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Protection against feline infectious peritonitis by intranasal inoculation of a temperature-sensitive FIPV vaccine

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Cats vaccinated intranasally (i.n.) with a temperature sensitive feline infectious peritonitis virus (ts-FIPV) vaccine were protected against an FIP-inducing challenge. Seventeen of 20 vaccinated cats (85%) survived a rigorous virulent FIPV challenge that caused FIP in 12 of 12 non-vaccinated cats (100%), 10 (83%) of which died. Intranasal vaccination stimulated serum IgG and serum and salivary IgA antibody responses (measured by ELISA), FIPV-neutralizing antibody (VN), and a cell-mediated immune (CMI) response as measured by lymphocyte proliferation. The serum antibody response to vaccination was not associated with protection. In fact, the IgG, IgA and VN titres were much higher in control cats than in vaccinated cats following challenge suggesting an immune-mediated pathogenesis. In contrast, stimulation of a mucosal IgA response to vaccination was related to protection. The in vitro proliferation of peripheral blood lymphocytes in response to virulent FIPV was observed in vaccinated cats, in vaccinated and challenged cats but not in non-vaccinated challenged cats.

Keywords: FIP; ts-FIPV; vaccine; intranasal

Introduction

Feline infectious peritonitis virus (FIPV) is a coronavirus related to transmissible gastroenteritis virus (TGEV) of pigs, enteric coronavirus of dogs and a respiratory coronavirus of man. There is also a feline enteric coronavirus (FECV) that is antigenically related to FIPV but replicates mainly in the intestine and causes only a mild diarrheal disease1.

The natural route of FIPV infection is unknown, although cats can be experimentally infected upon oral, nasal or intratracheal inoculation of FIPV. The FIPV multiplies first in the epithelial cells of the upper respiratory tract and intestine following these routes of infection2,3. Clinically apparent FIP occurs after the virus crosses the mucosal barrier, and spreads throughout the cat in infected macrophages and monocytes.

Primary FIP may be mild, consisting of a febrile response and a slight nasal and ocular discharge. Secondary FIP may develop following the primary infection and appears in two forms. The exudative, or wet form, is characterized by peritonitis and pleuritis with asciates and pleural effusion. The dry form is characterized by granulomatous inflammation of various organs and no, or little, exudate. Both forms may appear together. Cats may become anemic and neutrophilia may occur. Increased concentrations of immunoglobulin as well as fibrinogen are found in most cases. Increased urea and creatinine are indicative of renal damage. Disseminated intravascular coagulation also can occur4.

Immune pathogenesis of FIP may be postulated1. The FIPV, expressed on the surface of infected cells, including macrophages, stimulates T-helper lymphocytes and B-lymphocytes to produce virus-specific antibody. Circulating antibody enhances the infection of macrophages which may increase their synthesis and release of complement. Anti-FIPV antibody:FIPV immune complexes could activate the complement cascade causing release of anaphylatoxin resulting in cytolysis. The dying macrophages may release more virus which in turn could infect more macrophages or be phagocytized as immune complexes. Previous attempts at protective immunization against FIP have not been successful. Indeed, immunization often led to more severe disease symptoms5-8.

We show in this report that intranasal (i.n.) administration of a temperature sensitive (ts)-FIPV vaccine, with its ability to grow at the temperatures present in the nasopharynx and its inability to grow at the temperature present parenterally9, protects cats against FIPV infection and subsequent FIPV-induced immune-mediated pathology.

Materials and methods

Vaccine

The original FIP virus was isolated upon post-mortem examination of a cat and adapted to tissue culture on the Norden Laboratories Feline Kidney (NLFK) cell line (Norden Laboratories, Lincoln, NE, USA). This virulent strain (DF2-FIPV) was attenuated by passage on NLFK cells for 99 passages of which passages 61-99 were propagated at 31°C. The 99th passage was made temperature sensitive by exposure to ultraviolet (UV) irradiation. The ts-FIPV used to vaccinate cats was plaque purified, propagated on NLFK cells for eight more passages and lyophilized. The characteristics of the ts-FIP vaccine virus have been described by Christianson et al.9. Briefly, the ts-FIPV, unlike its virulent DF2-FIPV parent, propagated at 31°C (permissive temperature) but...
not at 39°C (non-permissive temperature). In cats, the virus replicated only at the lower temperature in the upper respiratory tract and not at systemic sites where higher temperatures (39°C) exist. The ts-FIPV was more thermolabile than the parent DF2 wild-type FIPV (wt-FIPV) and Western blot analysis revealed a difference in the envelope proteins of the two viruses. Viral structural proteins and RNA were synthesized at 39°C but infectious ts-FIPV was not produced at its non-permissive temperature.

