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Continuing Studies of Feline Sarcoma Virus Induced Tumors in Newborn Lambs and in Kittens: A Model to Study Comparative Neoplasia*

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Abstract—*Two-day-old lambs were inoculated with transformed sheep fetal (SF) cells replicating Feline sarcoma virus (FeSV), or cell-free FeSV derived from FeSV transformed SF cells. Some received autochthonous transformed SF cells replicating FeSV and others, allogeneic transformed SF cells and/or cell-free virus. Undifferentiated sarcomas occurred at every inoculation site. Tumors were usually characterized by a short latent period* (4–6 days) *rapid growth to peak size of* 2–8 cm *in maximum dimension* (10–15 days) *and rapid regression* (21–28 days *in most lambs). Variations in sizes and duration of tumors were seen in some lambs being noted even in dizygotic twins given similar inocula. Cell-free FeSV was as effective in tumor induction as were transformed allogeneic and autochthonous SF cells replicating virus. Lymphocytic infiltrations were consistently observed in all regressive tumors and regression was coincident with development of cytotoxic complement-dependent antibody and tumor associated transplantation antigen.*

INTRODUCTION

SEQUENTIAL immunological studies of the role of regional and distant nodes in the same sheep with a developing Feline sarcoma virus (FeSV) induced tumor have been reported [1, 2]. Before development of the FeSV tumor model in sheep, it was difficult to evaluate fully the immunological events that occur in the regional node after contact with malignant cells or antigens released from them. Unfortunately, FeSV induced tumors in adult sheep usually rapidly regress.

It was logical to use newborn lambs in FeSV tumor induction experiments to obtain progressive tumors because it is well recognized that newborn animals are more susceptible to the viral gene products involved in growth, invasiveness and metastasis. In previous work, FeSV purified from a kitten tumor induced undifferentiated sarcomas in sheep fetuses but not in newborn lambs which demonstrated that the sarcoma gene products in FeSV obtained directly from cats were non-transforming in newborn lambs [3]. Attempts to produce tumors in sheep with *in vitro* FeSV transformed sheep cells were successful and permitted for the first time immunological studies on sheep of various ages exposed to FeSV transformed sheep cells [2, 4, 5]. Tumors did not occur in all inoculates, however, and histologic confirmation was not always done in the early investigative research. Some of the tumors were produced in immunocompromised sheep. In one study newborn lambs were exposed to FeSV infected sheep fetal cells [6]. Five of these lambs received their own cells (autochthonous), transformed with FeSV, while 17 lambs received foreign transformed cells (allogeneic). None of the autochthonous group developed tumors while 14 out of 17 of the allogeneic group developed undifferentiated sarcomas. Nine of these regressed and five progressed to at least 10% of body wt at which time the lambs were euthanatized. Three of the five euthanized animals had been experimentally immunosuppressed. All inoculates developed antifeline oncornavirus antibody as well as

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antibody to feline oncornavirus associated cell membrane antigen (FOCMA) [7].

The present study was designed to standardize the production of FeSV-induced sarcomas in lambs with virus in FeSV transformed sheep fetal (SF) cells and to determine if inocula containing only cell free virus, produced in SF cells, would induce tumors. It was also important to repeat the experiment of inoculating FeSV transformed autochthonous SF cells versus FeSV transformed allogeneic SF cells because if tumors could be induced only with allogeneic FeSV transformed cells, it might support a hypothesis that tumors can be induced in a host in the presence of immune stimulation [8] rather than by the lack of the hosts ability to mount an immune response.

MATERIALS AND METHODS

Cell cultures

Primary sheep fetal (SF) cell cultures were initiated from either tail or ear tissue surgically removed *in utero* from 60-day-old fetuses. Approximately 0.2 gm of tissue was minced with scissors and suspended in 2 ml of Eagle's Minimum Essential Medium (MEM) containing 10% fetal bovine serum, 200 U/ml penicillin and 250 g/ml streptomycin (growth medium). This tissue suspension was repeatedly aspirated with a Pasteur pipette to further break up particles and then divided between three 25 cm^2 plastic tissue culture flasks each containing 3 ml of growth medium. The flasks incubated at 37°C were fluid changed after 24 h and every 3–4 days thereafter. Upon reaching confluency the cultures were subcultured after trypsinization.

