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Studies with Canine Sera That Contain Antibodies Which Recognize Human Immunodeficiency Virus Structural Proteins[1]

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Abstract

In a serological survey, using the immunoblotting technique, we found that substantial numbers of dog sera from both normal and diseased dogs, including dogs with neoplasia, reacted with one or more human immunodeficiency virus (HIV) recombinant proteins. A total of 144 dog sera were tested, and 72 (50%) of them reacted with one or more HIV recombinant structural proteins. Ten dog sera were also tested for reactivity with simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), and caprine arthritis encephalitis virus (CAEV). Six dog sera reacted with at least the major core protein of HIV, while one of the dog sera tested reacted with SIV core protein, and there were no reactions with the viral proteins of either FIV or CAEV. Cell extracts from canine peripheral blood lymphocytes cocultivated with human cells and an extract of human cells infected with HIV were immunoblotted against dog sera which previously tested positive or negative on HIV recombinant protein commercially available Western blot strips. Two lymphocyte lysates and the HIV-infected Hut cell lysate reacted with the Western blot strip-positive dog serum; however, no reactions were seen with the Western blot strip-negative dog serum.

Introduction

Retroviruses have been isolated from a wide variety of animal species including humans. Some retroviruses are associated with neoplasia, others with inflammatory diseases, and some with immunodeficiency syndrome. Retroviruses have also been implicated in a number of canine malignancies, although no conclusive evidence for the existence of a canine retrovirus has so far been reported.

Immunoblotting (Western blot) is routinely used for detection of antibodies in human sera against HIV[4] proteins (1). The same technique is also applied to detect antibodies to simian AIDS in monkey sera (2), antibodies to CAEV in goat sera,[5] and antibodies to FIV in cat sera (3).

Materials and Methods

Sera. Canine sera were obtained from the Veterinary Medical Teaching Hospital, University of California, Davis. Human control sera (positive and negative) were included in the Chiron Western blot kit. SIV, CAEV, and FIV sera were obtained from the University of California, Davis. Dog sera were usually tested at a 1:100 dilution; in some cases, however, the dog sera were additionally tested at dilutions of 1:20, 1:50, and 1:200.

Human cell lines Hut-78, CEMx174, Molt clone 8, and Tahr cells were grown in RPMI-1640 (Gibco) supplemented with 10% FBS, glutamine (Gibco), and antibiotics (Gibco). Canine PBL were derived from heparinized blood obtained from Veterinary Medical Teaching Hospital, University of California, Davis, patients and separated on Histopaque (Sigma) gradients. The PBL were cultivated in RPMI-1640 supplemented with 10% FBS, glutamine, antibiotics, 10^{-5} M mercaptoethanol (Sigma), 10 μg/ml of human recombinant interleukin 2 (Cetus Corporation), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (Gibco), and 5 μg/ml of Con A (Sigma). Fresh culture medium without Con A was added twice a week, and the cell concentration was adjusted to about 8×10^5 cells/ml whenever necessary. For cocultures, 1×10^6 canine PBL were cocultivated with 1×10^6 cells of the human T-cell lines Hut-78, CEMx174, Molt clone 8, and Tahr cells. Fresh cells were added to the cultures whenever needed.

Western Blot Analyses. Commercially available Chiron RIBA strips, (TM)-HIV 216 test system, recombinant immunoblot assay (Ortho Diagnostic System) (4, 5), or DuPont (HIV-1, Western blot, NEA-8025) strips were incubated 2 h at room temperature with test serum and then for 30 min with peroxidase-labeled anti-canine (Miles) or anti-human (Chiron) serum. Western blot strips of viral native proteins were made as described (6) using gradient-purified virus which was fractionated on a 4 to 12% sodium dodecyl sulfate-polyacrylamide gel and electrophoretically transferred to nitrocellulose (7). Individual strips were incubated first with test sera and then with appropriate peroxidase-labeled anti-canine (Miles), anti-monkey (BioRad), anti-cat (BioRad), anti-goat (BioRad), or anti-human (BioRad) sera. Reactive bands were stained with dimethylaminoazobenzene. Molecular weight standards (BioRad) were run to identify the positions of the bands.

