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Effect of dual-subtype vaccine against feline immunodeficiency virus infection

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Abstract

Dual-subtype feline immunodeficiency virus (FIV) vaccine, consisting of inactivated cells infected with subtypes A (Petaluma strain) and D (Shizuoka strain), was developed and tested for its vaccine efficacy against FIV infection in specific pathogen free (SPF) cats. Animals were monitored for proviral DNA by FIV-specific PCR and for FIV-specific antibody profiles by ELISA and virus-neutralization assays. In addition, blood from challenged cats was inoculated into naive SPF cats to confirm the viral status of the vaccinated cats. All cats immunized with Petaluma vaccine alone were protected against homologous Petaluma challenge, but only one of four cats was protected against heterologous Shizuoka challenge. More importantly, all cats immunized with the dual-subtype vaccine were protected against both Petaluma and Shizuoka challenges. These results suggest that a multi-subtype vaccine approach may provide the broad-spectrum immunity necessary for vaccine protection against strains from different subtypes.

Keywords: Feline immunodeficiency virus; AIDS; Inactivated cell vaccine; Dual-subtype vaccine

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1. Introduction

Feline immunodeficiency virus (FIV) was originally isolated in 1986 from a feline leukemia virus (FeLV)-negative cat with chronic opportunistic infections (Pedersen et al., 1987). FIV is a typical lentivirus with ultrastructural morphology, structural protein profile, and reverse transcriptase requirement resembling human and simian immunodeficiency viruses (HIV and SIV). The FIV isolates from domestic cats have been classified into five subtypes, designated A [USA (California) and Europe], B [Japan and USA (except California)], C (Canada), D (Japan) and E (Argentina) by comparing the 684-nucleotide sequences (variable regions V3 through V5) of the FIV env gene (Kakinuma et al., 1995; Pecoraro et al., 1996; Sodora et al., 1994). The hallmark of FIV infection in domestic cats is the immunodeficiency syndrome caused by the severe loss of $CD4^+$ T cells with a concomitant decline in the CD4/CD8 ratio (Ackley et al., 1990; Barlough et al., 1991; Hoffmann-Fezer et al., 1992; Novotney et al., 1990; Torten et al., 1991). These similarities in immunopathogenesis of FIV and HIV have placed FIV infection of cats as an important small animal AIDS model for testing therapeutic and prophylactic approaches (Bendinelli et al., 1995; Gardner, 1991; Johnson et al., 1994; Pedersen et al., 1989).

A variety of vaccine approaches has been evaluated using the FIV model and these include fixed infected-cell, inactivated whole virus, subunit, and recombinant vaccines (Gonin et al., 1995; Hofmann-Lehmann et al., 1995; Hosie, 1994; Hosie and Flynn, 1996; Hosie et al., 1992, 1995; Lombardi et al., 1994; Lutz et al., 1995; Matteucci et al., 1996; Siebelink et al., 1995; Verschoor et al., 1995; Yamamoto et al., 1991b, 1993). Of these vaccine approaches, fixed infected cell and inactivated whole virus vaccines have successfully protected cats against homologous FIV challenge (Yamamoto et al., 1991b). These vaccines were prepared from FL-4 cells, a feline T cell line chronically infected with prototype subtype A strain, FIV Petaluma (FIVPet) (Yamamoto et al., 1991a). Vaccine protection was observed also against challenge with slightly heterologous FIV Dixon (FIVDix), a subtype A isolate which differed from FIVPet by 9% at the amino acid sequence of the major surface glycoprotein (Env) (Yamamoto et al., 1993). In contrast, protection was not achieved against FIV Shizuoka (FIVShi), a distinctly heterologous isolate which belonged to subtype D (approximately 20% Env amino acid difference) (Johnson et al., 1994). Furthermore, a similar preparation of inactivated whole virus vaccine was ineffective against FIV-UK-8 (FIVUK-8), a subtype A isolate which differed from FIVPet by 11% at Env amino acid sequence and in virus neutralization profile (Hosie et al., 1995). In a recent study, cats immunized with fixed FIV M2-infected MBM cells (IL-2-dependent feline T-lymphocyte line) were protected against homologous challenge with infected cat plasma (Matteucci et al., 1996). In another study, cats immunized three times with an inactivated whole virus vaccine retained vaccine-induced immunity for at least 8 months (Hosie et al., 1995). These studies clearly suggest that vaccine protection against homologous challenge is achievable. However, these studies also demonstrate the difficulty in achieving vaccine protection against distinctly heterologous strains.