The primary SF cells were cultured in the presence of growth medium containing cell-free FeSV to induce morphologic transformation. The source of FeSV-containing medium was a primary sheep fetal cell (BCA-3) culture which had been inoculated with FeSV prepared from feline sarcoma tissue by a modification of a procedure described by Moloney [9]. Aliquots of each passage of FeSV transformed SF cell cultures were frozen at −70°C in growth medium containing 10% dimethyl sulfoxide.

Each inoculum consisted of 2×10^8 stored frozen cells and 5×10^7 actively growing cells of the same culture, which had not been frozen. Addition of frozen cells allowed for a standard tumor inducing inoculum larger than any used previously in sheep. Each cellular inoculum of 2.5×10^8 cells contained approximately 9×10^6 FFU of FeLV [10]. It was not determined how much FeSV was contained in each inoculum; however, the amount was considerably less than that of the injected FeLV [10, 11]. Pooled, quick-thawed aliquots of cells from each passage were washed three times in MEM by centrifugation to remove fetal calf serum before injection into experimental lambs. Cell-free FeSV inocula were obtained from pooled culture medium of FeSV transformed SF cell cultures by differential centrifugation. Clarification of this medium at 9846 ***g*** for 45 min in a Sorvall RC-5 Super Speed refigerated centrifuge was followed by pelleting of virus material at 70,000 ***g*** for 1 hr in a Beckman L-350 ultracentrifuge at 4°C. Each cell-free inoculum contained approximately 5×10^7 FFU of FeLV and one-half to one log less of FeSV.

Animal studies

Ten, time-dates, pregnant, 2–5-yr-old, crossbred ewes were purchased from a commercial breeder; the uterus was entered aseptically through a ventral midline abdominal incision under general halothane anesthesia to harvest ear or tail tissue from 60-day-old fetuses. After routine closure and recovery from anesthesia, ewes were held on irrigated pasture until lambing. Newborn lambs from these pregnancies were inoculated s.c. approximately 48 hr *post partum* with either their own FeSV transformed cells (autochthonous) or transformed cells derived from another fetus (allogeneic) or cell-free pellet from pooled media of several cell lines or multiple inoculations of allogeneic cells and cell-free pellet (Table 1). Two uninoculated lambs were used as contact controls. To confirm presence of tumorigenic activity within the transformed SF cells, five kittens, 5–10-weeks-old were inoculated with transformed SF cells; one kitten was inoculated with cells obtained from a culture derived from a lamb tumor.

Tumor induction

Lambs were palpated every other day for detection of tumors and superficial lymph node enlargement. Tumor growth was recorded as approximate length, width and thickness from which approximations of tumor volume were determined.

Table 1. Sources of inocula and quantity used per site in experimental lambs

Lambs receiving autochthonous cells	Dose of cells and source cell culture		Approximate FFU*
1A	2.5×10^8	1A/FeSV	5×10^6
2	2.5×10^8	2/FeSV	5×10^6
Lambs receiving allogeneic cells			
1B	2.5×10^8	2/FeSV	5×10^6
3B	2.5×10^8	3A/FeSV	5×10^6
7B	2.5×10^8	1A/FeSV	5×10^6
11A†			
Site A	4×10^8	1A/FeSV	N.D.
Site B	4×10^8‡	10/FeSV	N.D.
11B†			
Site A	4×10^8	1A/FeSV	N.D.
Site B	4×10^8	9A/FeSV	N.D.
12	2.5×10^8	1A/FeSV	5×10^6
13	2.5×10^8	3A/FeSV	5×10^6
Lambs receiving cell-free virus	**Amount of medium processed for cell-free inoculum**		
11A†	Site C 4 1		1×10^7
11B†	Site C 4 1		1×10^7
14	9 1		2×10^7

*FFU = focus forming units.
†Lambs 11A and 11B were inoculated with separate inocula at 3 different subcutaneous sites.
‡These were male cells but the cell culture established from the tumor (11A-T-B) was female in karyotype.

