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Humoral Immune Response of Asymptomatic Cats Naturally Infected with Feline Leukemia Virus

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The humoral immune response of cats that were naturally infected with the feline leukemia virus (FeLV) was examined after antigenic stimulation with the synthetic antigen poly(L-Tyr, L-Glu)-poly(DL-Ala)–poly(L-Lys). The primary humoral antibody response in FeLV-infected cats was both delayed and greatly reduced, compared with that seen in uninfected control cats. A similar discordance was observed after secondary stimulation with the antigen, in that FeLV-infected cats had both a delayed response and a reduced response, compared with uninfected cats. The levels of total immunoglobulins of the immunoglobulin G and immunoglobulin M classes in the sera of FeLV-infected cats were significantly higher (two- and threefold, respectively) than were those of the uninfected control animals. The presence of an impaired humoral immune response to newly presented antigens in the presence of elevated immunoglobulin levels has been thoroughly documented in the case of people with the acquired immunodeficiency syndrome. This further emphasizes the potential value of FeLV-infected cats as a model for human acquired immunodeficiency syndrome.

Infection with retroviruses has been associated with various parameters of clinical immunosuppression in mice, cats, and chickens (3, 6, 7, 9, 12). Yet, very little is known about how these viruses mediate this effect.

Feline leukemia virus (FeLV) is a horizontally transmitted cat retrovirus (16). It establishes a chronic, persistent infection that may ultimately result in either neoplastic or nonneoplastic diseases in cats. After natural or experimental infection, cats may develop leukemia or lymphoma which is usually of T-cell origin (2, 5, 17). Additionally, FeLV infection is characterized by a generalized susceptibility to various infectious agents and thus a greatly increased risk for development of disease caused by opportunistic bacterial, viral, or parasitic agents (14). Under conditions of natural infection with FeLV, the diseases associated with immunosuppression cause more cat deaths than does leukemia or lymphoma (15).

Most studies directed at understanding the mechanisms involved in immunosuppression by FeLV have concentrated on the cell-mediated immune response (1, 5, 8, 21). An early attempt to show that humoral immunity was depressed by FeLV infection was unsuccessful when infection was artificially induced (25). However, many infectious agents are controlled, at least in part, by the humoral immune system. Therefore, the aim of this study was to examine the humoral immune response of cats infected naturally with FeLV. The antigen used for immunization was the synthetic polypeptide multichain (L-tyrosine, L-glutamic acid)-poly(DL-alanine)–poly(L-lysine), denoted (T,G)-A–L.

MATERIALS AND METHODS

Antigens. (T,G)-A–L, synthesized as described by Sela et al. (26), was used for immunization.

FeLV-infected cats. Eight outbred adult cats that became persistently viremic with FeLV in their natural environment were used in this study. With the use of indirect fixed-cell immunofluorescence (16), each cat was confirmed to be viremic for at least 6 months before inoculation with antigen. The cats were judged to be clinically healthy, both before and during the course of the experiment.

Uninfected controls. Six uninfected healthy adult cats obtained from similar environments served as controls.

Immunization. Antigen (1 mg) dissolved in phosphate-buffered saline (PBS) was emulsified with an equal volume of complete Freund adjuvant and administered subcutaneously in equal amounts on both sides of the neck of the cat for primary stimulation. At 8 to 12 months after the first inoculation, seven of the FeLV-infected animals and five control cats were challenged with a second dose of antigen administered in a similar amount and manner as for primary stimulation.

Blood samples. Blood was taken from the jugular vein at day 0, on every second day until day 11, and at various intervals thereafter. Blood was again taken at the time of booster administration and thereafter at similar intervals as postprimary antigenic stimulation. The serum was separated by centrifugation and stored at −20°C until tested.

Antisera. Rabbit anti-feline immunoglobulin G (IgG) was the generous gift of Bruce Wilkie, and goat anti-cat IgM (μ chain specific) was a product of Pel-Freeze Biologicals (Rogers, Ark.).

