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Therapeutic effect of anti-feline TNF-alpha monoclonal antibody for feline infectious peritonitis

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ABSTRACT

Feline infectious peritonitis virus (FIPV) replication in macrophages/monocytes induced tumor necrosis factor (TNF)-alpha production, and that the TNF-alpha produced was involved in aggravating the pathology of FIP. We previously reported the preparation of a feline TNF-alpha (fTNF-alpha)-neutralizing mouse monoclonal antibody (anti-fTNF-alpha mAb). This anti-fTNF-alpha mAb 2–4 was confirmed to inhibit the following fTNF-alpha-induced conditions in vitro. In the present study, we investigated whether mAb 2–4 improved the FIP symptoms and survival rate of experimentally FIPV-inoculated SPF cats. Progression to FIP was prevented in 2 out of 3 cats treated with mAb 2–4, whereas all 3 cats developed FIP in the placebo control group. Plasma alpha1-glycoprotein and vascular endothelial growth factor levels were improved by the administration of mAb 2–4, and the peripheral lymphocyte count also recovered. These results strongly suggested that the anti-fTNF-alpha antibody is effective for the treatment of FIP.

1. Introduction

Feline infectious peritonitis virus (FIP virus: FIPV), a feline coronavirus (FCoV) of the family Coronaviridae, causes a fatal disease called FIP in wild and domestic cat species. Several organs, including the liver, lungs, spleen, serosae, kidneys, eyes, and central nervous system, are affected in cats that develop FIP, and the formation of lesions in these organs is accompanied by necrosis and pyogenic granulomatous inflammation (Pedersen, 2009). Pleural effusion and ascitic fluid was reported to accumulate in some cats. Macrophages/monocytes play an important role in the pathogenesis of FIP. For example, differences in the proliferation of virus in macrophages/monocytes were shown to be related to differences in pathogenicity between feline enteric coronavirus (FECV) and FIPV (Dewerchin et al., 2005; Stoddart and Scott, 1989). FECV and FIPV cannot be serologically distinguished from each other; however, FECV infection is normally asymptomatic in cats. It was recently reported that FECV and FIPV may be distinguished based on the base sequences of the ORF 2 (S gene) and 3c genes (Bank-Wolf et al., 2014; Licitra et al., 2013; Pedersen et al., 2012; Porter et al., 2014).

Tumor necrosis factor (TNF)-alpha binds to cell surface TNF receptors (TNFR) and induces various physiological activities (Vandenabeele et al., 1995; Reinhard et al., 1997). For example, when it binds to cell surface TNFR-1, caspase is activated and induces apoptosis, and when it binds to cell surface TNFR-2, transcription factors, such as NF-kB and c-Jun, are activated that promote cell proliferation and induce the expression of cytokines involved in immunity and inflammation (Faustman and Davis, 2010; Tracey et al., 2008). TNF-alpha plays a critical role in many aspects of immunity. However, the excessive production of TNF-alpha can lead to acute inflammation and immune system abnormalities in human and other animals. The involvement of TNF-alpha in aggravating the symptoms of rheumatoid arthritis, psoriasis, and inflammatory bowel disease has been reported (Kollias et al., 1999; Brotas et al., 2012; Wang and Fu, 2005). Moreover, previous studies have described aggravation of the pathologies of viral infections (such as human immunodeficiency virus, influenza A virus, herpes simplex virus (HSV) and dengue virus infections) due to increased TNF-alpha production (Fauci, 1993; Maury and Lähdevirta, 1990; Poli et al., 1990; Uchide et al., 2012; Yen et al., 2008).

We previously showed that virus replication in macrophages/monocytes induced TNF-alpha production, and that the TNF-alpha produced was involved in aggravating the pathology of FIP. TNF-alpha produced by FIPV-infected macrophages was involved in lymphopenia and an increase in the level of the cellular receptor of serotype II FIPV, aminopeptidase N (APN) (Takano et al., 2007a; Takano et al., 2007b). It is also reported that neutrophil apoptosis in cats with FIP was inhibited by TNF-alpha. This finding suggests that neutrophilia in cats with FIP due to TNF-alpha-induced neutrophil survival (Takano et al., 2009).

In humans, anti-TNF agents have been shown to therapeutic effect for TNF-related disease, such as rheumatoid arthritis and inflammatory bowel disease (Tracey et al., 2008). Anti-TNF agents were previously shown to be effective for the treatment of fatal viral infections. Boivin et al. (2013) reported that the survival rate of mice with HSV encephalitis was improved by the administration of an anti-TNF-alpha antibody.

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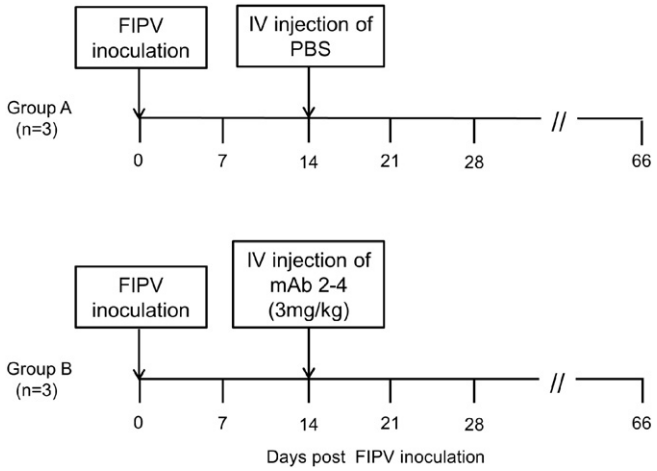

Fig. 1. The experimental schedule of the anti fTNF-alpha mAb (mAb 2–4) treatment for FIPV-infected cats.