Cell Lysates. Cells were lysed in 1% Triton X-100 (Sigma) in phosphate-buffered saline. Cell lysates of canine PBL cocultivated with Hut-78, CEMx174, Tahr cells, or HIV-infected Hut-78 cells were electrophoresed on 12% acrylamide slab gels in the presence of sodium dodecyl sulfate and mercaptoethanol and were then electrophoretically transferred to nitrocellulose. The strips were first incubated with canine HIV protein strongly positive or HIV protein-negative serum and then with peroxidase-labeled anti-dog serum.

Results

A total of 144 dog sera were tested on Chiron Western blot strips. Of these, 72 sera (50%) reacted with one or more HIV recombinant proteins (Table 1). Ten cell lysates were tested by Western blot analysis. Two dog cell lysates (PBL from a dog with immunological dysfunction cocultivated with Hut-78 and Tahr cells) and the HIV-infected Hut-78 cell lysate showed reaction with the HIV protein-positive dog serum, but no reaction could be seen with the HIV protein-negative dog serum (Fig. 1). Some background precipitation was also observed.

The results of the different reaction patterns are shown in Fig. 2. Ten dog sera were tested using Western blot analysis with sucrose gradient-purified virus. Viruses used were human HIV, monkey SIV(mac), cat FIV, and goat CAEV. The reaction

Table 1 *Reactivity of dog sera by recombinant HIV immunoblots*

Total no. of sera tested	Positive sera		% of sera positive for			
	n	%	gp120	gp41	p31	p24
144	72	50	21.5	23	22	43

[1] Presented at the "XIVth Symposium of the International Association for Comparative Research on Leukemia and Related Diseases," October 8–12, 1989, Vail, CO.

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[4] The abbreviations used are: HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; FIV, feline immunodeficiency virus; CAEV, caprine arthritis encephalitis virus; FBS, fetal bovine serum; PBL, peripheral blood lymphocytes; Con A, concanavalin A.

[5] N. East, personal communications.

