Effects of tacrolimus on infection of Friend murine leukemia virus to *Fv*-4 gene heterozygous mice[1]

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ABSTRACT

AIM: To investigate the effect of tacrolimus (FK506) on the infection of Friend murine leukemia virus (Friend MuLV) *in vivo*. **METHODS:** Three kinds of mice were used including Friend MuLV-sensitive BALB/c mice, Friend MuLV-resistant *Fv*-4 gene-homozygous mice (Fv-4 mice), and Friend MuLV-resistant *Fv*-4 gene-heterozygous mice (F1 mice). Tacrolimus was administrated ip to those mice in every 2 d. Those treated mice were inoculated ip with Friend MuLV once on d 3. The symptoms and viral proliferations in those mice were observed to recognize the Friend MuLV infection. The expression and genotype of *Fv*-4 gene that resistant against the infection of Friend MuLV were analyzed to confirm the genomic background and related mechanism of the resistance. **RESULTS:** BALB/c mice and F1 mice, but not Fv-4 mice, appeared obvious early death, spleenomegaly, and viral proliferation after both treatments of viral inoculation and tacrolimus administration, whereas the expression and genotype of *Fv*-4 gene was not changed in F1 mice and Fv-4 mice with treatment of tacrolimus. Compared to the virus-inoculated control, the Friend MuLV-sensitivity of tacrolimus-treated BALB/c mice and the Friend MuLV-resistance of tacrolimus-treated Fv-4 mice were the same as the controls, but only F1 mice became the symptoms and viral proliferation after both treatments. It suggested the Friend MuLV-resistant F1 mice could be converted to be Friend MuLV-sensitive by treatment of tacrolimus, and this conversion was not depended on the expression and genotype of *Fv*-4 gene. **CONCLUSION:** Tacrolimus could not inhibit the infection of Friend MuLV in all mice, furthermore, it could enhance the infection of Friend MuLV in F1 mice. The enhancement may be related to the immunosuppressive effect of tacrolimus.

INTRODUCTION

Tacrolimus (FK506), an immunosuppressive drug, has been widely used in the prevention of the organ transplantation rejection reaction and the therapy of autoimmune disease[1], and so on. During the resent years, many researches have indicated that this drug might have extensive potential effects in microbial infections, for example, promoting most bacterial and viral infections[2-5], but inhibiting the infections of some viruses, such as human immunodeficiency virus (HIV), cytomegalovirus (CMV), *etc*[6,7].

In the infection of human retrovirus HIV, tacrolimus showed inhibitory effect *in vitro* by inhibiting the activity of viral reverse transcriptase and the apoptosis induced by viral protein[8-10]. However, the

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inhibitory activity of tacrolimus has not been observed in the infection of Friend MuLV, an animal retrovirus, *in vitro* in our previous experiments (no published).

To further investigate the effects of tacrolimus on Friend MuLV *in vivo*, Friend MuLV-sensitive BALB/c mice, Friend MuLV-resistant *Fv*-4 homozygous mice, and Friend MuLV-resistant *Fv*-4 heterozygous mice were employed in this study. The symptoms and viral proliferations in the experimental mice were observed, and the genotype and expression of *Fv*-4 gene were detected to identify the genomic background and related resistance of the mice.

MATERIALS AND METHODS

Drug Tacrolimus was offered kindly by FUJISAWA Pharmacological Co, Japan. Tacrolimus was administerted (5.0 mg/kg, ip) to the mice in every 2 d.

Virus Friend MuLV was propagated in the sensitive BALB/c mice. The supernatant of spleen homogenant of infected mice was prepared as Friend MuLV pool. The viral titer was tested by UV-XC test on SC-1 cells as about 5.2 log focus forming unit [FFU] per 0.2 mL.

Animal Friend MuLV-sensitive BALB/c mice, Friend MuLV-resistant *Fv*-4 homozygous mice (Fv-4 mice), and Friend MuLV-resistant *Fv*-4 heterozygous mice (F1 mice) were used. The F1 mouse was the offspring of Fv-4 mouse and BALB/c mouse. The mice of the infection group were inoculated ip with 0.5 mL of viral pool once on d 3 after the administration of tacrolimus.