Microscopy

Cell cultures of SF cells transformed by FeSV and cell cultures derived from lamb tumors were fixed in 2% glutaraldehyde in Millonig's buffer and post fixed in 1% uranyl acetate and embedded in araldite. Ultrathin sections were cut on a Reichert ultramicrotome and stained with uranyl acetate and lead citrate. Grids were examined in a Philips EM-200 electron microscope; 100 cells from each culture were observed in detail. Needle biopsies of tumors were taken every other day beginning when tumors reached approximately 2 cm in largest dimension. Selected needle biopsies of tumor tissue were fixed in Karnovsky's solution, transfered to glutaraldehyde and processed as above. Other biopsy tissue was immersed in 10% neutral buffered formalin for 24 hr, embedded in paraffin, sectioned at 4–6 μm and stained with hematoxylin and eosin. Replicate sections were also stained with Giemsa, alcian blue, Masson's trichrome, and by Gordon–Sweet's reticulin method and by the periodic acid-Schiff reaction. Kartotypic analysis was conducted on lamb tumor cell culture, 11A-T-B, to determine the sex of the tumor cells. Ten metaphase spreads were examined.

Clinical pathology

White and red blood cell counts and serum biochemistry analysis were run every other day on all lambs beginning with pre-inoculation samples on the first day *post partum.*

Antibody studies

Serum from inoculated lambs and controls were collected every 2–3 days during induction, progression, regression and post-regression of tumors. It was stored at −70°C and later tested for levels of complement dependent cytotoxic antibody using the chromium-51 release test (CRT), antibody to feline oncornavirus cell membrane antigen

(FOCMA) by indirect immunofluorescence as previously described and by double immunodiffusion in agar gel using ether-disrupted feline leukemia virus (FeLV) as antigen [2, 9, 10, 11]. Seven percent agar was used with 0.004% Orange-G added for ease of visualization in the latter test.

RESULTS

Cell cultures

A normal sheep fetal cell culture, BCA-3 (Fig. 1) was transformed with FeSV obtained from a kitten tumor within 5–10 days post exposure (Fig. 2). Transformation was characterized by increasing diffuse haphazard arrangement of transformed cells, increased numbers of fusiform and rounded forms with focal piling up and with detachment of many cells into the growth medium. Upon transfer to new flasks, unattached cells rapidly formed new populations of adherent and non-adherent cells. Sheep fetal cell cultures from other fetuses exhibited similar rapid transformation when exposed to cell-free medium of BCA-3 transformed with FeSV. Cell cultures were readily established from explants of tumor tissue and appeared morphologically similar to the *in vitro* transformed sheep fetal cell cultures. Normal sheep fetal cell cultures underwent morphologic transformation within 7–14 days when cultured in the presence of cell-free growth medium from tumor derived cell cultures.

Tumor induction and growth kinetics

Tumors arose at the site of all FeSV inoculations. Table 1 contains the protocol for inoculations and in Table 2 the clinical course of induced tumors at various sites in experimental lambs is outlined. Typically, tumors were clinically palpable during the first week, reached maximum size in the second week (Fig. 3) and were completely regressed in the third to fourth week. Lamb 13 was euthanized when the tumor was just beginning to decrease in size at 18 days post inoculation. Animal 11A had relatively large tumors, at the three sites of inoculation, equal to 18% of body wt at necropsy. Its dizygotic twin, 11B, had small tumors at the inoculation site with FeSV transformed SF cells (sites A and B) and these tumors regressed quickly. The site of cellfree inoculation in each animal 11A and 11B, was designated 'Site C', and seemed to have delayed onset of tumor although this was possibly due to the fact that tumors produced by cell-free FeSV arose as flat diffuse thickenings in the subcutis and deep fascia rather than as discrete prominent nodules typical of

Table 2. Clinical course of tumors

Lamb	Type of inoculum*	First palpated (P.D.†)	Max dimension at cm	Max dimension at P.D.†	Totally regressed (P.D.†)
1A	Auto	5	4	11–13	24
1B	Allo	4	5	11–13	24
2	Auto	4	2	8	13
3B	Allo	6	8	11	18
7B	Allo	5	3	9	16
11A					
Site A	Allo	4	8	15	progressed
Site B	Allo	4	12	15	progressed
11B					
Site A	Allo	4	4	7	16
Site B	Allo	4	2	5	7
12	Allo	4	3	11–16	28
13‡	Allo	4	8	9–18	—
11A					
Site C	Virus	12	20	15	progressed
11B					
Site C	Virus	12	16	16–30	50+
14	Virus only	5	12	14–21	40+

*Auto = inoculum derived from FeSV transformed autochthonous cells: Allo = inoculum derived from FeSV transformed allogeneic cells.

†P.D. = post-innoculation day.

‡This lamb was orphaned at PD 9 and developed servere digestive upset and depression by PD 18 at which time the animal was euthanized having a tumor that was beginning to regress.

