Immune Response to Leukemia Virus and Tumor-associated Antigens in Cats

M. Essex, A. Sliski, W. D. Hardy, Jr., and S. M. Cotter

Summary

Cats represent an unusually valuable model for studying the role of the immune response to leukemia, lymphoma, and other mesodermal neoplasms. The agents that cause spontaneous feline leukemias, lymphomas, and fibrosarcomas, the feline leukemia and sarcoma viruses, are well characterized. A specific tumor cell membrane antigen, designated the feline oncornavirus-associated cell membrane antigen (FOCMA) has also been described. Feline leukemia and feline sarcoma viruses are antigenically indistinguishable, and FOCMA is common for both. Both laboratory-induced and spontaneous feline leukemias, lymphomas, and fibrosarcomas are available for study. A clear correlation has been shown between the resistance of cats to development of lethal tumors following inoculation of feline sarcoma virus and the presence of high humoral antibody titers to FOCMA. The geometric mean antibody titer to FOCMA for cats that resisted growth of fibrosarcomas was more than 20-fold higher than the mean for cats that succumbed to lethally progressing tumors. Cats with induced or spontaneous leukemia or lymphoma also have either no detectable FOCMA antibody or very low levels. Conversely, some cats resist development of leukemia or lymphoma following natural exposure to feline leukemia virus in leukemia cluster households, and these cats have high FOCMA antibody titers. These results support the concept of a natural immunosurveillance mechanism against leukemia or lymphoma development in an outbred mammalian species.

Introduction

Evidence that convincingly supports a viral etiology for most spontaneous human lymphoid tumors has not been obtained. Despite this, substantial evidence indicates that both oncornaviruses and herpesviruses can cause spontaneous lymphoproliferative tumors in certain animal species. In at least 2 species, the chicken and the cat, horizontally transmitted oncornaviruses cause spontaneous lymphoid leukemia and/or lymphoma. Oncornaviruses have been identified in numerous other outbred mammalian species, but oncogenicity by the particles has been difficult to demonstrate (13). Highly oncogenic oncornaviruses have been found in inbred laboratory mouse strains, but evidence is lacking that the same viruses cause spontaneous tumors in wild mice.

Our purpose here is to examine the role of the immune response in the development and progression of leukemia and lymphoma in cats and to speculate about what significance this may have concerning a role for the immune response in the development of lymphoid tumors of man. It is therefore necessary first to consider the natural history of lymphoid neoplasia of cats. Several features of the feline disease appear to make it an appropriate model for the study of lymphoid neoplasia of children. Cats are outbred like man, and leukemia occurs in all breeds with no evident genetic predisposition. An early age peak is seen in feline leukemia (13, 16, 42), similar to the peaks seen for acute leukemia and Burkitt's lymphoma of children. Cats develop both true lymphoid leukemia, with primary involvement of the blood and bone marrow, and localized lymphoma (13, 16). The histopathology of feline lymphoma is similar to that for any rapidly growing lymphoid tumor such as Burkitt's lymphoma (60).

A possible disadvantage of the model is that feline leukemia and lymphoma are caused by horizontally transmissible agents that replicate in a productive fashion in most cats with tumors, since comparable agents have not been found in acute leukemia of man. On the other hand, the seroepidemiology of FeLV appears similar to that of the Epstein-Barr virus, which is horizontally transmitted and which many believe will eventually be accepted as the cause of Burkitt's lymphoma (36, 45). Serological tests for evaluating the host immune response to both the etiological agent and the altered tumor cell are available for feline leukemia and lymphoma (13, 22, 23, 32, 34, 58). The opportunity to use such procedures for the study of individuals with either laboratory-induced or spontaneous tumors known to be caused by the same agents is unusual. Furthermore, healthy cats at high risk for development of leukemia, which were known to be exposed to FeLV under natural conditions, are also available for study (8, 9, 13, 14, 17–19, 31, 34).