Enzyme-labeled antibodies. Alkaline phosphatase (EC 3.1.3.1, type VII-S; Sigma Chemical Co., St. Louis, Mo.) was coupled to the serum immunoglobulins of rabbit anti-feline IgG with glutaraldehyde as described by Engvall and Perlman (10).

Enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assay was carried out on M 129A flat-bottom enzyme-linked immunosorbent microassay plates (Dynatech Laboratories, Inc., Alexandria, Va.) coated with 5 μg of (T,G)-A–L per ml in 0.05 M carbonate-bicarbonate buffer, pH 9.6, as follows. A sample (200 μl) of antigen solution was introduced into each well and incubated for 18 h at 4°C. After

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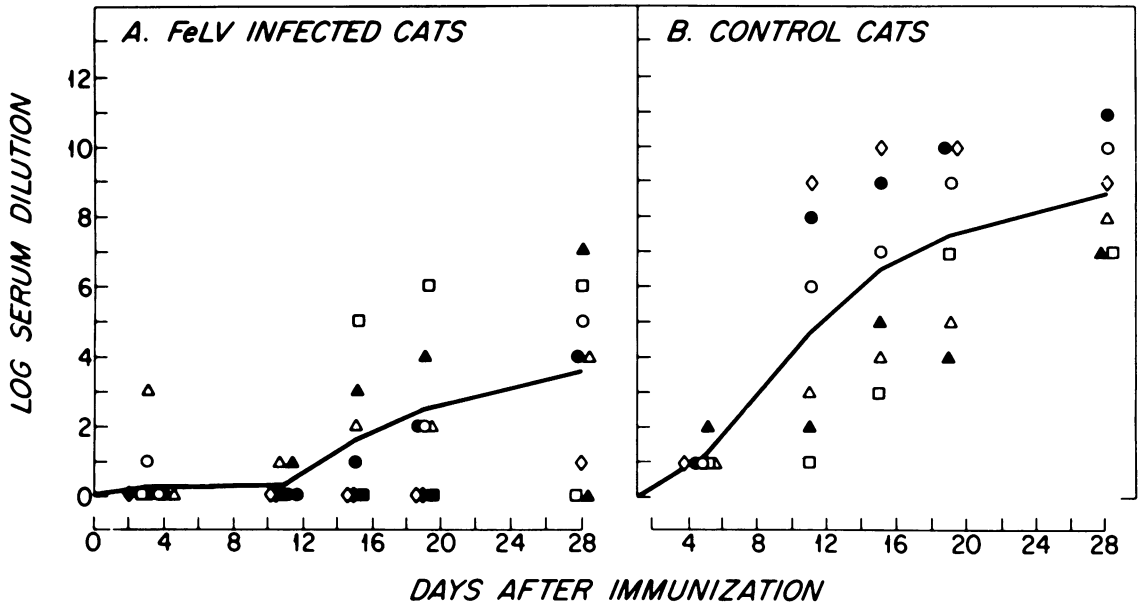

FIG. 1. Titers of antibodies against (T,G)-A–L elicited in FeLV-infected cats (A), and control cats (B) upon primary immunization as examined by enzyme-linked immunosorbent assay. Curves represent geometric means of individual responses. Each point represents the reciprocal of the highest twofold dilution of serum that gave a positive reaction.

incubation, plates were washed three times with PBS containing 0.5 ml of polysorbate (Tween 20) per liter. Coated plates were kept dry at 4°C for up to 3 months before use. Sera were diluted in PBS-Tween, and samples (200 μl) were incubated in the antigen-coated wells for 18 h at 4°C. After three washings with PBS-Tween, enzyme-labeled antibody diluted in PBS–0.05% Tween–0.2% bovine serum albumin was introduced into the wells in 200-μl portions and incubated for 2 h at 37°C. An additional identical washing step was carried out after incubation.

The substrate, disodium-4-nitrophenyl-phosphate hexahydrate (Fluka Chemical Corp., Hauppauge, N.Y.; 1 mg/ml in 0.05 M sodium carbonate buffer [pH 9.6] containing 1 mM $MgCl_2$) was then added to the wells in 200-μl portions and incubated for 90 min at room temperature. The reaction was stopped by the addition of 50 μl of 3 N NaOH to each well, and the absorbance was measured at 405 nm. All tests were carried out in duplicate. The titer represents the highest twofold dilution of serum that gave a positive reaction. Additional details on the assay system have been published (27).