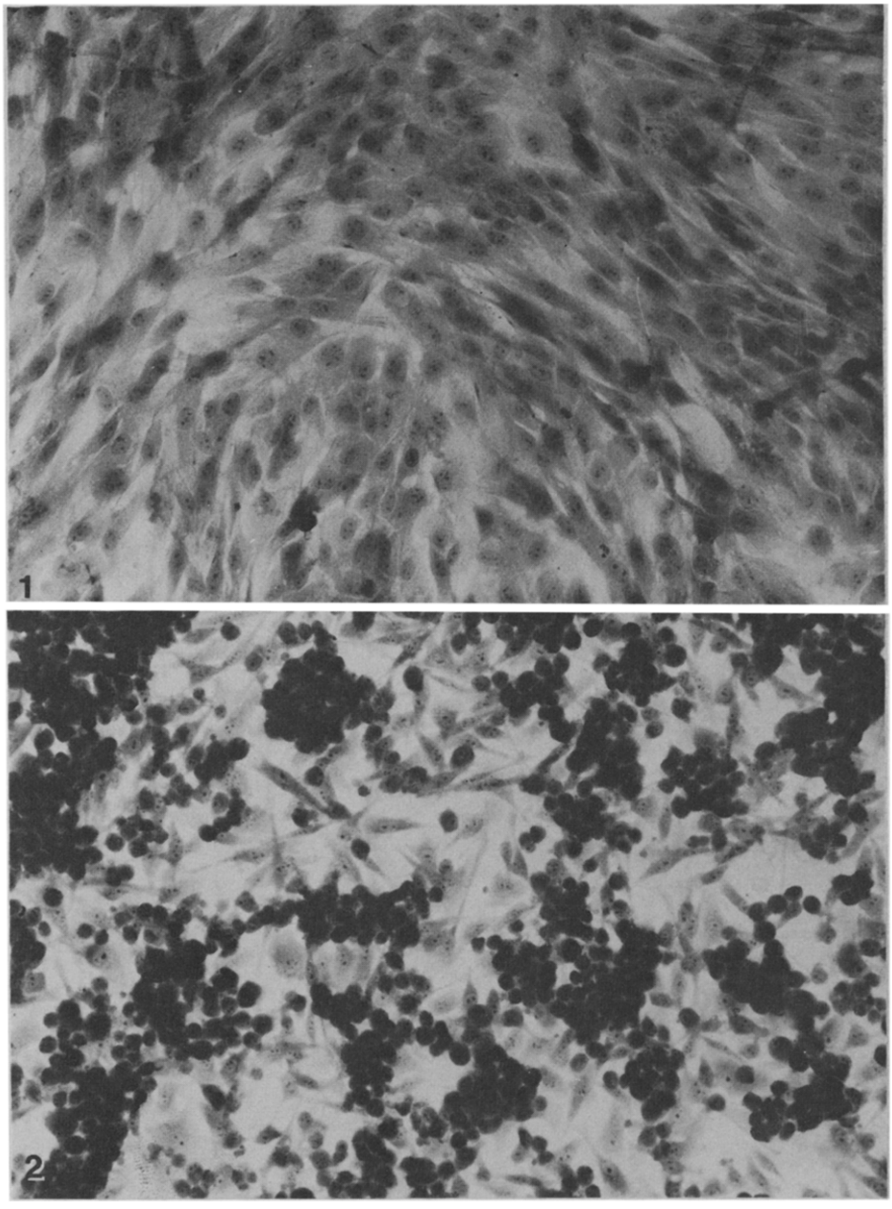

Fig. 1. *Normal sheep fetal cell culture, BCA-3. Note normal organization of the monolayer.* (*Giemsa stain;* ×540).

Fig. 2. *Sheep fetal cell culture BCA-3* 10 days *after inoculation with* 1.5×10^5 *FFU of FeLV containing FeSV derived from a kitten sarcoma. Note lack of normal organisation and rounding and piling up of cells.* (*Giemsa stain;* ×540).