Anti-TNF-alpha agents are also expected to improve the symptoms of FIP caused by viral infection (Addie and Ishida, 2008). We previously described the preparation of a feline TNF-alpha (fTNF-alpha)-neutralizing mouse monoclonal antibody (anti-fTNF-alpha mAb). This anti fTNF-alpha mAb exhibited high neutralizing activity against recombinant and natural TNF-alpha, and was confirmed to inhibit the following fTNF-alpha-induced conditions in vitro: i) an increase in the survival rate of neutrophils from cats with FIP, ii) APN mRNA expression in macrophages, and iii) apoptosis of a feline T-lymphocyte cell line (Doki et al., 2013).

In the present study, we confirmed the efficacy of fTNF-alpha mAb for the treatment of FIP in vivo. SPF cats were experimentally inoculated with FIPV and treated with the anti-fTNF-alpha mAb, and improvements in FIP symptoms and survival rate were investigated.

2. Materials and methods

2.1. Cell cultures and virus

Hybridoma cells producing mAb 2–4 were maintained in Dulbecco's modified Eagle's minimum essential medium supplemented with 10% FCS, 600 U/ml of benzylpenicillin potassium, and 240 μg/ml of streptomycin sulfate. WEHI-164 murine sarcoma cells (ATCC CRL1751) were maintained in RPMI 1640 growth medium supplemented with 10% FCS, 600 U/ml of benzylpenicillin potassium, 240 μg/ml of streptomycin sulfate, 50 μM 2-mercaptoethanol, and 2 μg/ml of polybrene. WEHI-164 murine sarcoma cells were obtained from the American Type Culture Collection. Type II FIPV strain 79-1146 was grown in *Felis catus* whole fetus-4 cells at 37 °C. FIPV strain 79-1146 was supplied by Dr. M. C. Horzinek of State University Utrecht, The Netherlands.

2.2. Monoclonal antibody

Anti fTNF-alpha mAb 2–4 has been described previously (Doki et al., 2013). The mAb 2–4 have neutralizing activity for recombinant fTNF-alpha and natural fTNF-alpha.

A phycoerythrin (PE)-conjugated anti-feline CD4 mAb (Southern Biotechnology Associates, Inc., U.S.A.) and fluorescein isothiocyanate (FITC)-conjugated anti-feline CD8 mAb (Southern Biotechnology Associates, Inc., U.S.A.) were used in the flow cytometry analysis to measure the numbers of CD4 + and CD8 + lymphocytes.

2.3. Purification of mAb 2–4

mAb 2–4 were purified from the hybridoma culture supernatant with Protein G Sepharose (GE Healthcare., U.S.A.) according to the product manual. After purification, the buffer of mAb 2–4 was exchanged to phosphate-buffered saline (PBS) (pH 7.4) by Amicon Ultra-15 centrifugal filter devices (NMWL 30,000; Millipore., U.S.A.). The concentrations of purified mAb 2–4 were assayed by the Bradford method. The neutralizing activity of purified mAb 2–4 were confirmed by neutralization test against TNF-alpha using WEHI-164 cells as previously described by Doki et al. (2013). Purified mAb 2–4 at 10 μg/ml neutralized 80% or more of the activity of 160 ng/ml recombinant fTNF-alpha.

2.4. Experimental schedule

Six SPF cats were randomly assigned to two experimental groups. The cats were bred in our own laboratory, and maintained in a temperature-controlled isolated facility. In accordance with the experimental schedule indicated in Fig. 1. FIPV strain 79-1146 (10^5 $TCID_{50}$/0.5 ml) was inoculated subcutaneously to cats. PBS or mAb 2–4 (3 mg/kg) were intravenously administered to groups A and B, respectively, 2 weeks after the virus inoculation. Cats were examined daily for clinical signs, and their body temperatures and weights were measured. Blood was collected weekly using a heparinized syringe after the virus inoculation, and complete and differential cell counts were measured.

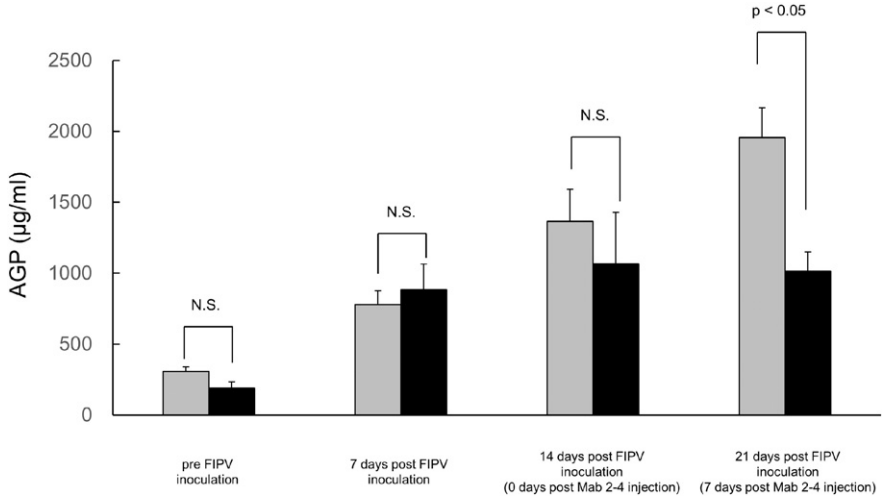

Fig. 2. The changes on plasma alpha1-acid glycoprotein (AGP) concentration of FIPV-infected cats treated with mAb 2–4. The normal value of AGP is 386 ± 60 μg/ml (quoted from the manufacturer's protocol, mean ± S.D.). Gray bar; group A, black bar; group B. N.S.: not significant.