One method to broaden vaccine immunity is to incorporate protective epitopes from multiple FIV subtypes. Since protective epitopes have yet to be defined for FIV, in this

study, whole virus antigens from two FIV subtypes has been used in place. To this end, fixed infected cell vaccine consisting of FIV strains from subtypes A and D has been developed. The overall objective of this study is to evaluate the immunogenicity and the protective efficacy of dual-subtype FIV vaccine against homologous FIV challenges. Such a study will be the first step in determining if the multi-subtype vaccine approach can be used to broaden vaccine efficacy.

2. Materials and methods

2.1. Preparation of FIV vaccines

FL-4 cells were used for the preparation of Petaluma vaccine and FeT-1 cells chronically infected with Shizuoka strain (FIV/Shizuoka-FeT cells) for the preparation of Shizuoka vaccine. Indirect membrane immunofluorescence assay using anti-FIV cat serum showed that 90% of the FL-4 cells and 60% of the FIV/Shizuoka-FeT cells were positive for FIV antigen. As previously reported (Yamamoto et al., 1991b), these cells were inactivated with 1.25% paraformaldehyde at 4°C for 24 h and washed with PBS three times, before preparing fixed infected cell vaccines. Fixed infected cells of Petaluma vaccine were mixed with an equal volume of fixed infected cells of Shizuoka vaccine and used as a dual-subtype vaccine. As a single-subtype vaccine of Petaluma strain, fixed infected FL-4 cells were used.

2.2. Vaccination and challenge infection of cats

Twenty-four specific-pathogen free (SPF) cats aged 3 months were divided into three groups (eight cats in each group): challenge control group, single-subtype vaccine (Petaluma vaccine) group, and dual-subtype vaccine (Petaluma + Shizuoka vaccine) group. The single- and dual-subtype vaccine groups were subcutaneously inoculated with each cell vaccine (2.5×10^7 cells) and adenyl–muramyl dipeptide (A–MDP) adjuvant (200 μg per dose) (Byars and Allison, 1987) three times at 4-week intervals. The challenge control group was inoculated with A–MDP adjuvant alone. Two weeks after the final vaccination, the cats were intraperitoneally challenged with Petaluma strain or Shizuoka strain at 10 50% animal infectious doses (ID_{50}) according to the categories shown in Table 1. The challenge viruses of Petaluma strain and Shizuoka strain had been proliferated in primary peripheral blood lymphocytes (PBL) of SPF cats.

2.3. Vaccine efficacy against FIV infection

The effects of vaccination on challenge were evaluated by detection of FIV proviral DNA in PBL by the polymerase chain reaction (PCR). PBL (1×10^6 cells) and interleukin-2 (IL-2)-dependent feline T lymphocyte cell line (Kumi-1 cells) (Hohdatsu et al., 1996) that is highly sensitive to FIV were co-cultured for 15 days. DNA isolated from the cultured cells was used for PCR. The FIV gag region (position 1036–1345) was amplified by the method of Hohdatsu et al. (1992b). The PCR was performed by

Table 1
Detection of FIV and FIV proviral DNA from vaccinated cats after FIV challenge