Determination of the infection of Friend MuLV The early death, spleenomegaly, and virus proliferation were observed as the parameters of Friend MuLV infection in these mice after the treatment of virus inoculation and tacrolimus administration. The viral proliferation among the spleenocytes was measured by UV-XC test on SC-1 cells, in brief, subconfluent SC-1 cells were mixed with 0.2 mL of spleen homogenate supernatant in 55-mm dishes, the culture fluid was removed on d 5, the cells were irradiated with ultraviolet (60 erg/mm^2 per second) for about 20 s, maintenance medium contained 1×10^6 of XC cells was added, the cultures were fixed with methanol and stained with hematoxylin on d 3 after addition of the XC cells. Plaques representing the virus quantity were read with dissection microscope.

Identification to *Fv*-4 genotype of the mice treated with Tacrolimus The *Fv*-4 genotype of the mice was identified with genomic Southern blot to confirm the genomic background of the mice. Briefly, 5 μg of genomic DNA extracted from mouse spleen was electrophoresesed through 1 % agarose gel after digestion with *EcoR I*, then transferred to nitrocellulose filters and hybridized with a [^{32}P] labeled *Fv*-4 DNA probe (700 bp) which contains the sequence of *Fv*-4 gene and linked cellular DNA partly[11].

Detection the expression of *Fv*-4 gene in tacrolimus-treated mice spleenocytes To determine changes of *Fv*-4 gene expression on the surface of the tacrolimus-treated mice spleenocytes, the spleenocytes of F1 mice and Fv-4 mice were incubated with anti-Fv-4 serum and FITC-conjugated IgG (rabbit against mouse), respectively, then analyzed by flow cytometry with indirect staining.

RESULTS

Early death of the Friend MuLV inoculated mice treated with Tacrolimus Three kinds of mice were treated with only Friend MuLV, only tacrolimus, and both Friend MuLV and tacrolimus, respectively. The early death of BALB/c mice treated with both Friend MuLV and tacrolimus was the same as the Friend MuLV-infected BALB/c mice control. Different from the Friend MuLV-infected F1 mice, most of F1 mice treated with both Friend MuLV and tacrolimus died in 28 d. No Fv-4 mice died in this experiment (Tab 1).

Spleenomegaly of the Friend MuLV-inoculated mice treated with tacrolimus To confirm inducement of erythroleukemia in F1 mice treated with both Friend MuLV and tacrolimus, the spleen weights of at least 3 dead F1 mice in each group was observed and compared with control. The spleenomegaly could be observed among the dead F1 mice, the spleen weights were the same as that of BALB/c mice after Friend MuLV inoculation only or treatments with both viruses and Tacrolimus. No spleenomegaly observed in Fv-4 mice with or without any treatment (Tab 2).

Viral proliferation in the Friend MuLV-inoculated mice treated with tacrolimus To measure the viral proliferation, the supernatant of spleen homogenate were prepared from each group of mice, and detected with UV-XC test on SC-1 cells, respectively. After the administration of tacrolimus, high viral titer could be measured in Friend MuLV-inoculated BALB/c and F1 mice, but not in Fv-4 mice. Among the control group, only BALB/c mice showed high virus proliferation (Tab 2).

Tab 1. Death of the Friend MuLV inoculated mice after treatment of tacrolimus.

Mice		Treatments		Number of dead mice after treatment		
Strain	Genotype	Fr MuLV	Tacro-limus	14 d	21 d	28 d
BALB/c	*Fv*-4 $^{-/-}$	–	–	0/10	0/10	0/10
		–	+	0/12	0/12	0/12
		+	–	1/12	8/12	12/12
		+	+	2/16	11/16	16/16
F1	*Fv*-4 $^{r/-}$	–	–	0/10	0/10	0/10
		–	+	0/12	0/12	0/12
		+	–	0/15	0/15	0/15
		+	+	1/17	8/17	11/17 [a]
Fv-4	*Fv*-4 $^{r/r}$	–	–	0/10	0/10	0/10
		–	+	0/11	0/11	0/11
		+	–	0/11	0/11	0/11
		+	+	0/12	0/12	0/12

[a] All of the 6 remained F1 mice treated with Fr MuLV and Tacrolimus died within the following 60 d.