**Virulent FIP challenge virus**

Virulent wt-FIPV (DF2 passage 10) was diluted to give a challenge titre equal to $10^{2.3}$ to $10^{2.8}$ TCID$_{50}$/ml. This optimal dilution for challenge was determined by an minimal lethal dose study in cats. One ml of the virus dilution was given orally.

**Clinical scoring system of virulent challenge**

The scoring system devised to judge the extent of disease is shown in Table 1. This scoring system included symptoms associated with FIPV-infected animals: (1) eosinopenia; (2) lymphopenia; (3) leukopenia; (4) Doehle bodies; (5) icterus; (6) vacuolated neutrophils; (7) decreased packed cell volume (PCV); (8) febrile response; and (9) death. The biweekly clinical score of each cat consisted of points from observations of blood clinical symptoms, of febrile responses monitored for 12 to 19 days, and deaths that occurred through 56 days postchallenge.

**Enzyme-linked immunosorbent assay (ELISA)**

The IgG and IgA serum antibody titres were determined by ELISA. The ELISA antigen was prepared by differential centrifugation of clarified DF2-FIPV fluids. Antigen was diluted to an optimum concentration with 0.1 M sodium tetraborate (pH 9.0) and added to the wells (100 µl/well) of a microtitre plate (NUNC, Roskilde, Denmark). After adsorption overnight at 4°C, the wells were emptied and a solution of 1% polyvinyl alcohol in phosphate buffered saline (PVA) (pH 7.2) was added and allowed to incubate 1 h at 37°C. This solution was decanted and 100 µl of each serum dilution in 1% PVA-5% fetal bovine serum (FBS), were added to duplicate wells. A known positive serum and a known negative serum were placed on each plate as controls. After incubation for 60 min at 37°C the plates were decanted and washed three times with PBS-Tween 20 (pH 7.2). A volume of 100 µl of diluted goat anti-cat IgG horseradish peroxidase (Kirkegaard-Perry, Gaithersburg, MD, USA) in PBS-FBS was added to each well and allowed to incubate for 60 min at 37°C. The contents of the plates were then discarded and washed three times with PBS-Tween 20. Then 100 µl of 2,2'-azinodi[3-ethyl-benzthiazoline sulphonate (ABTS) substrate (Kirkegaard-Perry, Gaithersburg, MD, USA) were added to each well. The plate was read spectrophotometrically with a MicroELISA Reader (Molecular Devices, Palo Alto, CA, USA) at a dual wavelength of 405–490 nm. Titres were reported as the reciprocal of the highest dilution which gave an absorbance reading that was 30% of the positive control 30 min after addition of substrate. The IgA ELISA titres were obtained in the same way as the IgG ELISA titres except that goat anti-cat IgA (Nordic Immunological Labs, El Toro, CA, USA) and rabbit anti-goat IgG horseradish peroxidase were used.

**Saliva IgA ELISA**

Saliva was collected on a cotton swab which was then placed in diluent containing 1% PVA, 5% FBS, 0.1% β-mercaptoethanol and 0.005% thimerosal in PBS (pH 7.2) for 1 h at 4°C. The swab was removed and the diluent centrifuged at 600g for 15 min at 4°C. The supernatant was removed and frozen at −20°C. The ELISA method was the same as described for the serum IgA ELISA. Absorbance was read when the serum IgA positive control reached an absorbance reading of approximately 1.000. The increase in specific IgA in saliva after vaccination and challenge was expressed as an IgA Response Index (lgARI). The lgARI was determined as follows: Absorbance$_{X}$/Absorbance$_{0}$ where X indicates the day post first vaccination.