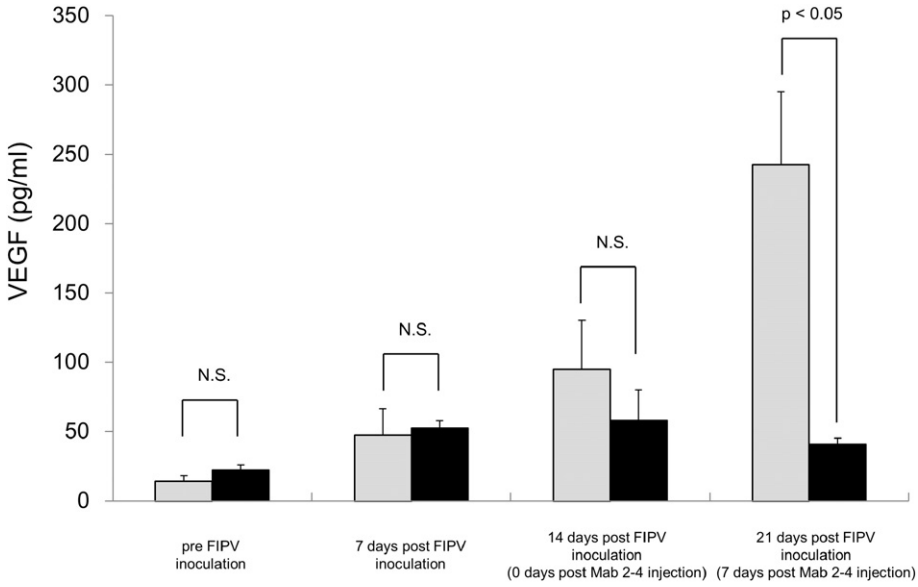

Fig. 3. The changes on plasma vascular endothelial growth factor (VEGF) concentration of FIPV-infected cats treated with mAb 2–4. Gray bar; group A, black bar; group B. N.S.: not significant.

FIP diagnoses were confirmed upon postmortem examination, revealing peritoneal and pleural effusions, and pyogranuloma in the major organs. The protocol for the experiments in the present study using cats was approved by the Ethics Committee of Kitasato University, School of Veterinary Medicine (Approval No. 13-087).

2.5. Plasma sample

Blood collected from cats using a heparinized syringe was centrifuged at 3000 rpm for 10 min, and the supernatant was used as a plasma sample. The plasma samples were stored at −30 °C until day of analysis.

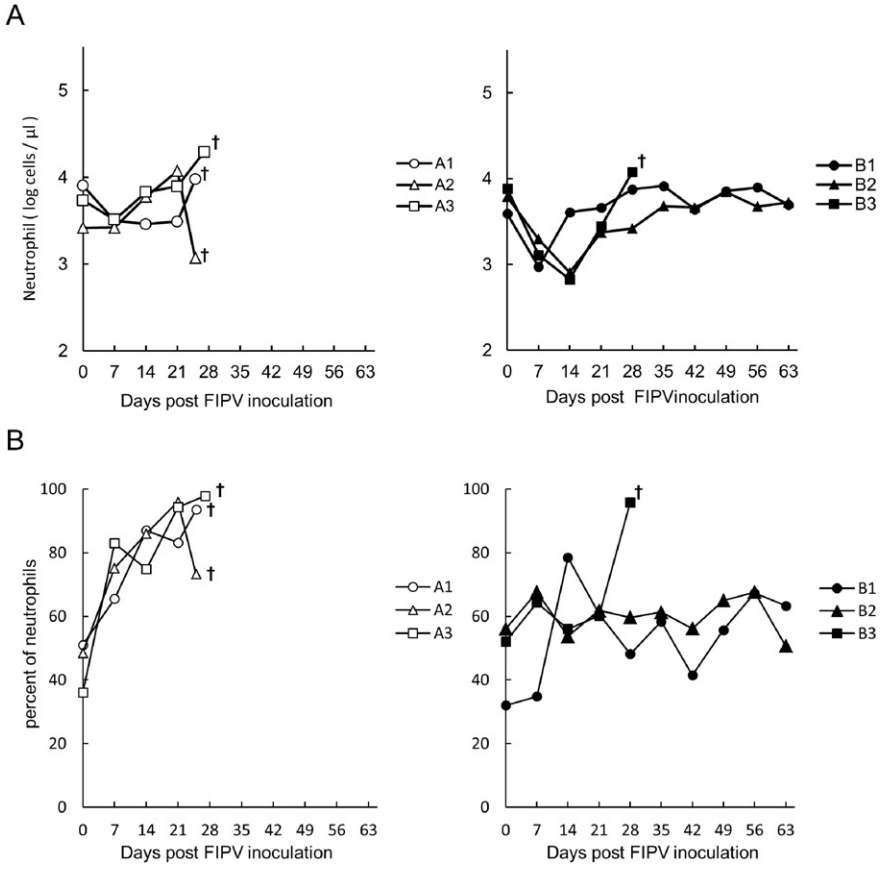

Fig. 4. The changes on neutrophil counts and percentages of neutrophils of FIPV-infected cats treated with mAb 2–4. (A) Neutrophil counts in peripheral blood. (B) Percentage of neutrophils on differential blood cell counting. †: Animal was euthanized because its critical condition reached the humane endpoint.

2.6. Measurement of plasma alpha1-glycoprotein (AGP)

Plasma concentrations of AGP were determined with feline alpha1 AG plate (The Institute for Metabolic Ecosystem Lab., Japan), according to the manufacturer's protocol.

2.7. Measurement of plasma vascular endothelial growth factor (VEGF) concentration

Plasma concentrations of VEGF were determined with human VEGF ELISA kit (R & D Systems, U.K.), according to the manufacturer's protocol. The ELISA kit detects primarily the feline VEGF isoform 164 (Koga et al., 2002).