Cat category and no.		Weeks after challenge									Detection of FIV by in vivo assay
		0	4	9	13	16	18	23	25	30 w	
Challenge control group											
Petaluma challenge	1	−	+	ND	+	+	+	+	ND	ND	ND
	2	−	+	+	+	+	+	+	ND	ND	ND
	3	−	+	+	+	+	+	+	ND	ND	ND
	4	−	+	+	+	+	+	+	ND	ND	ND
Shizuoka challenge	5	−	+	+	+	−	+	+	ND	ND	ND
	6	−	−	−	+	+	+	+	ND	ND	ND
	7	−	+	+	+	+	+	+	ND	ND	ND
	8	−	+	+	−	+	+	+	ND	ND	ND
Petaluma vaccine group											
Petaluma challenge	9	−	−	−	−	−	−	−	−	−	negative
	10	−	−	−	−	−	−	−	−	−	negative
	11	−	−	−	−	−	−	−	−	−	negative
	12	−	−	−	−	−	−	−	−	−	negative
Shizuoka challenge	13	−	−	ND	−	−	−	−	−	−	negative
	14	−	+	ND	+	+	+	+	+	+	positive
	15	−	−	ND	+	+	+	+	+	+	positive
	16	−	−	ND	−	−	−	−	+	−	positive
Petaluma + Shizuoka vaccine group											
Petaluma challenge	17	−	−	−	−	−	−	−	−	−	negative
	18	−	−	−	−	−	−	−	−	−	negative
	19	−	−	−	−	−	−	−	−	−	negative
	20	−	−	−	−	−	−	−	−	−	negative
Shizuoka challenge	21	−	−	−	−	−	−	−	−	−	negative
	22	−	−	−	−	−	−	−	−	−	negative
	23	−	−	ND	−	−	−	−	−	−	negative
	24	−	−	−	−	−	−	−	−	−	negative

+, PCR positive.
−, PCR negative.
ND, not done.

two-step amplification using the same primer pair (double PCR). As an in vivo assay to evaluate the effects of vaccination, 3 ml blood was collected 30 weeks after challenge and used to inoculate other SPF cats, which were examined for seroconversion to FIV.

2.4. Serological assays

Anti-FIV antibody was measured by enzyme-liked immunosorbent assay (ELISA) using core and transmembrane (TM) antigens of Petaluma virus and by the neutralization (NT) test using Kumi-1 cells. As the FIV core antigen, P25 antigen electrophoretically purified from detergent-disrupted FIV virions was used. As the TM antigen, a synthetic peptide was used. The amino acid sequence of the TM peptide (694-705) was QELGCNQNQFFC (Yamamoto et al., 1993). ELISA was performed by the method of Hohdatsu et al. (1992a). The NT test was performed using 96-well flat-bottomed

microplates. The NT titer for Petaluma strain was determined using the culture supernatant of FL-4 cells, and that for Shizuoka strain using Shizuoka virus after four passages in Kumi-1 cells. Serially diluted (2^n) test serum samples (50 μl) were reacted with an equal volume of 200 $TCID_{50}$ virus suspension at 37°C for 1 h, and 25 μl of 5×10^5 Kumi-1 cells was added. The mixture was allowed to react at 37°C for 1 h for viral adsorption. Then 175 μl of growth medium per well was added for incubation at 37°C. One day after incubation, half of the medium (150 μl/well) was replaced with fresh growth medium. Thereafter, the growth medium was similarly exchanged every 3 days. The supernatants of the test cultures at day 13 of incubation were tested by ELISA (FIV antigen detection kit; IDEXX, Portland, ME) for the production of FIV P25 antigen.

3. Results

In the vaccination group, all cats showed increases in antibodies against P25 antigen and TM antigen after three vaccinations (Figs. 1 and 2). After the three vaccinations, the