**Virus neutralization test (VNT)**

The VNT was a modification of the procedure described by Ingersoll and Wylie$^{10}$. Heat-inactivated serum samples were diluted twofold in medium followed by the addition of an equal volume of wt-FIPV (100 TCID$_{50}$/well). After incubation at 37°C for 1 h, 5 x 10$^4$ NLFK cells were added to each well. The plate was incubated for 4–5 days in a humidified 39°C incubator enriched with 5% CO$_2$. In each test a positive serum and negative serum were titrated as controls and the virus titrated to ensure that 100–200 infective virus particles per well were used. Titres were expressed as the reciprocal of the highest dilution of serum that inhibited 50% of viral propagation.

**Lymphocyte proliferation assay**

A lymphocyte proliferation assay was used to determine the cell-mediated immune (CMI) response of cats to wt-FIPV. Peripheral blood lymphocytes (PBL) were purified in the manner described by Tompkins et al.$^{11}$. Blood was obtained from anaesthetized cats via jugular
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Table 2 Summary results of immunogenicity tests and data reported

| Immunogenicity test | Vaccinated cats | Control cats | Data reported |
|---------------------|-----------------|--------------|---------------|
|                     | No. cats surviving/ No. cats challenged (%) | First challenge | Second challenge |               |
| I                   | 17/20 (85) | 16/17 (94) | 2/12 (17) | 2/6 (33) | Clinical scores, Serum IgG and IgA ELISA titres and VN titres Saliva IgA Lymphocyte proliferation |
| II                  | 15/20 (75) | 13/14* (93) | 2/10 (20) | 1/6 (17) |
| III                 | 8/10 (80) | ND | 2/5 (40) | ND |

*One of the 15 first challenge surviving vaccinated cats died from a ketamine overdose ND, not done.

puncture into EDTA-containing tubes and added to an equal volume of Minimum Essential Medium modified for suspension cultures (S-MEM) (GIBCO, Grand Island, NY, USA). This mixture was layered over a discontinuous Percoll gradient (Pharmacia, Piscataway, NJ, USA) and centrifuged at 800g for 20 min to yield a lymphocyte-rich band of cells. The band was removed, washed with S-MEM and resuspended in RPMI-1640 medium (GIBCO) without serum. Lymphocyte proliferative activity was measured in response to infectious wt-FIPV. The wt-FIPV or virus-free cell culture supernatant, used at a final dilution of 1:20, was added to microtitre plate wells. Then PBL, at a concentration of 2 x 10⁵ cells per well, and heat inactivated autologous cat serum, collected prior to vaccination, were added. After incubation in a CO₂-enriched incubator for 4 days, 2μCi of tritiated thymidine (New England Nuclear, Boston, MA, USA) were added to each well. The plates were incubated an additional 18 h after which the cells were collected on filter paper discs using a Skatron cell harvester (Skatron Titertek, Sterling, VA, USA). Lymphocyte responses were determined by liquid scintillation counting (Beckman Instruments, Palo Alto, CA, USA) and results expressed as a stimulation ratio (test counts per minute [c.p.m.]/control c.p.m.).

Vaccination and challenge of immunity

Specific pathogen-free (SPF) cats, 6 to 12 months of age, free of serum anti-coronavirus antibodies were obtained from Liberty Labs, Liberty, NJ, USA. Before challenge cats were housed in isolation cages (two cats/cage). At the time of challenge cats were placed in non-isolation cages (four to five cats/cage). Cats were vaccinated twice i.n. on days 0 and 21. Cats in the different experiments were challenged at either 23, 35 or 180 days post second vaccination. After challenge, vaccinated and control cats were monitored for disease symptoms. The surviving vaccinated cats in two experiments were challenged a second time to determine if they might be more susceptible than naive control cats to a second challenge.

Statistical evaluation

The Mann–Whitney U test was used to determine statistically significant differences between medians of the clinical scores of two populations having the same distribution. Statistically significant differences between

Results

Challenge of immunity

A list of the three immunogenicity tests referred to in this paper is given in Table 2. Pertinent data were selected from different immunogenicity tests since not all immune response parameters were measured in each test.

The clinical scores from the first immunogenicity test shown in Figure 1 are also representative of the other two challenge of immunity experiments presented in this paper. Cats vaccinated i.n. with ts-FIPV survived vaccinated cats were challenged twice. The number of cats surviving each challenge and the number of cats challenged is shown in Table 2, immunogenicity test I.

Figure 1 Mean clinical scores following wt-FIPV challenge of non-vaccinated cats □ and cats vaccinated twice i.n. with ts-FIPV ■. Surviving vaccinated cats were challenged twice. The number of cats surviving each challenge and the number of cats challenged is shown in Table 2, immunogenicity test I.

group means of the lymphocyte blastogenesis data was determined using a Student’s t test.