2.8. Measurement of $CD4^+$ and $CD8^+$ T lymphocyte counts using flow cytometry analysis

Heparinized blood (5 ml) was 2-fold diluted with PBS, and subjected to Ficoll-Hypaque (Axis-Shield PoC AS, Norway) density gradient centrifugation at 800 $\times g$ for 20 min. The PBMC layer was collected, washed twice with PBS, and resuspended with ice cold PBS containing 0.1% NaN_3 at 1×10^6 cells/ml. Cells were incubated with PE-conjugated anti-feline CD4 mAb and FITC-conjugated anti-feline CD8 mAb at 4 °C for 30 min. After washing, the cells were stored in fluorescence buffer prior to analysis on a flow cytometer (Cytomics FC500, Beckman Coulter, U.S.A.). The small lymphocyte and lymphoblast populations were gated on the basis of the cell size and granularity (forward and side scatter). For each sample 100,000 events were recorded, and the percentage of $CD4^+$ and $CD8^+$ T lymphocyte population was calculated. Absolute $CD4^+$ and $CD8^+$ T lymphocyte counts were determined from complete blood count, differential cell counts and percentage of $CD4^+$ and $CD8^+$ T lymphocyte population.

2.9. Statistical analysis

Data from two groups were analyzed by the Student's t test.

3. Results

3.1. Effect of anti fTNF-alpha mAb 2–4 on plasma AGP and VEGF level of cats inoculated with FIPV

Increases in plasma AGP and VEGF levels have been reported in cats that developed FIP (Duthie et al., 1997; Paltrinieri et al., 2007a; Paltrinieri et al., 2007b, Takano et al., 2011). In order to determine the therapeutic effects of anti-fTNF-alpha mAb 2–4, changes in plasma AGP and VEGF levels after the FIPV inoculation and administration of mAb 2–4 were investigated.

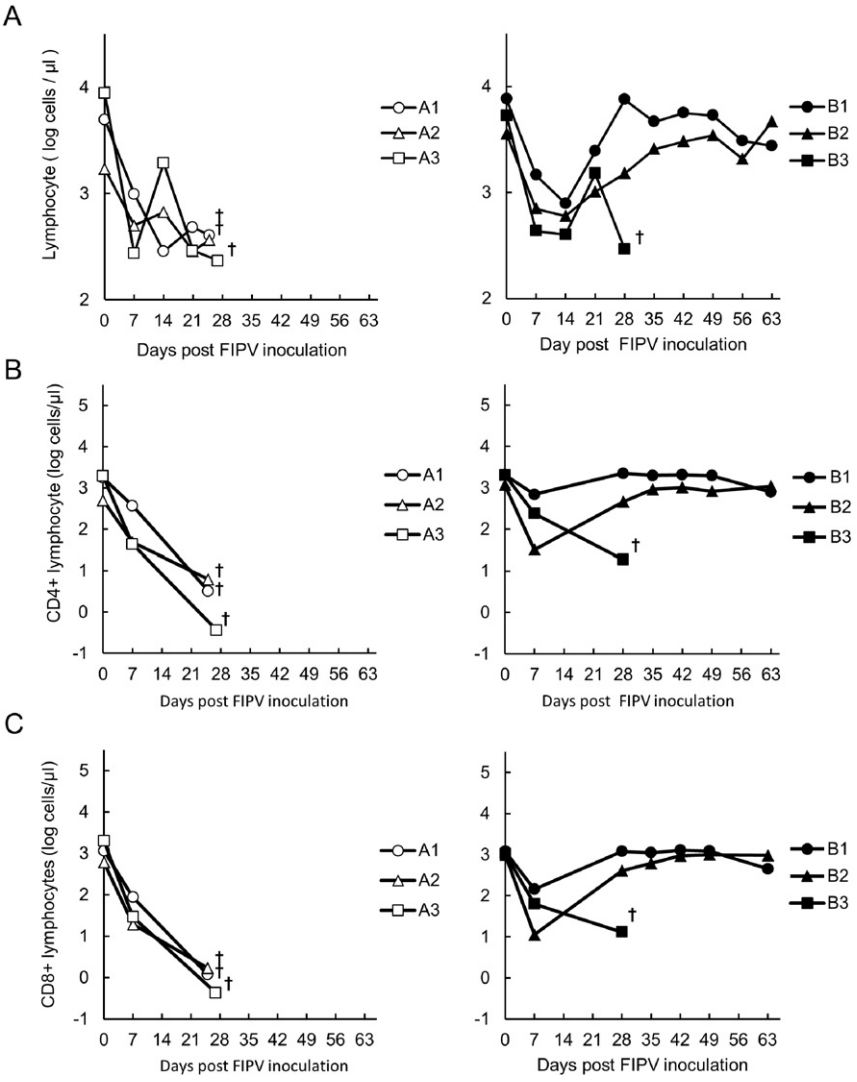

Fig. 5. The changes on lymphocyte counts and lymphocyte subsets of FIPV-infected cats treated with mAb 2–4. (A) Lymphocyte counts in peripheral blood. (B) CD4 + T lymphocyte counts in peripheral blood. (C) CD8 + T lymphocyte counts in peripheral blood. †: Animal was euthanized because its critical condition reached the humane endpoint.

Increases in AGP and VEGF levels were noted 7 and 14 days after the FIPV inoculation in placebo-treated (group A) and mAb 2–4-treated cats (group B) (Figs. 2 and 3). No significant difference was noted in AGP or VEGF levels between groups A and B until 14 days after the FIPV inoculation (0 days after the administration of mAb 2–4); however, these levels were significantly lower in group B than in group A 21 days after the FIPV inoculation (7 days after the administration of mAb 2–4).