Quantitative radial immunodiffusion. Quantitative radial immunodiffusion was carried out on feline sera for the determination of levels of immunoglobulins of the IgG and IgM classes. These were estimated by quantitative radial gel immunodiffusion (22) with anti-feline γ and μ chains.

RESULTS

Elicitation of antibodies to (T,G)-A–L in FeLV-infected cats upon antigenic stimulation. The humoral response to (T,G)-A–L in FeLV-infected healthy cats was significantly depressed, compared with that of uninfected controls (Fig. 1). Two of the eight infected cats did not respond to (T,G)-A–L at all, and a third had a barely detectable response by day 27 to 31. The major antibody response in such cats was both delayed and greatly reduced (Fig. 1). Whereas in normal cats significant antibody titers could already be detected on day 10, FeLV-infected cats exhibited low levels of antibodies by day 14 to 20 after immunization. By day 28, the geometric mean titer of antibodies had reached a constant level (Fig. 1) in both groups of animals and was approximately 100-fold lower in the eight FeLV-infected cats.

Secondary immune response to (T,G)-A–L in FeLV-infected cats. At the time of booster administration 8 months after the primary injection, the titer of antibodies to (T,G)-A–L in FeLV-infected cats was fourfold lower than that of the control group (Table 1). The rise in antibody titer of the uninfected control group increased significantly starting on day 3. Its peak value was reached on day 31 with a mean titer of 1:54,375 (arithmetic mean [Table 1]). The FeLV-infected cats exhibited no significant increase in antibody titer up to day 14 after the secondary inoculation. Hence a fivefold increase was observed, persisting for 2 weeks afterward (Table 1). The mean endpoint titer on day 31 was 1:23,857.

TABLE 1. Humoral antibody titers to (T,G)-A–L in FeLV-infected and uninfected control cats during the month after second immunization

Infection category	Antibody titer on postimmunization day[a]				
	0	3	8–9	14–16	28–31
FeLV infected					
Arithmetic mean	2,160	2,189	2,491	11,246	23,857
Geometric mean	1,038	1,161	1,280	6,163	8,343
Uninfected					
Arithmetic mean	5,888	11,648	29,216	33,620	54,375
Geometric mean	4,457	4,457	10,480	21,520	31,000

[a] Reciprocal of the highest twofold dilution of serum that gave a positive reaction. The differences between infected and control cats were significant at the following levels (day postimmunization): 0, $P < 0.025$; 3, $P < 0.05$; 8 to 9, $P < 0.025$; 14 to 16, $P < 0.2$; 28 to 31, $P < 0.0005$.