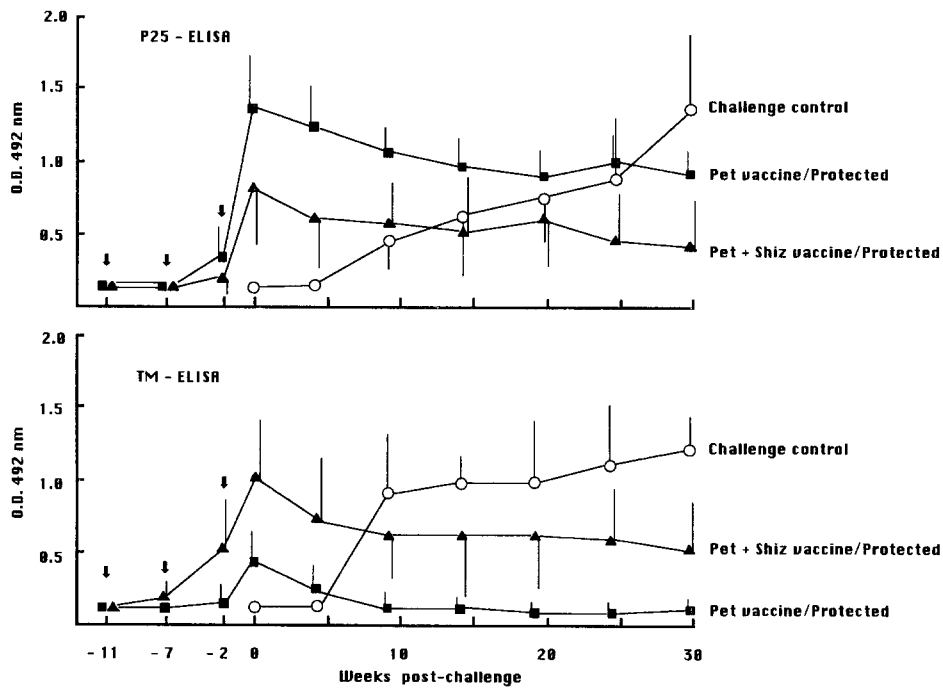

Fig. 1. Antibody levels in vaccinated and control cats before and after FIV Petaluma challenge. Sera collected at the indicated times were tested by ELISA using electrophoretically purified P25 antigen or TM peptide. Arrows indicate vaccine doses. ■, Means ± standard deviation (SD) of ELISA O.D. values in Petaluma vaccine immunized cats (No. 9–12) that were protected against challenge with Petaluma virus. ▲, Means ± SD of ELISA O.D. values in Petaluma + Shizuoka vaccine immunized cats (No. 17–20) that were protected against challenge with Petaluma virus. ○, Means ± SD of ELISA O.D. values in adjuvant control cats (No. 1–4) challenged with Petaluma virus.

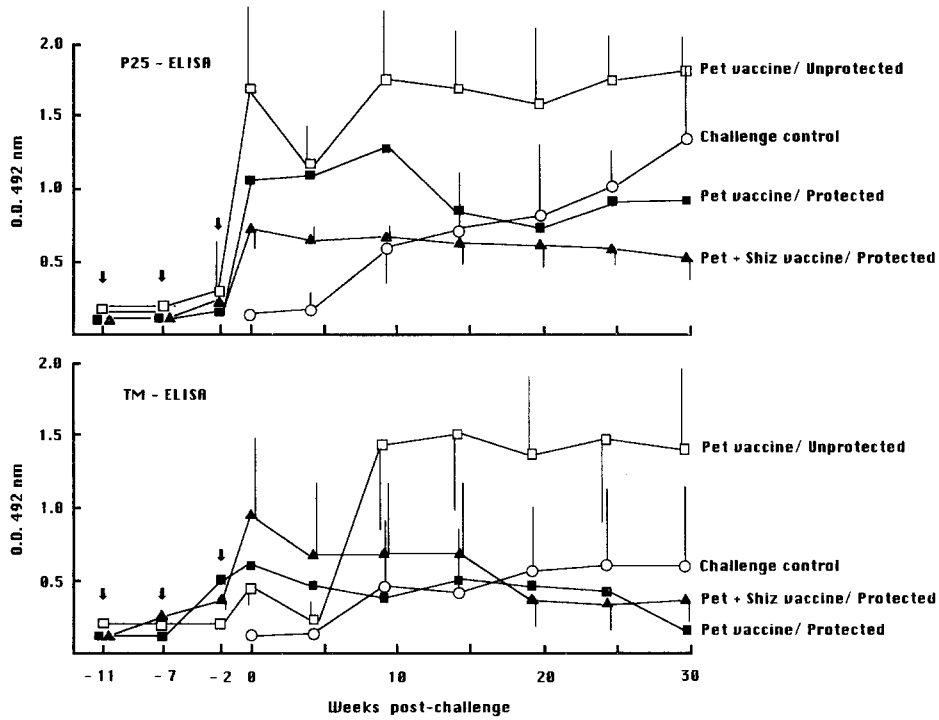

Fig. 2. Antibody levels in vaccinated and control cats before and after FIV Shizuoka challenge. Sera collected at the indicated times were tested by ELISA using electrophoretically purified P25 antigen or TM peptide. Arrows indicate vaccine doses. ■, ELISA O.D. value in a Petaluma vaccine immunized cat (No. 13) that was protected against challenge with Shizuoka virus. □, Means ± SD of ELISA O.D. values in Petaluma vaccine immunized cats (No. 14–16) that were not protected against challenge with Shizuoka virus. ▲, Means ± SD of ELISA O.D. values in Petaluma + Shizuoka vaccine immunized cats (No. 21–24) that were protected against challenge with Shizuoka virus. ○, Means ± SD of ELISA O.D. values in adjuvant control cats (No. 5–8) challenged with Shizuoka virus.