3.2. The changes on neutrophil counts and percentages of neutrophils of FIPV-infected cats treated with mAb 2–4

Changes in the neutrophil count after the FIPV inoculation and administration of mAb 2–4 were investigated. The neutrophil count was slightly decreased 7 days after the virus challenge, but recovered to almost normal levels after 14 or 21 days (Fig. 4A). The rate of neutrophils on differential cell counting is shown in Fig. 4B. In group A, the rate of neutrophils increased after the FIPV inoculation and was maintained at a high level at 21 days and thereafter. In contrast, in cat Nos. B1 and B2, the rate of neutrophils was increased 14 days after the FIPV inoculation, and then stabilized 7 days after the administration of mAb 2–4 (21 days after the FIPV inoculation) and thereafter. In cat No. B3, which was treated with mAb 2–4 but still developed FIP, the rate of neutrophils was stable until 21 days after the FIPV inoculation, but then rapidly increased 28 days after the FIPV inoculation.

3.3. The changes on lymphocyte counts and lymphocyte subset counts of FIPV-infected cats treated with mAb 2–4

Changes in the peripheral lymphocyte count after the FIPV inoculation and administration of mAb 2–4 were investigated. Peripheral lymphocytes markedly decreased in all cats 7 days after the virus challenge (Fig. 5A). In cat Nos. B1 and B2, the count started to increase 7 days after the administration of mAb 2–4 (21 days after the FIPV inoculation) and recovered to the pre-FIPV inoculation level 21 days after the administration of mAb 2–4 (35 days after the FIPV inoculation). In contrast, the peripheral lymphocyte count in cats that developed FIP in group A did not recover, even at 14 days after the virus challenge, and remained low until the development of FIP. In cat No. B3, which was treated with mAb 2–4 but still developed FIP, the peripheral lymphocyte count temporarily recovered 7 days after the administration of mAb 2–4 (21 days after the FIPV inoculation), but markedly decreased when FIP developed.

Of the lymphocyte subsets, CD4 + and CD8 + T-lymphocytes markedly decreased in cats that developed FIP. We investigated whether these cell counts recovered after the administration of anti-fTNF-alpha mAb 2–4. The CD4 + and CD8 + T-lymphocyte counts decreased 7 days after the virus inoculation in all cats in groups A and B (Fig. 5B,C), and these counts continuously decreased until the development of FIP in all cats in group A. In contrast, the CD4 + and CD8 + T-lymphocyte counts in cat Nos. B1 and B2 recovered to the pre-virus inoculation levels after the administration of mAb 2–4, but did not in cat No. B3, which developed FIP.

3.4. Effect of anti fTNF-alpha mAb 2–4 on body temperature, body weight, clinical sign and survival of cats inoculated with FIPV

Changes in body temperatures and body weights after the FIPV inoculation were measured (Fig. 6A). Fever developed after the FIPV inoculation in groups A and B. In the 4 cats that developed FIP (cat Nos. A1, A2, A3, and B3), a high body temperature persisted until the development of FIP. In contrast, although a high body temperature persisted in cat No. B2 until 28 days after the FIPV inoculation, no fever was observed thereafter, and the body temperature variation stabilized 35 days after the FIPV inoculation and thereafter.

No significant differences were noted in body weights between groups A and B until 14 days after the FIPV inoculation (Fig. 6B). Body weights continuously decreased in the 4 cats that developed FIP (cat Nos. A1, A2, A3, and B3). In contrast, although weight loss was noted until 14 days after the FIPV inoculation, body weights slightly increased after 21 days in 2 cats (cat Nos. B1 and B2).

Clinical sign (listlessness and loss of appetite) were observed from 15 days after the FIPV inoculation in both groups, but improved in group B after the administration of mAb. Animal was euthanized became its critical condition reached the humane endpoint. In group A, all 3 animals were euthanized 25 and 27 days after the FIPV inoculation; accordingly, survival decreased to zero (Fig. 7). In group B, one animal was euthanized at 30 days. However, cat Nos. B2 and B3 developed no clinical sign thereafter and survived until 65 days.

4. Discussion

In humans, molecular targeted therapy is performed to treat infectious disease, immune-mediated diseases, and tumors (Mitsuya et al., 1991; Tracey et al., 2008; Hainsworth, 2000). Targeted therapy is capable of exhibiting strong therapeutic effects while minimizing adverse reactions because it only targets the factors involved in diseases. Targeted therapy has not been performed in the veterinary field, and no molecular target drug has been prepared to treat intractable diseases