Whether immunosurveillance of developing malignant cell clones occurs under natural conditions is an issue that
is central to tumor immunology (46). Several observations support this concept. A substantially increased risk for tumor development occurs in organ-transplant patients and others subjected to therapy with immunosuppressive drugs (54). A similar increase in tumor risk occurs in patients with the rare inherited immunological deficiency diseases (29), and this is not due to an increased susceptibility of cells to the transforming effects of oncogenic agents (44). In laboratory animals the administration of immunosuppressive drugs results in increased incidences of tumor development, shorter latent periods, and increased rates of metastasis (2, 35, 37). An observation that is in apparent conflict with the immunosurveillance concept is the lack of increased risk for tumor development in nude (thymusless) mice (56, 63).

**Cell Membrane Antigens**

The antigen systems to be considered for an analysis of the immune response to feline leukemia can be operationally grouped into 2 categories: those found at the surface of the infected and/or transformed cells, and those that make up the virion particle. The main proteins that make up the virion particle are listed by molecular weight as p30, p15, p12, and p10 of the virus core. Considerable evidence suggests that the 3 major virion proteins, gp70, p30, and p15, can also be detected on the membranes of infected cells. In the case of gp70, it is logical to expect some degree of detection, because oncornavirus particles bud from the cell membrane, and the gp70 is at the surface of the virus particle (4). The detection of p30 and p15 at the cell membrane might not be as readily expected, however, because they appear to be internal virus proteins (4). In the case of p30, this has been demonstrated on cells replicating either feline or murine oncornaviruses using antisera from rabbits hyperimmunized with either disrupted virus or the p30 fraction (65).

An antigenic activity that appears to be the Friend-Moloney-Rauscher cell surface antigen has also been found in disrupted virus particles (25). In this case it appears to represent the p15 fraction (62). It is unlikely, however, that the Friend-Moloney-Rauscher cell surface antigen is a single cross-reacting entity that always occurs as the predominant antigenic activity on infected cells. The degree of cross-reactivity between Friend, Moloney, and Rauscher tumor cells varies widely with different sera and different serological procedures (7, 64).

In addition to virus core and envelope proteins, it is at least theoretically possible that antigenic determinants that are not virion structural proteins are also found at the surface of infected and/or transformed cells. These could include depressed embryonic antigens (12, 41) as well as virus-directed non-virion antigens that might or might not be tumor specific (12, 47).

A question that still remains concerning the virus core components (especially p30) at the cell surface is whether or not they are immunogenic in the autologous host naturally exposed to that virus. Such virus core antigens have generally been considered nonimmunogenic in autologous hosts (40). If they are immunogenic, the question still remains of whether the autologous immune response to the determinants can result in cytostasis or cytolysis of tumor cells. In most instances where immunogenicity to the virus core “group-specific” (gs) antigens has been detected, it has been associated with a non-complement-fixing humoral response, which is not correlated with tumor development or progressions (52, 59).

The issue of antigens in the virus particle that result in virus neutralization seems simple by comparison. The gp70 is the primary virion envelope antigen, and antisera to this component results in virus inactivation (4, 61). Antiserum to core proteins is ineffective because such a reaction cannot occur without disruption of the envelope.

We proposed the term feline oncornavirus-associated cell membrane antigen (FOCMA) for the specific reactivity found at the surface of FeLV-infected cells. FOCMA does not cross-react to any significant degree with similar antigens induced by other oncornaviruses, including those induced by the endogenous oncornaviruses of cats (6, 12, 13, 20, 24, 53). The target cell most commonly used for detection of feline antibody to the FOCMA antigen by either membrane fluorescence or cytotoxicity tests is the FI 74 culture of feline lymphoma cells (12, 13, 22, 23). This cell produces FeLV of all 3 of the virus subgroups that have been described in cats (57). The virus subgroups are each antigenically distinct on the basis of their virus envelope antigens. The presence of the virus core antigens on the surface of the FI 74 cell must also be considered.