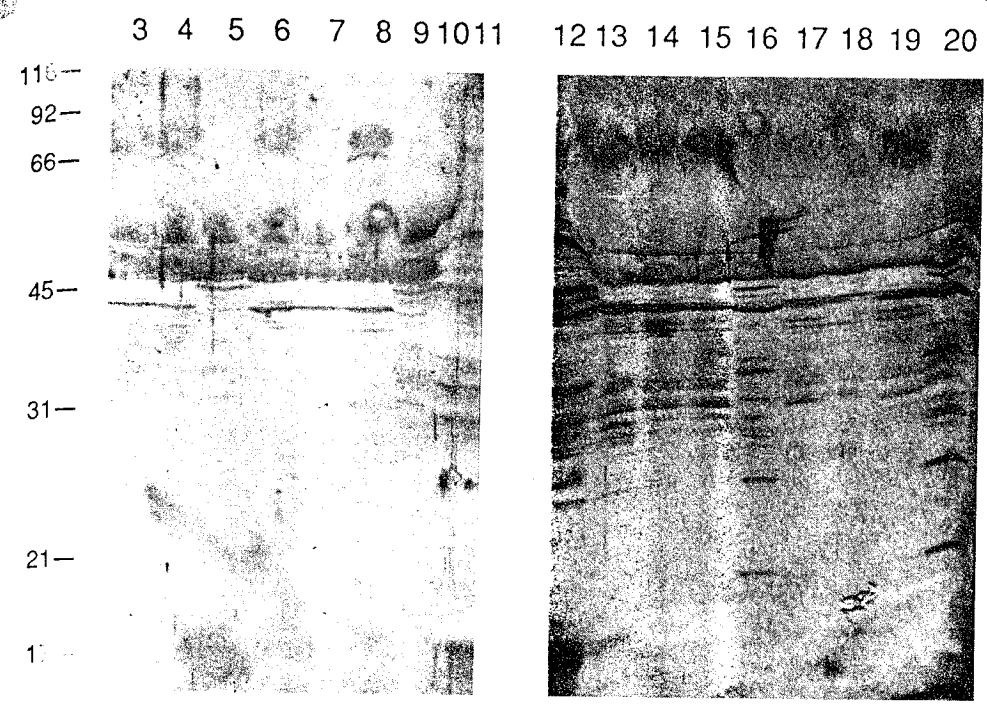

Fig. 1. Reactivity of HIV protein-positive (*Lanes 12* to *20*) and -negative (*Lanes 3* to *11*) dog serum on immunoblotted cell lysates. Dog sera were diluted 1:100; peroxidase-labeled anti-dog serum was diluted 1:750. *Lanes 3* and *13*, 154293 (PBL/Molt clone 8); *Lanes 4* and *14*, 154243 (PBL/Molt clone 8); *Lanes 5* and *15*, 13993 (PBL/Molt clone 8); *Lanes 6* and *16*, 239547 (PBL/CEMx174); *Lanes 7* and *17*, 543 (PBL/Tahr); *Lanes 8* and *18*, 543 (PBL/Tahr); *Lanes 9* and *19*, 543 (PBL/Molt clone 8); *Lanes 10* and *20*, 230547 (PBL/Tahr); *Lanes 11* and *12* (HIV/Hut-78).

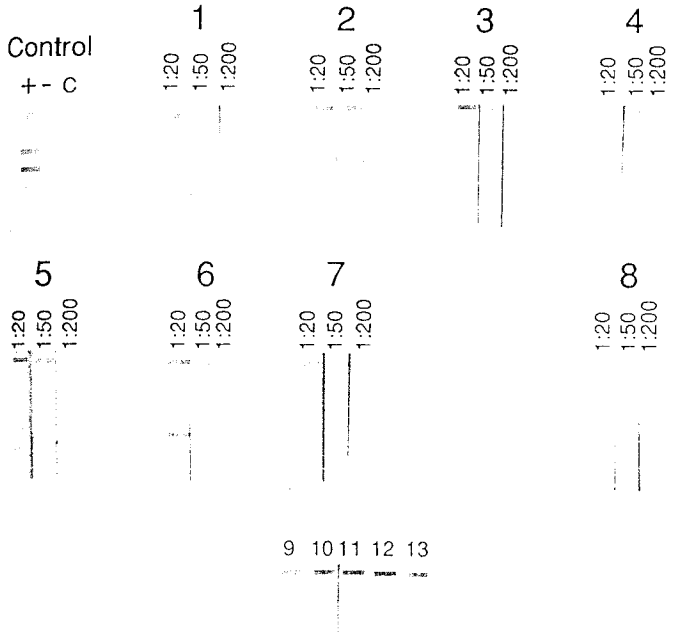

Fig. 2. Reactivity of 13 different canine sera (*lane groups* 3 to 13) on Chiron strips. Sera 1 to 8 were diluted 1:20, 1:50, and 1:200; sera 9 to 13 were diluted 1:100. Serum 8 represents a negative serum. Peroxidase-labeled anti-dog serum (Miles) was diluted 1:500. Human HIV-positive and negative sera (Chiron) were used as control, as well as peroxidase-labeled anti-dog serum. The four HIV proteins on the Chiron strips are, *top* to *bottom*, gp120, gp41, p31, and p24. Comparative results were obtained with commercially available DuPont strips.