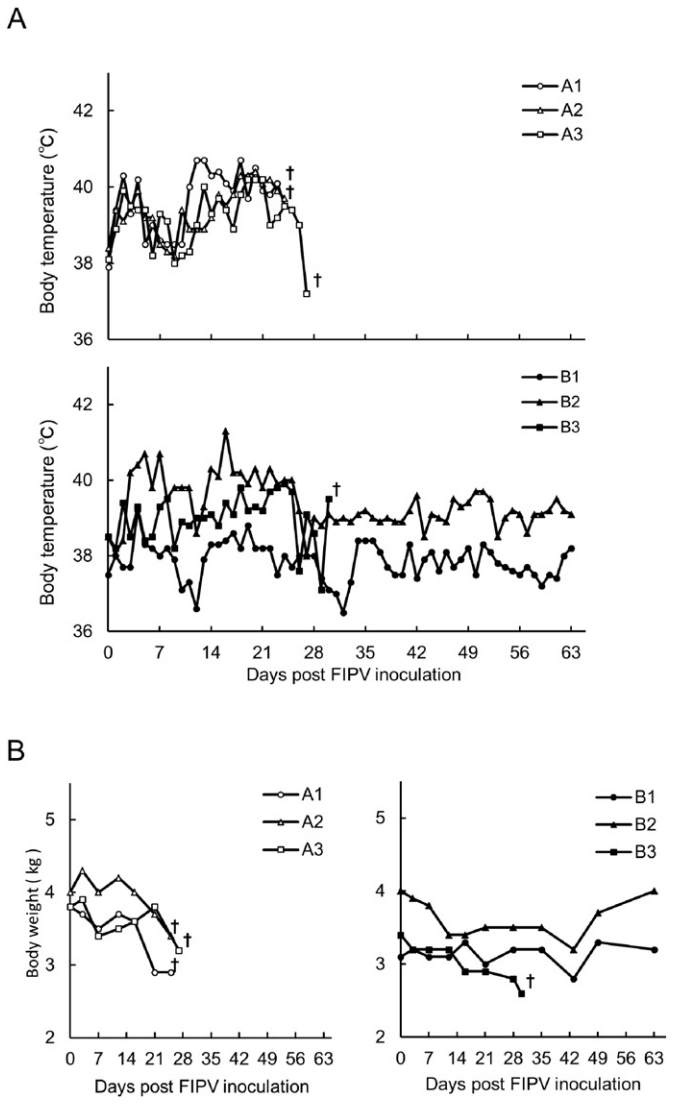

Fig. 6. The changes in body temperature and body weight of FIPV-infected cats treated with mAb 2–4. (A) Body temperature. (B) Body weight. †:Animal was euthanized because its critical condition reached the humane endpoint.

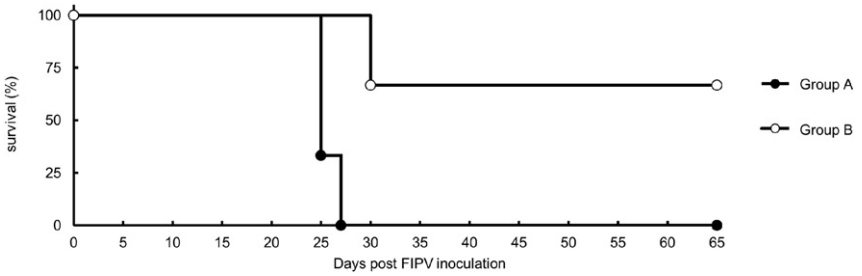

Fig. 7. Survival rate of FIPV-infected cats treated with mAb 2–4. Animals which reached the humane endpoint with no prospect of survival were euthanized.

in cats. In the present study, we investigated whether the antibody neutralizing TNF-alpha, an FIP pathology-aggravating factor, actually exhibited a therapeutic effect in cats that developed FIP, i.e., antibody therapy targeting TNF-alpha was performed in cats with FIP and improvements in symptoms were investigated.

Anti-fTNF-alpha mAb 2–4 was administered 14 days after cats were inoculated with FIPV strain 79-1146. The highly pathogenic FIPV 79-1146 was chosen for this reason: the high probability of inducing FIP by the use of FIPV strain 79-1146 may be important to reduce the number of animals used to perform the experiment (to reliably induce FIP). Fewer than 50% of Type I FIPV-inoculated cats develop FIP. In our previous experiment, when the type I FIPV strain KU-2 was orally, subcutaneously, and intraperitoneally administered, the incidences of FIP were 0, 20, and 50% (unpublished data), respectively, suggesting that when type I FIPV is used, the incidence is not stable and the therapeutic effect cannot be accurately judged. It may also be difficult to reproduce FIP development by challenging field fecal materials derived from cats with type I FIP. This point remains to be further investigated.

In our previous experiment, TNF-alpha, VEGF, and AGP levels were measured in plasma collected over time, and significant increases were noted in all factors 7–14 days after the FIPV inoculation. Furthermore, clinical signs (fever, listlessness, and jaundice) developed 6–8 days after the FIPV inoculation in all groups. The timing of the administration of mAb 2–4 was based on a comprehensive judgment of these findings. To use a mouse mAb as a placebo, it would be necessary to confirm that it does not affect the virus, feline tissue, or the immune system. Since no such mouse mAb was available, we used the solvent of anti-fTNF-alpha mAb, PBS, as a placebo. Since the single administration of anti-fTNF-alpha mAb caused no adverse reaction to mAbs, we consider that the use of PBS as a placebo was appropriate.

Progression to FIP was prevented in 2 out of the 3 cats treated with mAb 2–4, whereas all 3 cats developed FIP in the placebo control group. In mAb 2–4-treated cats, plasma VEGF levels improved after the administration of the antibody and the lymphocyte count also recovered. In addition, a plasma AGP level exceeding the diagnostic criterion of FIP, 1500 μg/ml (Addie et al., 2009), was observed on day 21 in the placebo group, whereas the level was significantly lower in mAb 2–4 treated cats. These facts strongly suggested that mAb 2–4 alleviated FIP symptoms by inhibiting TNF-alpha activity.

It is suggested that FIPV-specific CD4+ and CD8+ T-lymphocytes contribute to virus elimination (de Groot-Mijnes et al., 2005). We previously reported that TNF-alpha produced by FIPV-infected macrophages efficiently induced the apoptosis of T-lymphocytes, particularly CD8+ T-lymphocytes (Takano et al., 2007a). In the present study, decreases in the numbers of CD4+ and CD8+ T-lymphocytes were noted from 7 days after the FIPV inoculation, but recovered in 2 out of the 3 cats treated with mAb 2–4. The TNF-alpha-induced apoptosis of CD4+ and CD8+ T-lymphocytes may have been reduced by mAb 2–4, i.e., a cellular immune response against FIPV may have been induced and maintained.