Results

Challenge of immunity

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The clinical scores from the first immunogenicity test shown in Figure 1 are also representative of the other two challenge of immunity experiments presented in this paper. Cats vaccinated i.n. with ts-FIPV were protected against two oral challenges with wt-FIPV. The clinical scores of control cats following the first challenge were significantly higher than vaccinated cats (p < 0.01). Seventeen of 20 vaccinated cats (85%) survived a wt-FIPV challenge that caused FIP in 12 of 12 control cats (100%), ten (83%) of which died. The two control cats that did not die had severe FIP symptoms and were euthanized. In addition to the three vaccinated cats that died, three other vaccinated cats showed transient symptoms of FIP after challenge. One had a febrile response and a decreased PCV, another lymphopenia.
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Humoral immune response

As shown in Figure 2, all vaccinated cats developed a VN titre following first vaccination (geometric mean titre [GMT] = 1:40) that increased following the second vaccination (GMT = 1:86). The GMT of vaccinated cats increased moderately following challenge, peaking at 1:314 at 28 days postchallenge and then dropping to 1:170 at 56 days postchallenge. The VN titres of the three vaccinated cats that died were 1:160, 1:3072 and 1:4096 prior to death. In contrast to the vaccinated cats, control cats developed extremely high VN titres following challenge [GMT = 1:2631 (n = 6) and 1:4096 (n = 2) at 28 and 56 days postchallenge, respectively].

Vaccination also stimulated the development of serum IgG- and IgA-specific FIPV antibodies as measured by ELISA (Figures 3 and 4). In contrast to the virus neutralizing antibody response, which was to peplomer antigen, the ELISA titre was representative of the entire repertoire of IgG and IgA antibodies to wt-FIPV epitopes since whole purified wt-FIPV was used as the solid phase antigen. The first dose of vaccine stimulated the production of IgG antibody (GMT = 1:1077) that increased only slightly following the second vaccination (GMT = 1:1270). At 28 days postchallenge the GMT had increased to 1:11 404, dropping to 1:5077 at 56 days postchallenge. Again the control cats tended to develop higher anti-FIPV antibody titres than vaccinated cats postchallenge. The GMT of control cats at 28 and 56 days postchallenge were 1:39 481 and 1:33 779, respectively.

Low levels of serum IgA antibody were stimulated by vaccination (Figure 4). Geometric mean titres were 1:52 at 28 days and 1:64 at 56 days postchallenge. Like IgG ELISA titres, the serum IgA titres of vaccinated cats increased moderately following challenge (GMT = 1:449 and 1:364 at 28 and 56 days postchallenge, respectively). Again, the IgA titres of control cats were higher at 28 days (GMT = 1:951) and 56 days (GMT = 1:5382) following challenge.

Local IgA response

In a second immunogenicity study (Table 2) it was demonstrated that vaccination stimulated a local immune response. This was shown by the presence of specific IgA anti-FIPV antibody in saliva (Figure 5). The mean IgA response peaked 21 days post first vaccination, decreased slightly on day 42 (21 days post second vaccination) and then declined at the time of challenge on day 58. The level and eosinopenia and the other a transient decrease in PCV. Vaccinated cats were challenged a second time 56 days postchallenge to determine if the first exposure to virulent wt-FIPV made the cats more susceptible to subsequent FIPV exposure. This was clearly not the case. Of 17 vaccinated cats 16 (94%) that survived the first wt-FIPV challenge survived the second challenge. In contrast, four of six naive challenge control cats (67%) developed FIP and died. The clinical scores of the control cats were significantly higher than those of the vaccinated cats (p < 0.01). The one vaccinated cat that did die following second challenge was the cat that had shown lymphopenia and eosinopenia following first challenge. The other two vaccinated cats showed transient symptoms following first challenge showed fewer symptoms following second challenge. A transient lymphopenia and decrease in PCV was noted, but no febrile response.

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of specific IgA in saliva following vaccination was related to protection (Figure 5). There was no increase in specific IgA in the saliva of five vaccinated cats that did not survive challenge. The IgA level in their saliva was no greater than the IgA level in the saliva of control cats. In contrast, there was an increase in specific IgA in the saliva of the 15 vaccinated cats that survived challenge.