Tab 2. Infection of Friend MuLV to the mice after the treatment of tacrolimus. Mean±SD. $^{c}P<0.01$ *vs* A and B control groups of BALB/c mice, but not C control group of BALB/c mice. $^{f}P<0.01$ *vs* all control groups of F1 mice.

Mice		Treatment		Infectious sign of the mice	
Strain	Genotype	Fr MuLV	Tacro-limus	Spleen weight/mg	Viral titer[1)]
BALB/c	*Fv*-4 $^{-/-}$	–	–	100±9	N
		–	+	106±11	N
		+	–	650±50	4.5±0.5
		+	+	690±60^{c}	5.0±0.4^{c}
F1	*Fv*-4 $^{r/-}$	–	–	110±5	N
		–	+	106±10	N
		+	–	108±7	N
		+	+	480±60^{f}	3.0±0.5^{f}
Fv-4	*Fv*-4 $^{r/r}$	–	–	102±11	N
		–	+	106±8	N
		+	–	110±7	N
		+	+	105±8	N

1) log FFU per 0.2 mL. FFU: focus forming unit; N: negative.

The *Fv*-4 genotype of the mice treated with tacrolimus Genomic Southern blot was employed to check the genomic background of the mice treated with Friend MuLV using *Fv*-4 gene cellular flanking DNA probe. The result showed that a 1.5 -kb fragment could be observed in the DNA extraction from BALB/c mice (*Fv*-4$^{-/-}$), while a 5.2-kb fragment was detected in the DNA extraction from Fv-4 mice (*Fv*-4$^{r/r}$). Both fragments could be observed in the DNA extraction from F1 mice (*Fv*-4$^{r/-}$) (Fig 1).

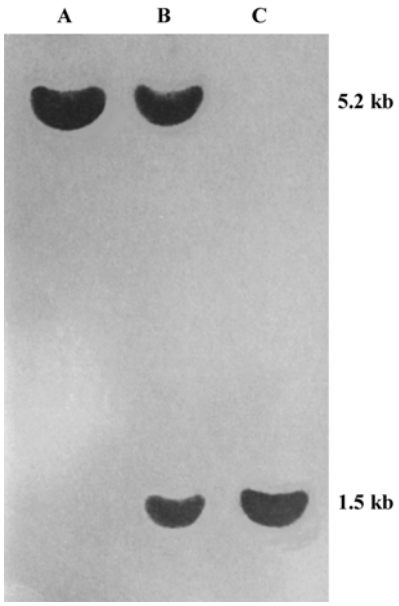

Fig 1. Identification of *Fv*-4 genotype of the mice treated with tacrolimus. A) *Fv*-4 gene homozygous mouse (Fv-4 mouse); B) *Fv*-4 gene heterozygous mouse (F1 mouse); C) BALB/c mouse.

Expression of *Fv*-4 gene in the spleenocytes of the tacrolimus-treated mice To investigate the related mechanism, the expression of *Fv*-4 gene in the spleenocytes from F1 mice and Fv-4 mice treated with tacrolimus were detected by Flow cytometry analysis. The data showed that the amount of *Fv*-4 protein on the surface of F1 mice spleenocytes was about 10-fold lower than that of Fv-4 mice. No obvious change of expression of *Fv*-4 gene was observed in the F1 mice and Fv-4 mice with or without the treatment of tacrolimus (Fig 2).