Various drugs to treat FIP have been investigated. Since FIP is a viral disease, treatments with antiviral agents (anti-FIPV drugs) have been examined (Hartmann and Ritz, 2008). On the other hand, treatments with anti-inflammatory drugs, represented by steroids, have also been tested because FIP is an inflammatory disease. However, existing anti-FIPV and anti-inflammatory drugs only transiently improve FIP symptoms, and do not improve survival times or quality of life (Hartmann and Ritz, 2008). MAb 2–4 improved the survival time and quality of life of cats with FIP; however, one of the 3 mAb 2–4-treated cats developed FIP. Thus, further investigations are necessary to determine its applicability as a therapeutic drug for FIP. We considered the combination of the above anti-FIPV drugs and mAb 2–4 to have improved therapeutic effects on FIP. The therapeutic effects of the combination of an anti-TNF-alpha antibody and anti-HSV drugs on HSV infection were previously reported to be high (Boivin et al., 2013). Regarding HSV infection, anti-HSV drugs such as acyclovir that only target the factors essential for viral replication have been developed. This type of drug has recently been developed for FIPV, for which future studies are expected (Hsieh et al., 2010). The treatment of FIP with a combination of these drugs warrants further investigation.

In the present study, we administered the mouse monoclonal antibody mAb 2–4 to cats only once, and their symptoms improved. However, it may be necessary to investigate the multiple administration of mouse mAb 2–4 to cats in which a therapeutic effect is only temporarily exhibited, as noted in cat No. B3. Since administration of mouse mAb 2–4 leads to anti-mouse mAb response to cats, it may cause anaphylactic reactions as an adverse effect, reductions in the therapeutic effects, and shortening of the half-life from second administration of mouse mAb 2–4. Umehashi et al. (2002) reported that the administration of a mAb with a constant region converted to the feline form (a mouse-feline chimeric antibody) did not induce an anaphylactic reaction. Based on this finding, we modified mAb 2–4 to a mouse-feline chimeric antibody, and are now evaluating its effects. Furthermore, we will need to prepare Fab-fragment which removed Fc portion of mAb 2–4.

In conclusion, the results of the present study suggest that the anti-feline TNF-alpha mAb is effective for the treatment of FIP in vivo. The modification of mAb 2–4 to a mouse-feline chimeric antibody and the therapeutic effects of its combination with anti-FIPV agent(s) need to be examined in more detail, with the aim of its application as an effective therapeutic drug for FIP.

Acknowledgments

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References

Addie, D.D., Ishida, T., 2008. Feline infectious peritonitis: therapy and prevention. In: Bonagura, J.D., Twedt, D.C. (Eds.), Kirk's Current Veterinary Therapy XIV. Saunders, Elsevier, pp. 1295–1299.

Addie, D., Belák, S., Boucraut-Baralon, C., Egberink, H., Frymus, T., Gruffydd-Jones, T., Hartmann, K., Hosie, M.J., Lloret, A., Lutz, H., Marsilio, F., Pennisi, M.G., Radford, A.D., Thiry, E., Truyen, U., Horzinek, M.C., 2009. Feline infectious peritonitis. ABCD guidelines on prevention and management. J. Feline Med. Surg. 11, 594–604.