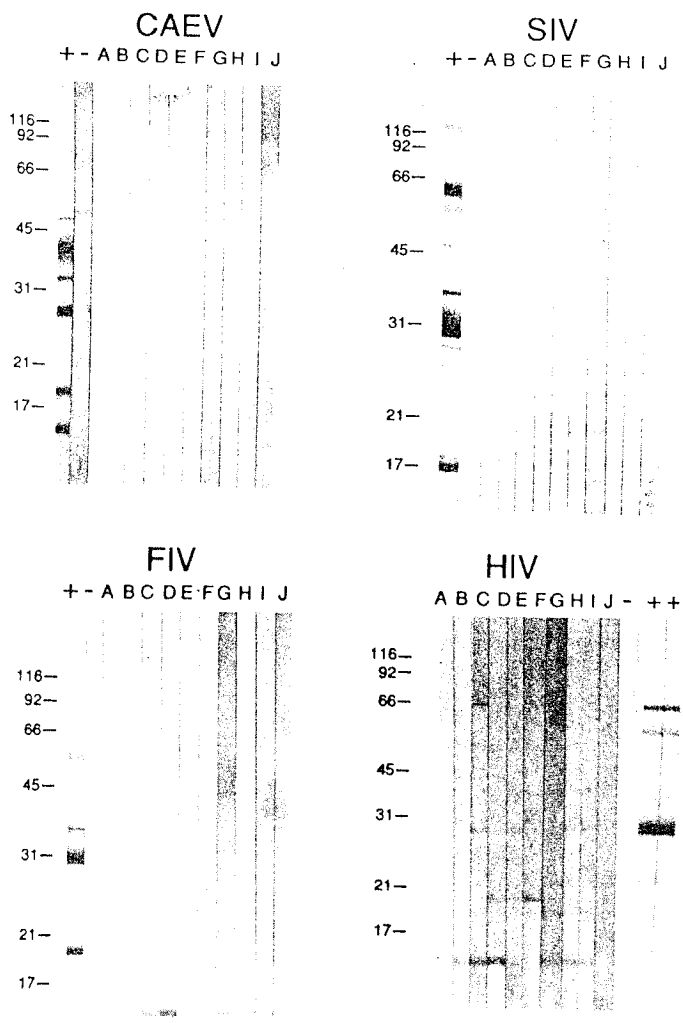

Fig. 3. Reactivity of dog sera to purified native viral proteins. Canine and appropriate immune sera were diluted 1:100. Peroxidase-labeled anti-dog serum (Miles) was diluted 1:750; anti-human, 1:2000; anti-goat, 1:2000; anti-cat, 1:750; and anti-monkey, 1:750.

Six of the 10 dog sera (*Lanes C, E, F, G, H,* and *I*) reacted with at least the major core protein of HIV. One dog serum, *Lane I*, reacted with SIV (mac) core protein. There were no reactions with the viral proteins of either CAEV or FIV.

patterns are shown in Fig. 3. Ten cell lysates were tested by Western blot analysis. Two dog cell lysates (PBL from a dog with immunological dysfunction cocultured with Hut-78 and Tahr cells) and the HIV-infected Hut-78 cell lysate showed reaction with the HIV protein-positive dog serum, but no reaction could be seen with the HIV protein-negative dog serum (Fig. 1). Some background precipitation was also observed.

Discussion

Serological results using the immunoblot technique suggest that dogs may be infected with an agent, which is antigenically related to lentiviruses, especially HIV. Some canine sera contain antibodies that cross-react with different HIV structural proteins and to a limited degree with SIV (mac) core protein. The majority of the sera reacted with the HIV core protein, although some sera reacted with more than one protein, which indicates a specific rather than a nonspecific reaction. No distinct pattern relating to age, breed, clinical or pathological illness, or neoplasia has yet been established. The reactivity of dog sera with lysates of HIV-infected Hut-78 cells provides additional evidence of a lentiviral infection in dogs. Lentiviruses are closely related to each other, which gives additional importance to these findings (8).

The origin of lentiviruses has not yet been established. Our results are provocative, although not conclusive, suggesting the possibility that dogs might be an important link in our understanding of interspecies lentivirus relatedness and perhaps of their pathogenesis.

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