**Experimentally Induced Tumors**

Antibody to FOCMA was first detected in cats that had been given laboratory inoculations of FeSV (21, 22, 24). Both the animals that developed tumors that subsequently regressed and those that showed no palpable tumors at all had significant levels of humoral antibody to FOCMA (Table 1). Of 41 “regressor” or “no tumor” animals, all developed titers of 4 or higher. More than two-thirds had titers of 16 or higher and four-fifths had titers of 8 or higher. The geometric mean antibody titer for this group was more than 20-fold higher than the geometric mean for cats that developed progressing tumors. Of 57 cats that developed rapidly progressing fatal tumors only one-half had any detectable antibody to FOCMA, and only 1 cat had a titer of 8 or higher.

A strong correlation was also seen between the presence of maternally transmitted FOCMA antibody in the serum of neonatal kittens and their ability to resist doses of FeSV that would otherwise be lethal (21, 24). Although protection against tumor development in the kittens was regularly associated with the presence of passively acquired FOCMA antibody, whether or not virus-neutralizing antibody was also present in the same kittens was not determined. It is thus possible that neutralization of FeSV inoculum by virus-neutralizing antibody, if it was also present, could have been responsible for preventing tumor induction.

Both virus-neutralizing and FOCMA antibody levels have been determined for many of the regressor and progressor cats listed in Table 1. The presence of significant titers of
Table 1

<table>
<thead>
<tr>
<th>Tumor status</th>
<th>No. with detectable antibody</th>
<th>No. with titers ≥4</th>
<th>No. with titers ≥8</th>
<th>Geometric mean titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progressor</td>
<td>29/57 (51)</td>
<td>13/57 (23)</td>
<td>1/57 (2)</td>
<td>0.87</td>
</tr>
<tr>
<td>Regressor or no tumor developed</td>
<td>41/41 (100)</td>
<td>41/41 (100)</td>
<td>33/41 (80)</td>
<td>20.30</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

Table 2

<table>
<thead>
<tr>
<th>Health status</th>
<th>No. with detectable antibody</th>
<th>No. with titers ≥4</th>
<th>No. with titers ≥8</th>
<th>Geometric mean titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoid leukemia</td>
<td>23/45 (51)*</td>
<td>7/45 (16)</td>
<td>1/45 (2)</td>
<td>0.77</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>14/44 (32)</td>
<td>2/44 (5)</td>
<td>0/44 (0)</td>
<td>0.34</td>
</tr>
<tr>
<td>Anemia</td>
<td>35/81 (43)</td>
<td>8/81 (10)</td>
<td>3/81 (4)</td>
<td>0.63</td>
</tr>
<tr>
<td>Healthy exposed</td>
<td>113/138 (82)</td>
<td>94/138 (68)</td>
<td>66/138 (49)</td>
<td>5.77</td>
</tr>
<tr>
<td>FeLV positive</td>
<td>53/66 (80)</td>
<td>42/66 (64)</td>
<td>24/66 (36)</td>
<td>3.51</td>
</tr>
<tr>
<td>FeLV negative</td>
<td>61/72 (85)</td>
<td>52/72 (72)</td>
<td>44/72 (61)</td>
<td>8.81</td>
</tr>
<tr>
<td>Healthy, presumably unexposed</td>
<td>3/120 (3)</td>
<td>1/120 (1)</td>
<td>1/120 (1)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Collected at the Angell Memorial Animal Hospital, Boston, Mass.
* Numbers in parentheses, percentage.
* Collected at previously identified leukemia-lymphoma cluster households in the Boston area where several FeLV-positive neoplastic cats were known to have resided. See Refs. 8, 9, 18, and 19.
* Specific-pathogen-free or "minimal disease status" cats maintained in experimental colonies.

Although a lower number of animals that were injected with FeLV were available for study, the same trend was observed (12–14). Those that developed clinical leukemia had either no detectable FOCMA antibody or very low levels, while those that remained healthy after receiving the same virus doses had high FOCMA antibody titers.

Since FeLV is known to be spread efficiently in a horizontal fashion under both laboratory and field conditions (13, 15, 19, 23, 31, 34), we determined FOCMA antibody titers for healthy cats that were contact exposed to FeLV or FeSV cagemates in the laboratory (17). Of 20 such cats that remained healthy without developing tumors, 18 developed FOCMA antibody titers ranging up to 128. The geometric mean for all 20 was 8.11.