Bank-Wolf, B.R., Stallkamp, I., Wiese, S., Moritz, A., Tekes, G., Thiel, H.J., 2014. Mutations of 3c and spike protein genes correlate with the occurrence of feline infectious peritonitis. Vet. Microbiol. 173, 177–188.
Boivin, N., Menasria, R., Piret, J., Rivest, S., Boivin, G., 2013. The combination of valacyclovir with an anti-TNF alpha antibody increases survival rate compared to antiviral therapy alone in a murine model of herpes simplex virus encephalitis. Antivir. Res. 100, 649–653.
Brotas, A.M., Cunha, J.M., Lago, E.H., Machado, C.C., Carneiro, S.C., 2012. Tumor necrosis factor-alpha and the cytokine network in psoriasis. An. Bras. Dermatol. 87, 673–681.
de Groot-Mijnes, J.D., van Dun, J.M., van der Most, R.G., de Groot, R.J., 2005. Natural history of a recurrent feline coronavirus infection and the role of cellular immunity in survival and disease. J. Virol. 79, 1036–1044.
Dewerchin, H.L., Cornelissen, E., Nauwynck, H.J., 2005. Replication of feline coronaviruses in peripheral blood monocytes. Arch. Virol. 150, 2483–2500.
Doki, T., Takano, T., Nishiyama, Y., Nakamura, M., Hohdatsu, T., 2013. Generation, characterization and therapeutic potential of anti-feline TNF-alpha MAbs for feline infectious peritonitis. Res. Vet. Sci. 95, 1248–1254.
Duthie, S., Eckersall, P.D., Addie, D.D., Lawrence, C.E., Jarrett, O., 1997. Value of alpha 1-acid glycoprotein in the diagnosis of feline infectious peritonitis. Vet. Rec. 141, 299–303.
Fauci, A.S., 1993. Multifactorial nature of human immunodeficiency virus disease, implications for therapy. Science 262, 1011–1018.
Faustman, D., Davis, M., 2010. TNF receptor 2 pathway: drug target for autoimmune diseases. Nat. Rev. Drug Discov. 9, 482–493.
Hainsworth, J.D., 2000. Monoclonal antibody therapy in lymphoid malignancies. Oncologist 5, 376–384.
Hartmann, K., Ritz, S., 2008. Treatment of cats with feline infectious peritonitis. Vet. Immunol. Immunopathol. 123, 172–175.
Koga, L., Kobayashi, Y., Yazawa, M., Maeda, S., Masuda, K., Ohno, K., Tsujimoto, H., 2002. Nucleotide sequence and expression of the feline vascular endothelial growth factor. J. Vet. Med. Sci. 64, 453–456.
Kollias, G., Douni, E., Kassiotis, G., Kontoyiannis, D., 1999. The function of tumour necrosis factor and receptors in models of multi-organ inflammation, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease. Ann. Rheum. Dis. 58 (Suppl. 1), I32–I39.
Licitra, B.N., Millet, J.K., Regan, A.D., Hamilton, B.S., Rinaldi, V.D., Duhamel, G.E., Whittaker, G.R., 2013. Mutation in spike protein cleavage site and pathogenesis of feline coronavirus. Emerg. Infect. Dis. 19, 1066–1073.
Maury, C.P., Lähdevirta, J., 1990. Correlation of serum cytokine levels with haematological abnormalities in human immunodeficiency virus infection. J. Intern. Med. 227, 253–257.
Mitsuya, H., Yarchoan, R., Kageyama, S., Broder, S., 1991. Targeted therapy of human immunodeficiency virus-related disease. FASEB J. 5, 2369–2381.
Paltrinieri, S., Giordano, A., Tranquillo, V., Guazzetti, S., 2007a. Critical assessment of the diagnostic value of feline alpha1-acid glycoprotein for feline infectious peritonitis using the likelihood ratios approach. J. Vet. Diagn. Investig. 19, 266–272.
Paltrinieri, S., Metzger, C., Battilani, M., Pocacqua, V., Gelain, M.E., Giordano, A., 2007b. Serum alpha1-acid glycoprotein (AGP) concentration in non-symptomatic cats with feline coronavirus (FCoV) infection. J. Feline Med. Surg. 9, 271–277.
Pedersen, N.C., 2009. A review of feline infectious peritonitis virus infection, 1963–2008. J. Feline Med. Surg. 11, 225–258.
Pedersen, N.C., Liu, H., Scarlett, J., Leutenegger, C.M., Golovko, L., Kennedy, H., Kamal, F.M., 2012. Feline infectious peritonitis: role of the feline coronavirus 3c gene in intestinal tropism and pathogenicity based upon isolates from resident and adopted shelter cats. Virus Res. 165, 17–28.
Poli, G., Kinter, A., Justement, J.S., Kehrl, J.H., Bressler, P., Stanley, S., Fauci, A.S., 1990. Tumor necrosis factor alpha functions in an autocrine manner in the induction of human immunodeficiency virus expression. Proc. Natl. Acad. Sci. U. S. A. 87, 782–785.
Porter, E., Tasker, S., Day, M.J., Harley, R., Kipar, A., Siddell, S.G., Helps, C.R., 2014. Amino acid changes in the spike protein of feline coronavirus correlate with systemic spread of virus from the intestine and not with feline infectious peritonitis. Vet. Res. 45, 49.
Reinhard, C., Shamoon, B., Shyamala, V., Williams, L.T., 1997. Tumor necrosis factor alpha-induced activation of c-jun N-terminal kinase is mediated by TRAF2. EMBO J. 16, 1080–1092.
Stoddart, C.A., Scott, F.W., 1989. Intrinsic resistance of feline peritoneal macrophages to coronavirus infection correlates with in vivo virulence. J. Virol. 63, 436–440.
Takano, T., Hohdatsu, T., Hashida, Y., Kaneko, Y., Tanabe, M., Koyama, H., 2007a. A "possible" involvement of TNF-alpha in apoptosis induction in peripheral blood lymphocytes of cats with feline infectious peritonitis. Vet. Microbiol. 119, 121–131.
Takano, T., Hohdatsu, T., Toda, A., Tanabe, M., Koyama, H., 2007b. TNF-alpha, produced by feline infectious peritonitis virus (FIPV)-infected macrophages, upregulates expression of type II FIPV receptor feline aminopeptidase N in feline macrophages. Virology 364, 64–72.
Takano, T., Azuma, N., Satoh, M., Toda, A., Hashida, Y., Satoh, R., Hohdatsu, T., 2009. Neutrophil survival factors (TNF-alpha, GM-CSF, and G-CSF) produced by macrophages in cats infected with feline infectious peritonitis virus contribute to the pathogenesis of granulomatous lesions. Arch. Virol. 154, 775–781.
Takano, T., Ohyama, T., Kokumoto, A., Satoh, R., Hohdatsu, T., 2011. Vascular endothelial growth factor (VEGF), produced by feline infectious peritonitis (FIP) virus-infected monocytes and macrophages, induces vascular permeability and effusion in cats with FIP. Virus Res. 158, 161–168.
Tracey, D., Klareskog, L., Sasso, E.H., Salfeld, J.G., Tak, P.P., 2008. Tumor necrosis factor antagonist mechanisms of action, a comprehensive review. Pharmacol. Ther. 117, 244–279.
Uchide, N., Ohyama, K., Bessho, T., Takeichi, M., Toyoda, H., 2012. Possible roles of proinflammatory and chemoattractive cytokines produced by human fetal membrane cells in the pathology of adverse pregnancy outcomes associated with influenza virus infection. Mediat. Inflamm. 2012, 270670.
Umehashi, M., Imamura, T., Akiyama, S., Tokiyoshi, S., 2002. Development and safety of mouse–cat chimeric antibody against the feline calicivirus. J. Japan Vet. Med. Assoc. 55, 293–297.
Vandenabeele, P., Declercq, W., Beyaert, R., Fiers, W., 1995. Two tumour necrosis factor receptors, structure and function. Trends Cell Biol. 5, 392–399.
Wang, J., Fu, Y.X., 2005. Tumor necrosis factor family members and inflammatory bowel disease. Immunol. Rev. 204, 144–155.
Yen, Y.T., Chen, H.C., Lin, Y.D., Shieh, C.C., Wu-Hsieh, B.A., 2008. Enhancement by tumor necrosis factor alpha of dengue virus-induced endothelial cell production of reactive nitrogen and oxygen species is key to hemorrhage development. J. Virol. 82, 12312–12324.
Hsieh, L.E., Lin, C.N., Su, B.L., Jan, T.R., Chen, C.M., Wang, C.H., Lin, D.S., Lin, C.T., Chueh, L.L., 2010. Synergistic antiviral effect of Galanthus nivalis agglutinin and nelfinavir against feline coronavirus. Antiviral Res. 88, 25–30.