The specific IgA level in the saliva increased in both protected and non-protected vaccinated cats and in control cats following challenge. The increase was greater in vaccinated cats indicating an IgA memory response. Unlike serum IgA and IgG levels of protected vaccinated cats, which declined following challenge, the level of IgA in the saliva of vaccinated cats remained high following challenge. The IgA level in the saliva of vaccinated cats increased further after a second virulent wt-FIPV challenge (data not shown). All but one of the vaccinated cats that survived the first challenge survived a second challenge that killed five of six naive control cats. The serum IgA, IgG and VN responses of vaccinated and control cats were similar to the responses of cats in the previous immunogenicity tests (data not shown).

Lymphocyte proliferation response
It was shown in a third immunogenicity test (Table 2) that vaccination stimulated a CMI response. Vaccination induced a specific lymphocyte proliferative response to wt-FIPV in ten cats that was still detectable 137 days postvaccination (Table 3). At day 14 postchallenge, the proliferative response of vaccinated cats, which had increased an average of tenfold over the prechallenge levels, was significantly higher than that of the control cats (p < 0.01). By day 42 postchallenge, the lymphocyte proliferative response had declined to near the prechallenge level. Of the 10 vaccinated cats 8 (80%) survived challenge, showing no evidence of FIP. In contrast, four of five control cats (80%) developed FIP and three died (60%). Only one control cat (number 11) showed a proliferative response postchallenge. This cat, although it developed symptoms of FIP (decreased PCV, lymphopenia, eosinopenia, increased numbers of Doehle bodies), recovered. None of the remaining four control cats, three of which died, showed a lymphocyte proliferative response to wt-FIPV. The serum IgA, IgG and VN responses of vaccinated and control cats were similar to the responses of cats in the previous immunogenicity tests (data not shown).

Discussion
An effective FIPV vaccine should stimulate a strong mucosal immune response to stop systemic spread of the

Table 3 Proliferative response of peripheral blood lymphocytes to wt-FIPV

| Group/Cat No. | Time (days)* | Results of challenge* |
|--------------|--------------|------------------------|
|               | 0  | 14  | 42  | FIP | Death |
| Vaccinated cats |    |     |     |     |       |
| 1             | 1.9 | 4.4 | 3.4 | -  | -     |
| 2             | 2.0 | 2.5 | 1.4 | -  | -     |
| 3             | 1.8 | 81.6| 11.0| -  | -     |
| 4             | 2.5 | 8.0 | 3.7 | -  | -     |
| 5             | 1.8 | 7.7 | 1.8 | -  | -     |
| 6             | 2.7 | 6.5 | 2.3 | -  | -     |
| 7             | 1.9 | 39.0| 2.4 | -  | -     |
| 8             | 1.7 | ND  | 2.0 | -  | -     |
| 9             | 1.6 | ND  | 2.0 | +  | +     |
| 10            | 3.0 | ND  | 2.0 | +  | +     |
| Mean          | 2.1 | 21.4| 3.5 | +  | +     |
| Control cats  |    |     |     |     |       |
| 11            | 1.2 | 2.5 | 1.7 | +  | -     |
| 12            | 1.2 | 1.4 | 0.9 | +  | -     |
| 13            | ND  | 0.1 | 0.1 | +  | +     |
| 14            | 1.4 | 1.7 | 0.8 | +  | +     |
| 15            | 0.8 | 0.6 | 0.6 | +  | +     |
| Mean          | 1.2 | 1.4 | 1.2 | +  | +     |

*Samples collected 137 days post second vaccination and 43 days before challenge (day 0) and at 14 and 42 days postchallenge

*No FIP (-); FIP (+); died (+); survived (--).

ND, not done

Figure 5 Mean salivary IgA Response Index (IgARI) of non-vaccinated cats (---A---), and of protected (---0---) and non-protected vaccinated (---x---) cats. Cats were vaccinated on days 0 and 21 and challenged on day 58 with wt-FIPV.
virulent FIPV and a CMI response that will immediately halt the spread of the virus if it does cross the mucosa. Stimulation of a nasal mucosal immune response is best done by i.n. administration of a vaccine. Mucosal B-lymphocytes, stimulated by i.n. vaccination to secrete anti-FIPV IgA antibody, will migrate to the gut mucosa and also confer local gut immunity. The ts-FIPV, because it is given i.n., and because of its ability to grow at temperatures present in the nasopharyngeal region, stimulated a local immune response as indicated by IgA antibody in saliva. In addition, the ts-FIPV vaccine stimulated a CMI response to FIPV following two vaccinations. The CMI response was still detectable 137 days postvaccination, and was amplified following challenge. A CMI response to FIPV did not occur in non-vaccinated cats infected with virulent FIPV.