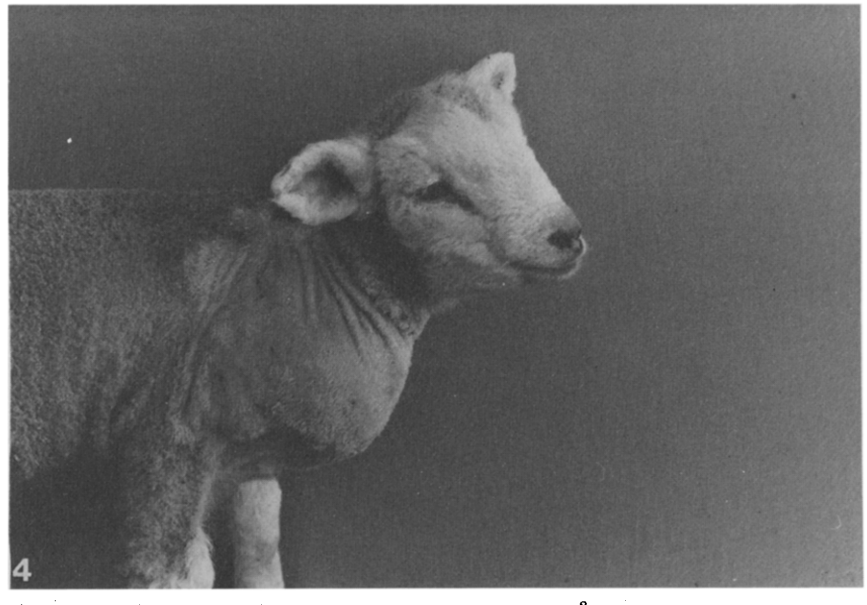

Fig. 3. *Lamb* 1*B at* 12 days *post-inoculation with* 2.5×10^8 *FeSV transformed sheep fetal cells. The firm s.c. mass was* 5 cm *in diameter, dome-shaped and surrounded by edematous subcutaneous tissue. The tumor of lamb* 1*A, given a similar dose of FeSV transformed autochthonous cells was almost identical. Both tumors regressed spontaneously.*

Fig. 4. *Lamb* 14 *at* 21 *days post-inoculation with cell-free FeSV. The nodular tumor which later regressed spontaneously measured approximately* 12 cm *in largest dimension and was approximately* 6 cm *thick. The inoculum was given at the uppermost part of the shaved area. The prescapular lymph node was involved in the tumor at this time.*

sites where FeSV transformed SF cells were inoculated.

The physical characteristics of the tumors varied with time. Early nodules tended to be flat and very firm, and surrounding tissues were usually edematous. Extensions and thickenings of the growing tumors were likewise firm. Lamb 14 given cell-free virus developed three nodules simultaneously, one at the inoculation site, one at the thoracic inlet and one in the prescapular lymph node. These all enlarged to form one nodular mass (Fig. 4). Softening was notable as tumors decreased in size. Disappearance was complete in most animals but those with a protracted course, 11B and 14, had persistent firm nodules in the subcutis at the cell-free inoculum sites which, histologically were found to be fibrous tissue.

Two lambs maintained as contact controls were examined for signs of clinical illness or tumor development as well as serologic evidence of infection with FeSV. There was no clinical illness nor was there any detectable complement-dependent cytotoxic antibody in these control lambs.

The five kittens, inoculated with 10^6 cells from FeSV transformed cell cultures BCA-3/FeSV, 1A/FeSV, 2/FeSV, 5/FeSV, 10/FeSV, respectively, and the one kitten inoculated with lamb tumor derived cells grown in culture developed tumors at the inoculation site and multiple metastases. Histologic examination of these neoplasms revealed them to be typical fibrosarcomas as previously described [12].

Microscopy

Transformed primary SF cells used as inocula contained budding and/or free type C particles in four of five cultures examined. Two of seven tumor-derived cell cultures contained type C particles. Examination of 100 cells was done from each tumor cell culture. One tumor biopsy (lamb 3B) had possible mature and budding type C particles while in three other tumors, virions were not found in single sections of 100 tumor cells.

All tumors induced in lambs were histologically similar to FeSV induced, undifferentiated sarcomas in other heterospecies [13, 14]. As in other studies, the regressing tumor was heavily infiltrated by lymphocytes. The progressive tumors of lamb 11A had no lymphocytic infiltrates. Details of pathology are to be reported elsewhere.

Electron microscopy of tumor and tumor derived cell cultures revealed a consistent popluation of cells with fairly uniform characteristics. The nucleus was round to elongate, had a fine chromatin pattern with condensation along the nucleolemma and usually a single nucleolus. Cytoplasm had a background of fine fibrils and microtubules with few profiles of rough endoplasmic reticulum, and a golgi apparatus. The cytoplasmic membrane was slightly ruffled.