Immune Surveillance Under Field Conditions

Although evidence for an association between an efficient immune response to FOCMA and protection from tumor growth under laboratory conditions was quite convincing, it was still necessary to determine whether the same type of relationship occurred under natural conditions. In an attempt to answer this question, 2 major groups of cats were studied serologically: (a) those that developed spontaneous leukemia or lymphoma; and (b) those that were known to be exposed to FeLV under natural conditions and that were still healthy.

Cats that develop spontaneous lymphoid neoplasia or nonregenerative anemia all have low or negative humoral antibody levels to the FOCMA antigen (10, 13, 14, 16) (Table 2). Anemia is included with this group because it has been shown to be caused by FeLV under experimental conditions (38), and a high percentage of the spontaneous nonregenerative anemias occur in FeLV-infected cats (10, 16, 27, 34). The geometric mean antibody titer was only 0.77 in cats with spontaneous lymphoid leukemia and only 0.34 in cats with lymphoma. Only 9 of 89 cats with lymphoid neoplasia had titers of 4 or higher and only 1 had a titer of 8 or higher. The low levels of FOCMA antibody were found in lymphoproliferative disease irrespective of such factors as pathological form, geographical location, whether or not the cat was viremic with FeLV, age at the time of diagnosis, or breed (10, 16). These results indicated that the immune status of cats with spontaneous lymphoproliferative anemia was the same as that for cats with laboratory-induced progressive sarcoma or leukemia, at least in respect to the apparent poor humoral antibody response to FOCMA.

To determine the serological status of cats that remained healthy despite being constantly exposed to FeLV, we concentrated the study on about 140 cats in 2 leukemia-lym-
phoma cluster households. In one of these houses 11 cases of lymphoid leukemia had occurred over a 39-month period among a population-at-risk of 35 (8, 9, 19). In the other house, between 140 and 150 cats had resided over an 18-month period, during which time 10 cases of lymphoma were recorded (18, 19).

The possibility that other cases had also occurred could not be eliminated because several cats died of unknown causes when an autopsy was not possible. FeLV could be detected in most of the cases of leukemia and lymphoma in each of the houses, and one-third to one-half of the healthy cats were also FeLV-positive, as determined by the presence of gs antigen in circulating leukocytes and platelets (8, 18, 19).

Because of evidence that both laboratory-inoculated and naturally infected cats excrete FeLV in oral and nasal excreta (28, 43), it seemed safe to assume that more of the cats in these 2 houses were regularly exposed to FeLV.

Serological examination of the healthy cats in these 2 houses indicated that 82% had detectable FOCMA antibody. Since virus-positive cats were more likely to be antibody negative, 92% of all the cats had either detectable virus or detectable FOCMA antibody, or both. The geometric mean FOCMA antibody titer for the FeLV-negative healthy cats in the 2 houses was 8.81. This is 10- to 20-fold higher than the geometric mean titers for cats with either spontaneous or induced leukemia, or induced fibrosarcoma that progresses to kill the host.

The serological status of cats in these high-exposure environments was followed in a prospective sense at intervals of 3 to 4 months for up to 2 years. During this time 14 previously healthy cats developed leukemia or lymphoma. Of those that developed such diseases, none had FOCMA antibody titers higher than 4 for up to 5 months prior to leukemia development, and the geometric mean titer for cats that subsequently developed leukemia or lymphoma was 5- to 10-fold lower than the mean for healthy cats living in the same environments.

It was clear that FeLV-negative cats with high FOCMA antibody titers represent the group at lowest risk for tumor development. Even cats that are FeLV positive do not appear to have an increased risk for tumor development if their FOCMA antibody titers remain high. Cats in the latter category are negative for free virus-neutralizing antibody (30). On the other hand, cats that test as FeLV positive and have FOCMA antibody titers of less than 4 over a 3- to 4-month period are at greatly increased risk for tumor development. As far as these 2 cluster households are concerned, about one-half of the cats in this category will die from leukemia or another disease within a 2-year period after 1st detection.

