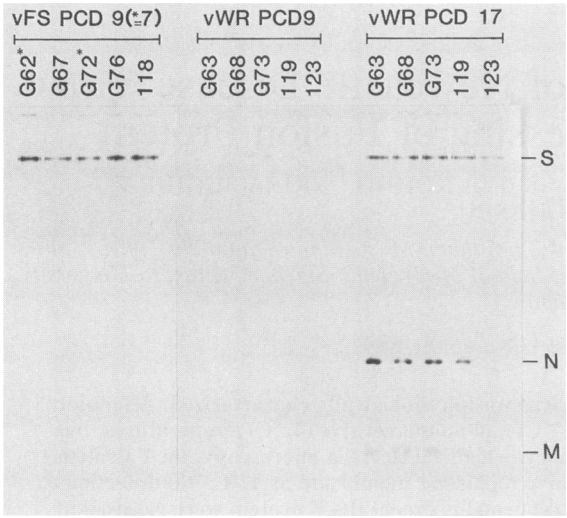


FIG. 2. Immunoprecipitation analysis of sera of experimentally infected kittens. Sera of vaccinia virus-immunized kittens were tested in an RIP assay with labeled FIPV proteins. Three panels of five lanes each are shown. The vaccinia virus used for immunization, vFS or vWR, and the PCD on which sera were taken are indicated at the top of each panel. Each lane is marked with the code of the corresponding kitten. FIPV structural proteins are indicated. Sera from PCD 7 or 9 of vFS immunized kittens immunoprecipitated the FIPV spike protein. Sera from PCD 9 of control kittens did not immunoprecipitate viral antigen. Sera from PCD 17 of control kittens immunoprecipitated all FIPV structural proteins.

were detectable in sera of the control kittens. Titers remained unchanged on PCD 3, but by PCD 7 or 9 all sera had increased neutralizing activity (Table 2). Except for kitten G76, all kittens immunized with vFS had titers that were significantly higher than those of control kittens. From PCD 17 onward, titers of control kittens increased to high levels.

The sera were then used in an RIP assay with labeled FIPV proteins to demonstrate antigen specificity. No response was detected in this assay with sera from PCD 3 and before. Sera from vFS-immunized kittens on PCD 7 or 9 precipitated the S protein but none of the other structural proteins (Fig. 2). Immunoprecipitation of N and M with these sera was found only when goat anti-cat immunoglobulin M antibodies were added to the incubation mixture before immune complexes were bound to Pansorbin (data not shown). With the standard RIP assay, which detects only immunoglobulin G antibodies, sera from control kittens of PCD 9 did not show an FIPV-specific response. Hence, the antibodies in the sera from the control kittens of PCD 9 detected in the neutralization assay were probably of the IgM isotype, as would be expected in a primary response. Immunoprecipitation of all structural proteins was obtained with sera from the control kittens from PCD 17 onward.

The data presented in this report show that immunization of kittens with recombinant vaccinia virus vFS, expressing the spike protein of FIPV, 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 in vitro neutralizing antibodies. The S antigen produced in the initial rounds of replication boosted the primed S response to a relatively high level of immunoglobulin G 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 after passive transfer of anti-FIPV antiserum (12) indicated that early death can be caused by ADE. Furthermore, ADE of FIPV infectivity in vitro in cultured feline macrophages has recently been reported (7). The data presented here show that an immune response against the viral spike protein alone can trigger early death syndrome after challenge with FIPV. Preliminary data (not shown) indicate that the M and N protein do not induce early death after challenge, with an approach similar to that used for the S protein.

In a more general perspective, our data exemplify clearly that in cases where the phenomenon of early death is known to exist and/or ADE has been demonstrated in vitro, extreme care should be taken with immunization strategies. Since ADE in vitro has recently been described for human immunodeficiency virus type 1 (5, 8), current vaccination trials, particularly those aimed at raising an immune response against the viral envelope protein, should be reconsidered until more is known about the role of ADE in vivo.

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