The host origin of the tumors was confirmed by karyotypic analysis of the tumor in one lamb inoculated with allogeneic cells of the opposite sex. The tumor cells were of the host's own sex and caused by FeSV [6].

Clinically pathology

Red blood cell and white blood cell counts were variable with each lamb and among experimental lambs and control lambs, and did not conform to a clinically significant pattern. Examination of lamb sera for selected electrolyte, serum enzyme, bilirubin, glucose and cholesterol levels revealed no apparent alteration except for calcium and alkaline phosphatase. In animal 11A, calcium levels were normal (9–11 mg/dl) up to 8 days post inoculation. By postinoculation day 12, the value was 16 mg/dl and by day 14, 19.2 mg/dl; both samples were in the hypercalcemia range. The animal was euthanatized *in extremis* at this time. Serum alkaline phosphatase (SAP) values at these same times were 448, 490 and 132 U/l, respectively. Serum alkaline phosphatase values fluctuated in the control animals but were between 800 and 2000 U/l during the first 3 weeks of life. In other experimental animals the SAP value reached its lowest level (well below that of the controls) when tumors were largest.

Antibody studies

All inoculated lambs developed rabbit complement-dependent cytotoxic antibodies. Cell lysis was induced in FeSV-transformed SF cells, cultured cells derived from lamb tumors and FL-74 cells, a cat lymphoblastoid cell suspension line replicating feline leukemia virus [15]. Using the chromium-51 release test, cytotoxic antibody was first detected as the tumors reached maximum size [16]. The ability of sera to lyse cells increased during the tumor regression period. All inoculates developed antibody for tumor associated transplantation antigen that may be the same cell membrane antigen described as FOCMA. This antibody was detectable by indirect im-

munofluorescence to at least a serum dilution of 1 : 125. Four of 10 lambs had group specific anti-FeSV antibody detectable in double immunodiffusion plates using undiluted serum against ether-disrupted FeLV while two of the six negative sera were weakly positive when sera were concentrated according to a previously published technique [2].

DISCUSSION

Transformation of sheep fetal cell culture by FeSV from experimental kitten tumor or from SF culture medium took 5–10 days in contrast to 4–6 weeks previously reported [5, 6]. This may be a matter of dose as 1.5×10^5 FFU of FeLV was used, which we estimated to be about ten times the amount used by us previously. Ten ml of cell-free medium from cell culture BCA-3/FeSV was sufficient to transform other SF cell cultures almost as quickly as virus obtained directly from cat tumors indicating that sheep cell cultures will readily produce transforming FeSV. Transformation of SF cell culture 9A differed from others in having a slower growth rate and less pronounced morphologic changes which indicates a possible cellular genetic component exists in regard to sheep cell transformation. This culture, 9A/FeSV, did produce a histologically confirmed tumor in site B of lamb 11B indicating that the replicating virus contained genetic information that resulted in tumor growth in a lamb despite its unusual characteristic in cultured cells.

Morphology and pattern of growth of cultures established from sheep tumors was similar to that of FeSV-transformed sheep fetal cultures, with characteristic rounding of cells that divided from the FeSV transformed fibroblastic cells and became free floating. A similar appearance has been reported for Snyder–Theilen strain-FeSV transformed non-producer mink lung cells (Mu/Lu) while other strains of FeSV produced different morphologic patterns in non-producer Mu/Lu cells [17]. These findings give evidence that each strain of virus is responsible for different types of transformed cellular morphology. The normal sheep fetal cell cultures appeared morphologically as fibroblasts as previously described [5]. Most feline sarcoma virus induced tumors in animals are fibrosarcomas or undifferentiated sarcomas [2, 4, 13, 14, 18, 19]. Reasons for histological differences are probably explained by species variations of host cell–virus interaction. It can be concluded from reports of others and from this study that a mesenchymal type cell including the fibroblast is "target" for transforming FeSV. EM studies of tissue cultures derived from lamb tumors or FeSV-transformed fetal cells revealed a paucity of virions when compared with FeSV transformed cat cells reported earlier [12]. However, all FeSV transformed SF cell cultures contained sarcomagenic gene products for growth, invasion and metastasis of tumors in cats because all inoculated kittens developed metastasizing fibrosarcomas.