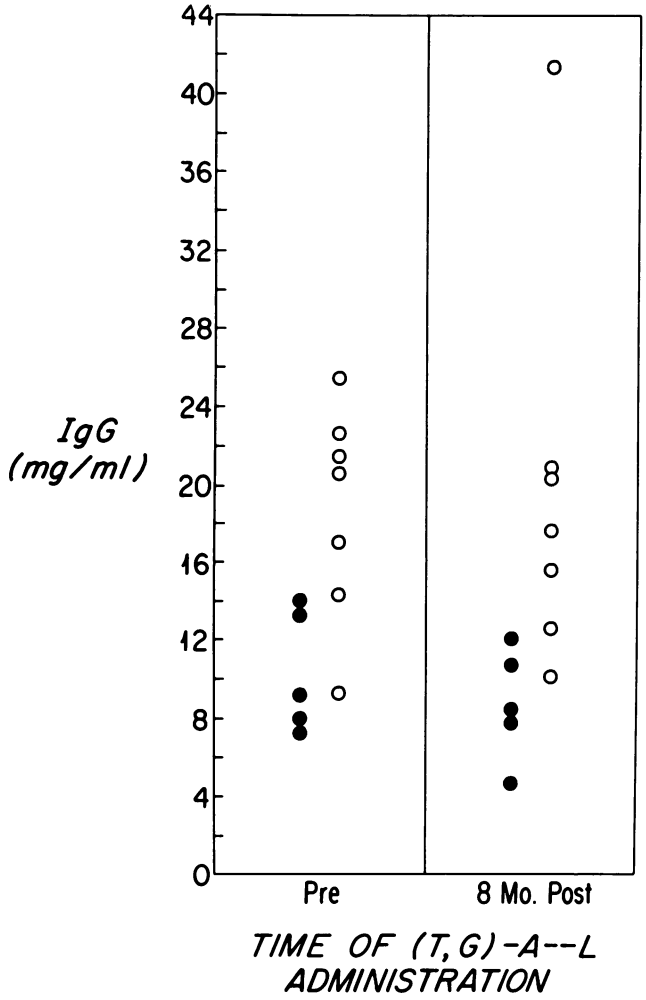

FIG. 2. Comparison of IgG levels in serum of FeLV-infected (○) and control (●) cats before initial (T,G)-A–L administration (zero time) and at 8 months afterward.

Thereafter, a gradual parallel decrease was observed in the antibody titers of both groups.

Levels of immunoglobulins of the IgG and IgM classes in the sera of FeLV-infected and normal cats. The levels of immunoglobulins of IgG and IgM classes in the sera of seven FeLV-infected cats and five normal controls were measured at the time of primary immunization and at the time of booster administration 8 months later (Fig. 2 and 3). In normal cats, the mean values of IgG levels in serum at these times were 10.34 ± 1.24 and 8.82 ± 1.15 mg/ml, respectively, whereas those of FeLV-infected cats were 18.67 ± 1.95 and 19.9 ± 3.6 mg/ml.

The difference in mean values between the two groups is highly significant ($P < 0.005$ in the first set of sera, and $P < 0.01$ in the second bleeding, according to Student's t-test). The results indicate an almost twofold higher mean IgG level in serum of the FeLV-infected cats, compared with that of the control group.

The mean value of IgM in serum of normal cats was 1.66 ± 0.12 mg/ml at the time of primary immunization and 1.79 ± 0.16 mg/ml at the time of booster administration (Fig. 3). The mean levels of IgM in serum of the FeLV-infected cats at these two times were 4.97 ± 0.94 and 4.63 ± 0.8 mg/ml, respectively. The difference between the two groups is highly significant ($P < 0.01$, according to Student's t-test). Here a threefold higher level of IgM was observed in the infected cats, compared with controls.

DISCUSSION

Our results indicate that the humoral antibody response of cats that are naturally infected with FeLV is both delayed and depressed. The immune impairment thus reflected is expressed in both the primary and secondary antibody responses obtained upon antigenic stimulation with (T,G)-A–L.

After both primary and secondary administration of antigen, the uninfected control animals responded with increased levels of antibodies within 3 days after inoculation. However, in the FeLV-infected cats a lower and a delayed level of antibody production was detected upon primary antigenic stimulation, and a second challenge with antigen evoked an increase of antibody production only after 9 days. It is thus clearly indicated that the humoral antibody response of cats naturally infected with FeLV is diminished when they are exposed to a newly encountered antigen. The suppression of the secondary response was much more dramatic in three of the infected cats. A similar observation