Humoral immunity is reported to enhance rather than protect against experimental FIPV infection. Our studies suggest that a humoral response to FIPV, per se, is not detrimental. The ts-FIPV vaccinated cats developed IgG, IgA and virus neutralizing antibody titres without the cats being sensitized to FIPV. However, it should be noted that the geometric mean VN titre of vaccinated cats was less than 10% of the geometric mean titre of the non-vaccinated control cats following challenge. A high level of VN antibody to peplomer epitopes may play a significant role in the pathogenesis of the disease. Indeed, Vennema et al. reported that an immune response to peplomer epitopes may be related to disease. They found that cats vaccinated with a recombinant peplomer vaccine developed FIP and died sooner than non-vaccinated control cats.

Although stimulation of the humoral immune response is often associated with disease and high serum antibody titres are sometimes used as an aid in diagnosing FIP, stimulation of the mucosal immune system, as indicated by specific IgA in the saliva, was related to protection. In a second challenge of immunity study, all 15 of the 20 vaccinated cats (75%) that survived challenge developed an IgA response to wt-FIPV following vaccination. In contrast, the postvaccination level of IgA in the saliva of non-protected vaccinated cats was no different from the mean IgA level in the saliva of ten control cats, eight of which died (80%). The specific IgA level in the saliva of the protected vaccinated cats increased postchallenge. The level of IgA in the saliva of three of the five non-protected vaccinated cats also increased following challenge. However, the increase in the level of local IgA may have either developed too late to prevent infection or may have been too low before challenge to inhibit the spread of virulent FIPV. It is also possible that these cats did not develop a CMI response to FIPV which, in conjunction with a mucosal immune response, is necessary for protection against FIP.

A cell-mediated immune response to FIPV is important, especially if FIPV evades the mucosal defence system. Pedersen and Floyd showed that FIPV stimulated a lymphocyte proliferation response in 17 cats that recovered from an FIPV infection but not in seronegative, uninfected cats. Stoddart et al. also reported that cats which survived an FIPV challenge developed a lymphocyte proliferation response to FIPV. The stronger the lymphocyte proliferation response, the longer the cats survived. In contrast, cats that died in 16–18 days postchallenge developed no response to FIPV. The present study showed that two i.n. vaccinations with the ts-FIPV vaccine stimulated a lymphocyte proliferation response to FIPV that was still detectable 137 days postvaccination. A peripheral blood lymphocyte response to wt-FIPV following challenge correlated with the ability of the cat to survive FIPV infection. The lymphocyte proliferation response of vaccinated cats had increased by an average of tenfold by 14 days postchallenge indicating that vaccination had stimulated an immune memory cell response. Although none of the three control cats that died developed a proliferative response to wt-FIPV postchallenge, one of the two control cats that recovered after exhibiting signs of FIP showed a weak lymphocyte proliferative response at 14 days postchallenge. Since the lymphocyte proliferation assay only measures the ability of lymphocytes to respond to FIPV and not the nature of the response, more studies of the cell-mediated response to FIPV need to be done.

Parenterally-vaccinated cats have been reported by others to develop FIP earlier than non-vaccinated cats following challenge. In contrast to parenterally administered vaccines, i.n. administered ts-FIPV vaccine did not predispose the vaccinated cats to FIP. The i.n. route of administration and the characteristics of the ts-FIPV, which include the inability of the vaccine virus to replicate outside of the nasal/pharyngeal area and the absence of ts-FIPV structural proteins on the surface of infected cells at 39°C, are thought to be the reasons vaccine-induced hypersensitivity does not occur.

In summary, a ts-FIPV vaccine, administered i.n., stimulated a mucosal immune response to wt-FIPV that correlated with immunity to two rigorous challenges. In addition, the i.n. administered vaccine stimulated a long lasting CMI response as measured by lymphocyte proliferation to wt-FIPV.

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