When all the above results are taken together, it is difficult to escape the conclusion that immunosurveillance is operative against feline leukemia under natural conditions and that FOCMA is the target of the immunosurveillance mechanism. Several key questions concerning this response still remain to be answered, however. One concern is the possible role of thymus-derived T-cell or classical cell-mediated immunity, since many tumor immunologists feel that T-cell functions are generally most important.

B-cell functions have been shown to be correlated with tumor regression or prevention in some systems, however, including the Moloney murine leukemia-induced fibrosarcoma of mice (48, 49) and malignant melanoma of man (50). It is possible that the humoral antibody activity regularly correlated with regression or tumor prevention in the feline system may merely be paralleling the activity of the cell-mediated immune response. It is clear, however, that the FOCMA antibody is not acting as a blocker for cell-mediated immunity. The possibility that FOCMA antibody can either act alone (with complement) to inhibit or lyse tumor cells or facilitate cytotoxicity by immune effector cells must be considered.

Another obvious question concerns the nature of the antigenic determinant to which the FOCMA antibody of regressor cats is directed. In reference to the apparent virus-negative leukemias of man, it would be interesting to know whether the FOCMA determinant is also a virion structural component and, if so, whether it is present only at the cell membrane and immunogenic when complete virus is also being produced. Our observations on the lack of correlation with FOCMA antibody and virus-neutralizing antibody suggest that it is not a component of the virion envelope. Since we know that p15 and p30 can be expressed at the cell surface, the possibility that the regression-related FOCMA antibody of cats is directed to either or both of the antigens must remain open. Equally likely, however, is the possibility that FOCMA might include a true tumor antigen determinant, one which would not necessarily also be a virion structural component but would represent the determinant which is immunogenic in cats that resist tumor development.

Role of Immunosuppression

As mentioned earlier, immunosuppression greatly increases the risk for development of lymphoproliferative neoplasms in both man and lower animals. Since the Friend and Moloney leukemia viruses are known to be immunosuppressive under laboratory conditions (11, 51), it is logical to ask whether the same phenomenon occurs in cats following natural exposure to FeLV. Perryman et al. have shown that cats inoculated with the Rickard strain of FeLV are also immunosuppressed while incubating leukemia (55). Several reports have also described premature atrophy of the thymus in both induced and spontaneous leukemia or lymphoma of cats (3, 39). Additionally, a higher-than-expected number of cats with at least 1 particular nonneoplastic disease, infectious peritonitis, were observed to be concurrently infected with FeLV (33).

With this background, we decided to test cats presenting with nonneoplastic infectious diseases at the Angell Memorial Animal Hospital for evidence of FeLV infection (10, 16). Most cases examined were either hemobartonellosis, infectious peritonitis, septiemia, pneumonia, cystitis, or stomatitis. Of 110 cats with these diseases, 64, or 58%, were also infected with FeLV, and less than 2% of the healthy cats in the same environment were FeLV positive (10, 13). Healthy cats in the 2 leukemia cluster households mentioned above were also studied for the development of these infectious
diseases. It was determined that FeLV-positive healthy cats had a 5-fold increased risk for the development of non-neoplastic diseases when compared to FeLV-negative cats from the same houses. Despite the fact that FeLV-positive healthy cats had hematocrit values that were indistinguishable from those for FeLV-negative cats, the mean absolute lymphocyte count for healthy FeLV-positive cats was severely depressed when compared to FeLV-negative cats from the same environments (18). These observations suggest that immunosuppression by FeLV occurs under natural conditions and that healthy cats that are incubating leukemia are at a high risk for development of other infectious diseases.

Since more than one-half of the cases of random Boston cats with severe nonneoplastic infectious diseases were found to be infected with FeLV, the possibility must be considered that FeLV kills more cats by predisposing to these other diseases than by inducing leukemia. It is also possible that extensive variation exists among cats in their general immune responsiveness and that poor responders have increased sensitivity to persistent infections with both FeLV and other infectious organisms. Nevertheless, if some forms of leukemia in man are eventually shown to be caused by oncornaviruses (5, 26), the question of whether these same agents also act as immunosuppressants, even in individuals that have not developed leukemia, must also be addressed.

References

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