cats were challenged with Petaluma or Shizuoka strain according to the categories shown in Table 1. In the challenge control group inoculated with A–MDP adjuvant alone, FIV proviral DNA was detected in PBL 4 weeks after challenge in all cats except Cat No. 6 (Table 1), and the ELISA O.D. values for P25 antigen and TM peptide could be detected by 9 weeks post challenge and continued to rise (Figs. 1 and 2). In the Petaluma vaccine group, no FIV proviral DNA was detected in the four cats challenged with Petaluma strain (Cat No. 9–12). However, FIV proviral DNA was detected in three of the four cats challenged with Shizuoka strain (Cat No. 14–16), showing 25% protection. In the dual subtype vaccine group, no FIV proviral DNA was detected in any cat challenged with the Petaluma or Shizuoka strain. To confirm the PCR detection of proviral DNA, blood taken 30 weeks after challenge was inoculated into other SPF cats, which were examined for the production of anti-FIV antibody due to FIV infection. An increase in anti-FIV antibody was confirmed only in cats inoculated with blood obtained from Cat No. 14, 15, and 16 positive for PCR (Table 1). In these cats (Pet vaccine/un-

Table 2
FIV neutralizing antibodies in vaccinated cats on the day of challenge

Cat category and no.		Neutralizing antibody titers		Protection
		Petaluma	Shizuoka	
Petaluma vaccine group				
Petaluma challenge	9	640	< 10	Yes
	10	40	< 10	Yes
	11	40	< 10	Yes
	12	40	< 10	Yes
Shizuoka challenge	13	20	< 10	Yes
	14	20	< 10	No
	15	80	< 10	No
	16	< 10	< 10	Yes
Petaluma + Shizuoka vaccine group				
Petaluma challenge	17	640	< 10	Yes
	18	20	< 10	Yes
	19	20	< 10	Yes
	20	< 10	< 10	Yes
Shizuoka challenge	21	80	< 10	Yes
	22	640	< 10	Yes
	23	< 10	< 10	Yes
	24	< 10	< 10	Yes
Positive control[a]				
	anti-Petaluma	320	< 10	–
	anti-Shizuoka	< 10	40	–
negative control				
	SPF-1	< 10	< 10	–
	SPF-2	< 10	< 10	–

[a]Serum of an experimental infected cat.

protected), the mean ELISA O.D. values for P25 antigen and TM peptide remained high until at least 30 weeks after challenge (Fig. 2). On the other hand, the mean ELISA O.D. values for P25 antigen and TM peptide gradually decreased in Cat No. 13 in the same group as well as in the protected cats in other groups (Figs. 1 and 2). Table 2 shows the neutralizing antibody titers for Petaluma and Shizuoka strains on the day of challenge. The neutralizing antibody titer for Petaluma strain was 1:20–1:640 in all cats except Cat No. 16, 20, 23, and 24, but that for Shizuoka strain was < 1:10 in all cats including those in the Petaluma + Shizuoka vaccine group.

4. Discussion

Inactivated vaccines derived from FL4 cells have been reported to be ineffective against challenge with Shizuoka strain in which 20% of the amino acid sequences in the V3–V5 region differs (Johnson et al., 1994). In this study, the inactivated cell vaccine

showed 100% protection against challenge with Petaluma virus (a homologous strain), but only a 25% against Shizuoka virus (a heterologous strain). However, when an inactivated cell vaccine of Shizuoka strain was added to this inactivated cell vaccine of FL4 cells, 100% protection was also observed against challenge with Shizuoka strain. These results suggest the usefulness of FIV multi-subtype vaccines. In this study, we performed not only PCR, which is frequently used for the evaluation of the effects of vaccines and serological analysis but also an in vivo assay by passage of blood 30 weeks after challenge to other cats. This in vivo assay indicated the presence of virus in cat No. 16, which was positive by PCR at only 25 weeks. In the FIV system, ID_{50} measured using cats is about 10–100 times as high as $TCID_{50}$ measured using feline T-lymphocytes (J.K. Yamamoto, personal communication). This suggests that the in vivo assay is more sensitive than the PCR used in these studies and useful for evaluating the effects of FIV vaccines.

