LETTER TO THE EDITOR

Endogenous feline retrovirus RD-114 does not elicit neutralizing antibodies in dogs

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At least two retroviruses, endogenous feline leukemia virus (FeLV) and RD-114 virus, are assumed to be integrated in the genome of domestic cats (*1*). Several feline cell lines have been shown to produce RD-114-related viruses (*2*). In Japan, feline cell lines are widely used to produce vaccines for dogs, particularly against canine parvovirus. The level of infectious RD-114 virus found in canine virus vaccines has been reported to be as high as 1,800 $TCID_{50}$/ml (*3*). In earlier work, we confirmed the contamination of canine vaccines with the infectious RD-114 virus (*4*). In preliminary studies, *in vivo* infectivity, acute and sub-acute pathogenicity, and propagation of RD-114 virus by experimental infection in SPF dogs, were assessed. Over the course of these studies (51 days), none of the dogs inoculated with RD-114 virus showed clinical signs, significant increases in rectal temperature or abnormal blood characteristics (*4*). Importantly, infectious RD-114 virus was not recovered from leukocytes (*4*).

Neutralizing antibodies against feline immunodeficiency virus (FIV) were demonstrated in cats naturally or experimentally infected with FIV, using a focus reduction assay in Crandell-Rees feline kidney cells (*5*). Neutralizing antibodies are an important component of protection against many viral pathogens, such as FeLV, and their appearance generally correlates with the anti-viral immunity (*6*, *7*). Neutralization assays are often considered the standard, against which other serologic assays should be measured (*8*). In our study, reported here, we investigated the presence of neutralizing antibodies against RD-114 virus.

Nine SPF beagles (10-month-old) were divided into three groups. Dogs were inoculated subcutaneously in the neck with 1 ml of either RD-114 virus stock ($10^{5.3}$ $TCID_{50}$/ml), inactivated RD-114 virus, or cell culture medium (negative control) (*4*). Blood samples were collected prior to inoculation (day 0), and at 4 and 7 weeks post inoculation (p.i.). Sera were heat-inactivated at 56°C for 30 min, and aliquots stored at -80°C.

Positive control sera were collected after 2 weeks from guinea pigs (Hartley, 300 g, females) immunized four times at 2-week intervals with 1 ml of RD-114 virus stock. Negative control sera were collected from untreated Guinea pigs. Sera were heat-inactivated and stored at -80°C. Guinea pigs were euthanized and whole blood, sternal bone marrow, axillary lymph nodes, liver, heart, spleen, mesenteric lymph node, and ovaries were collected for PCR assays (*9*).

Neutralization assays were performed using the LacZ marker rescue assay procedure (*9*). Serial four-fold dilutions of sera were incubated with an equal volume of RD-114 virus. After incubation at 37°C/5% CO_2 for 60 min, TE671 (LacZ) cells were inoculated with the serum-virus mixture. Inoculated cells were passaged 1 day later and supernatants used to infect TE671 cells (*9*). Virus in the culture supernatant was titrated on TE671 cells. A 50% reduction in the number of virus-positive cells compared with controls was considered the neutralization titer of the antibody. The neutralizing titer was expressed as the reciprocal of the endpoint serum dilu-

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Abbreviations: FeLV = feline leukemia virus; FIV = feline immunodeficiency virus; p.i. = post inoculation

Table 1. Neutralizing antibody titers in dogs and guinea pigs against RD-114 virus

Dogs infected with	Guinea pigs infected with	Neutralizing antibody titer (weeks p.i.)		
		0	4	7
Virus RD-114		<2	<2	<2
Virus RD-114 inactivated		<2	<2	<2
Cell culture medium (negative control)		<2	<2	<2
	Virus RD-114(positive control)		16	
	Nil (negative control)		<2	

tion. The globulin concentration was determined in blood samples obtained from dogs in each group.

In the neutralization assay, serum from any of the nine dogs did not indicate the presence of neutralizing antibodies. However, positive control sera from guinea pigs showed a neutralizing antibody titer of 16 (Table 1). RD-114 provirus was not detected by PCR in genomic DNA extracted from guinea pig tissues. The globulin concentration in each dog was within normal ranges, with no significant difference seen in the three groups.

Sera from dogs experimentally infected with RD-114 did not show any evidence of neutralizing antibodies against the virus. Guinea pigs exhibited a greater immune response compared with dogs. Neutralizing antibodies were detected using the aforementioned neutralization assay, demonstrating its applicability. As the PCR results from guinea pig tissues were negative, this suggested that RD-114 virus did not exist as a provirus in these animals. Guinea pigs did not show any clinical signs of infection; therefore, RD-114 virus likely does not infect or is not transmissible to guinea pigs.

In contrast, neutralizing antibodies were produced in guinea pigs. However, this was likely a product of hyperimmunization in a small animal with a large amount of antigen and frequent inoculation. Thus, if dogs were inoculated with a large enough dose of RD-114 virus or repeatedly inoculated, neutralizing antibodies would likely be produced. It is important to note that the amount of inoculum used in this study was approximately 1,000-fold greater than live RD-114 virus typically found in manufactured canine vaccines. Therefore, neutralizing antibodies are not likely to be detected using this assay, even if dogs were inoculated every year with canine vaccines containing live virus.

Because neutralizing antibodies were not detected in the virus- or inactivated virus-infected groups, we are unable to say whether RD-114 virus infected or propagated in dogs. If neutralizing antibodies were shown to increase in the virus group as opposed to the inactivated virus group, this would suggest that neutralizing antibodies were induced as a result of virus propagation. Immunoglobulin M is produced early during infection, is detectable within a few days from the onset of the illness and is more virus-specific than immunoglobulin G (*10*). Our data related to the globulin levels suggest that a significant antibody response did not occur in inoculated dogs. However, RD-114 virus has been previously shown to efficiently infect and propagate in canine cells *in vitro* (*11*). The possibility that RD-114 virus infects dogs *in vivo* under other conditions cannot be excluded.

In our study, we have shown that the RD-114 virus has little or no virulence in dogs. Thus, it is difficult to ascertain the risk of RD-114 virus in canine vaccines causing adverse events. It is clear that RD-114 virus is introduced into vaccine batches using raw material of feline origin (*12*). Improvement in the manufacture of these vaccines is recommended, where the replacement of cell lines, introduction of good manufacturing practices allowing clearance of the virus, and the inactivation of RD-114 virus might limit the potential risks of canine infection.

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