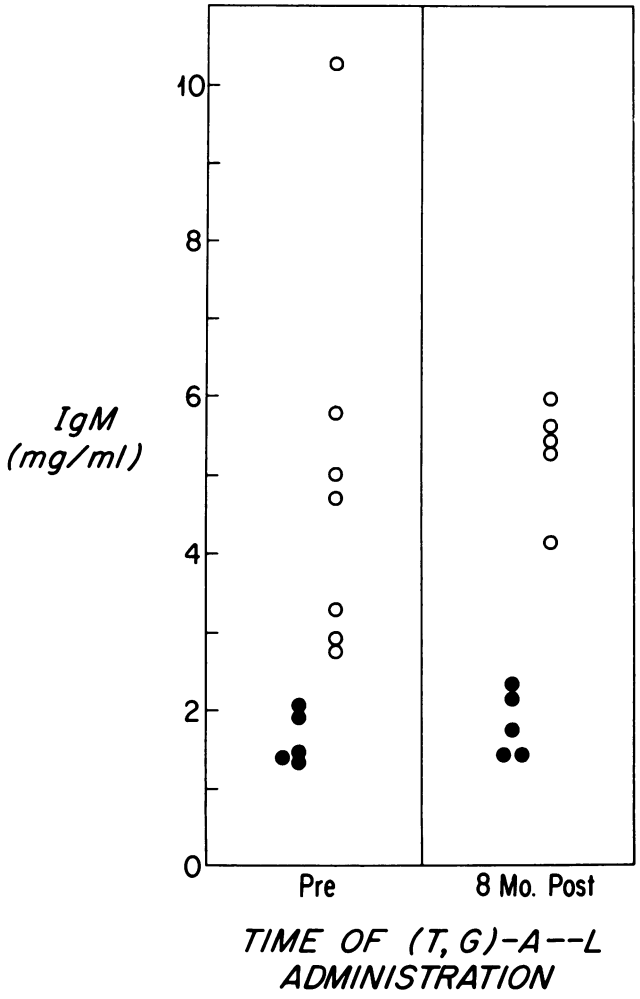

FIG. 3. Comparison of IgM levels in serum of FeLV-infected (○) and control (●) cats before initial (T,G)-A–L administration (zero time) and at 8 months afterward.

was reported earlier, where a subset of infected cats failed to mount a significant humoral antibody response (27).

Despite this apparent impairment in the humoral response to new antigens, the FeLV-infected cats exhibited hypergammaglobulinemia, which was twofold higher for mean levels of IgG and threefold higher for levels of IgM, compared with the uninfected controls. These findings do not necessarily indicate a normal function of the immune response and may be an expression of an intense polyclonal B-cell activation leading to high serum immunoglobulin levels, similar to observations made on acquired immunodeficiency syndrome patients (20).

In the case of human acquired immunodeficiency syndrome patients infected with HTLV-III/LAV, the cell-mediated immune response is severely impaired. Infection with FeLV has also been associated with immunosuppression expressed by prolongation of allograft rejection (25), thymic atrophy (1, 18), depletion of paracortical lymphoid tissues (18, 19), depressed peripheral blood lymphocyte counts (12), and diminished mobility of lymphocyte membrane capping (8).

The mechanism(s) involved in immunosuppression by FeLV is unknown. Possibilities include the formation of immune complexes (15), the action of an exfoliated protein from the viral envelope of FeLV (p15E) (4, 23), and the involvement of a virus-induced suppressor cell population (21). Still another possibility is that the virus infection induces an impairment of the T helper cells, as in human acquired immunodeficiency syndrome. (T,G)-A–L is a T-cell-dependent antigen (24). The mitogenic stimulus provided by activated T helper cells is necessary for vigorous B-cell proliferation, when the triggering antigen is T cell dependent (9). In both the primary and the secondary immune responses, the B cells from FeLV-infected cats are capable of synthesizing antibodies to (T,G)-A–L after a considerable delay. Thus, a defect in the production or the release of helper factor which normally would stimulate B-cell proliferation can also be considered. On the other hand, the observation that uninfected animals maintain a relatively high level of antibodies against (T,G)-A–L for more than 8 months after the first immunization, while the FeLV-infected animals do not seem to have this ability to the same extent, is compatible with the mechanism by which longevity of B cells would be impaired.

Cats infected with FeLV have a substantial increase in risk for infection with certain protozoan, viral, bacterial, and fungal agents (11–13). They also have increased rates of both hematopoietic (11) and nonhematopoietic tumors (28); only the former is more directly attributable to the oncogenic activities of the virus itself. When taken with the current results, this information suggests that studies of the pathogenesis of FeLV-induced immunosuppression might provide a valuable model for a better understanding of human acquired immunodeficiency syndrome.

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