Induction of FIV-specific humoral immune responses such as neutralizing antibody and cell-mediated immune responses such as $CD8^+$ cytotoxic T lymphocyte (CTL) responses has been reported to be important for protection against FIV infection (Flynn et al., 1996; Hosie and Flynn, 1996; Yamamoto et al., 1991b, 1993). However, the types of immune effectors responsible for the vaccine-induced protection observed in this study remain unclear. As shown in Table 2, the neutralizing antibody titer for Shizuoka virus on the day of challenge was $< 1{:}10$ even in the cats immunized with the Petaluma + Shizuoka vaccine. The protection against challenge with Shizuoka virus was not correlated with the neutralizing antibody titer. The neutralizing antibody for Petaluma virus was detected in 12 vaccinated cats. However, Cat No. 20 showing a neutralizing antibody titer $< 1{:}10$ was also protected against challenge with Petaluma virus. Studies have shown inconsistent results on the correlation between protection against FIV infection and the neutralizing antibody titer at the time of challenge (Hosie and Flynn, 1996; Lombardi et al., 1994; Matteucci et al., 1996; Siebelink et al., 1995; Yamamoto et al., 1991b, 1993). Hosie and Flynn (1996) found that protection against challenge was correlated with the neutralizing antibody titer measured using a CrFK cell system but not with that measured in a NT assay based on the feline T cell line MBM. The neutralizing antibody titer measured using a CrFK cell system was reported to be higher than that measured using a feline T cell system (Baldinottl et al., 1994). In the feline T cell system, the neutralizing antibody titer was reported to depend on the passage history of the virus used (Baldinottl et al., 1994). Though the neutralizing antibody titer differs according to the methods used, protection against FIV infection is possible by passive antibody transfer, suggesting the importance of humoral immunity in FIV infection (Hohdatsu et al., 1993; Pu et al., 1995). To evaluate the relationship between the effects of FIV vaccines and neutralizing antibody, further studies are needed on the effects of different neutralizing antibody measurement systems.

In an SIV vaccine study, the protection observed in macaques immunized with SIV grown in human cells appears to be related more closely with an immune response directed to cell membrane antigens rather than a virus-specific immune response (Stott, 1991). However, in the FIV vaccine system, protection was reported to result from virus-specific immune responses and is not associated with immune responses against vaccine-derived cell membrane antigens (Hohdatsu et al., 1993; Hosie et al., 1995;

Matteucci et al., 1996). In this study also, antibodies against FeT-1 cell membrane antigens as well as virus-specific antibody were detected after vaccination (data not shown).

FIV-specific CTL activity has been detected 6–9 weeks after experimental FIV infection or in vaccinated cats that were protected against challenge (Flynn et al., 1996; Hosie and Flynn, 1996; Song et al., 1992; Yamamoto et al., 1991b). Gag-specific CTL activity as well as env-specific CTL activity have been detected (Flynn et al., 1995; Hosie and Flynn, 1996). This finding suggests that unlike neutralizing epitopes, CTL epitopes are present not only in the FIV env region but also in the gag region. If gag-specific CTL is effective for protecting against FIV infection, induction of marked gag-specific CTL activity may allow production of FIV vaccines that are also effective against infection with more FIV strains. However, CTL epitopes resulting in recovery from FIV infection have not been identified. Since FIV-specific CTL activity in this study was not examined, its association with protection could not been clarified.

5. Conclusion

In conclusion, the mechanism of protection by FIV inactivated vaccines is still unclear, but a dual-subtype vaccine, produced by the combination of paraformaldehyde-fixed cells infected with Petaluma or Shizuoka strain, showed 100% protection against challenge of each virus. These results suggest the usefulness of multi-subtype FIV vaccines. All of the subtypes of FIV isolates have geographic-specificity. Selection of FIV strains for multi-subtype vaccines considering this district-specificity may be important to produce more effective FIV vaccines. Studies on multi-subtype FIV vaccines will be also useful for the development of multi-subtype HIV vaccines.

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