CONCLUSION

Friend MuLV, a NB tropic and ecotropic mouse retrovirus, can infect BALB/c mouse and show obvious viral proliferation and erythroleukemia with symptoms of early death and spleenomegaly, *etc*. The infection of Friend MuLV was influenced with many host genes, such as *Fv-1*, *Fv-2*, and *Fv*-4 gene. The resis-

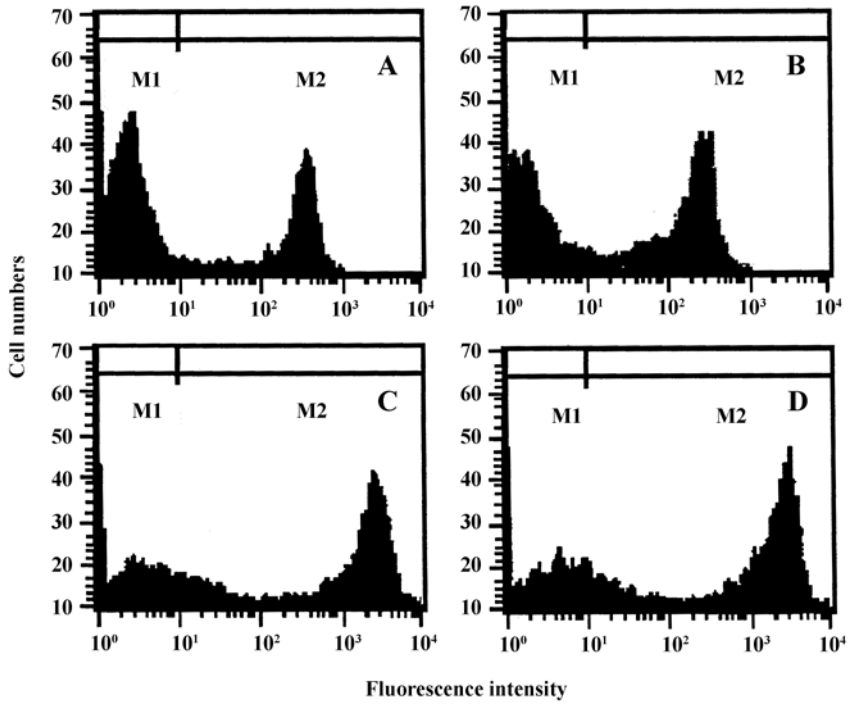

Fig 2. Detection of *Fv*-4 gene expression product on the spleenocyte from the mouse by Flow cytometry with indirect staining method. (A) Normal *Fv*-4 gene heterozygous mouse (F1 mouse); (B) Tacrolimus treated F1 mouse; (C) Normal *Fv*-4 gene homozygous mouse (Fv-4 mouse); (D) Tacrolimus treated Fv-4 mouse.

tance effect of *Fv*-4 gene against Friend MuLV had been proved in the *Fv*-4 gene transgenic mouse or congenic mouse (Fv-4 mouse)[12,13]. Therefore, the infection of Friend MuLV was used usually as the retrovirus infection model for searching the potential medicine or establishing new treatment method.

According to the inhibitory effect of tacrolimus on the infection of human retrovirus, the effect of tacrolimus on the infection of Friend MuLV was investigated to confirm the anti-retrovirus activity of the drug in this experiment. Whereas the results showed that tacrolimus could not inhibit or promote the infection of Friend MuLV in the BALB/c mouse and Fv-4 mouse, but converted the F1 mouse from Friend MuLV-resistant to Friend MuLV-sensitive, and showed the parameters of Friend MuLV infection and occur of erythroleukemia in the tacrolimus-treated F1 mouse. It suggested that tacrolimus had different effects on retrovirus infection through different mechanisms.

As a result of tacrolimus promoting the infection of Friend MuLV in the *Fv*-4 gene heterozygous mice, the inhibition effect of tacrolimus on retroviral reverse transcriptase activity was not measured in this experiment, but the *Fv*-4 genotype and expression could be detected with genomic Southern blot and flow cytometry in the tacrolimus-treated mice, suggesting that the treatment of tacrolimus could not interfere the genotype and expression of the *Fv*-4 gene in the mice. Since the immunosuppressive properties of tacrolimus is dependent on its ability of blocking the transcription of lymphokine gene of activated-T cells through the formation of complex with FK506-binding protein 12, which inhibits the phosphatase activity of calcineurin[14-16], the mechanism of tacrolimus on the infection of Friend MuLV may be related with immunosuppressive activity rather than the regulation of *Fv*-4 gene expression.

Because of the controversy activities of it, tacrolimus should be carefully used in clinical application to avoid the potential dangers.

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