In this study several different types of inocula produced biologically different sarcomas in sheep. In a pilot study, lambs inoculated with their own cells (autochthonous) transformed with FeSV did not develop sarcomas but lambs inoculated with FeSV transformed allogeneic sheep fetal cells developed tumors [6]. This suggested that an immune response related to histocompatibility differences might be contributing to tumor induction, which supported a very intriguing hypothesis in which it was suggested that an immune response enhanced neoplasia [8, 20]. In the present study, however, autochthonous as well as allogeneic cells, and cell-free virus inocula produced histologically confirmed tumors in lambs. A possible explanation for the difference in our two studies might be that we used about 2.5 times as many cells in this experiment and each inoculum of 2.5×10^8 cells contained approximately 9×10^6 FFU of FeLV. This suggests a virus dose response relationship although it does not completely rule out tumor enhancement by immune stimulation [6, 8, 20].

Another important finding was the occurrence of sarcomas at every inoculation site, indicating the reliability of these inoculation methods in the production of FeSV induced sarcomas in 2-day-old lambs. In the animals with multiple site inoculations of larger doses (lambs 11A and 11B, Tables 1 and 2) tumors were usually larger, again signifying a dose response relationship, as reported earlier in cats [18].

Tumors in lambs occurred very early after inoculation with transformed SF cells replicating FeSV. These inocula contained considerably less FeSV than the cell-free preparation (Table 1) and yet with the cell-free virus, tumors seemed to take longer to develop. It is possible that cell-associated virus contains greater amounts of transforming genetic information than cell-free virus preparations which would be one explanation for the quicker tumor development.

Two control lambs in constant contact with some of the inoculated lambs developed no tumors, nor antibody against feline oncornaviruses or tumor associated transplantation antigens. This would indicate that FeSV induced tumor in sheep is a self limiting disease and this is probably true for other heterospecies as well.

Younger animals tend to develop larger tumors as a rule [1]. However, among lambs in this experiment, of the same age, given the same number of FeSV-infected cells with associated virus, there was variation in tumorigenic potency of the inocula. With the exception of the cell-free virus inoculates, regression was usually complete at about 3–4 weeks post inoculation as in other species, regardless of size of tumor induced. Efficient host anti-tumor activity is thus present or develops in these lambs as in most other species. This activity appears to parallel the influx of round cells into tumors as well as the occurrence of antibodies in sera as determined by a variety of methods. Antibodies are directed against internal viral antigens, envelope antigens and virus-induced antigens on cell surfaces (probably FOCMA) [1, 4].

The immunological role of cell mediated immunity has been studied during regression of massive FeSV induced tumors in sheep, but all immunological events are not fully understood [2]. In these studies tumor regression coincided with appearance of anti-FeSV antibody directed against viral antigens and nonviral antigens on the cell surface. Cell surface antigens were probably tumor associated transplantation antigens. Previously, we demonstrated the regional lymph node is the first line of defense against establishment of FeSV induced tumors in adult sheep although the response became generalized, and cell mediated as well as humoral factors played a role in tumor rejection [1]. Tumor regression of FeSV induced tumors in heterospecies is expected [2–6, 9]. Regression does not seem to be consistent, however, in marmosets (*Sanguinus fascicollis*) [19]. Regression usually doesn't occur in kittens inoculated before 12–16 weeks of age unless a protective antibody has been passed from dam to offspring. It has been recorded in our laboratory that dams obtain antigens from their FeSV inoculated kittens which results in an immune response in the dam which is passed to subsequent litters of kittens generally giving them an immunity to FeSV challenge [21, 22]. This protective antibody has been referred to as feline oncorna cell membrane antigen (FOCMA). It appears that the same type of biological process is working in tumor regression of sheep tumors. There is now active research in our laboratory (unreported results) as well as other laboratories trying to identify this protective protein or protein species [23, 24].

In two previous studies, karyotypic analysis has consistently verified the host origin of tumors; i.e., lambs received FeSV transformed cells of the opposite sex and induced tumors were of the inoculated lamb's own sex [2, 6]. Karyotypic analysis with one tumor examined in this study agreed with previous observations thereby demonstrating the induced tumor was autochthonous and of viral origin, not a transplant. All tumors were not karyotyped since it would have been unnecessary repetition of previous work. The induction of tumors in lambs with cell-free, sheep-adapted FeSV also verified that it is the sheep-adapted FeSV which is tumorigenic in contrast to FeSV purified directly from cat tumors [3].

There is no correlation of most clinical pathological values and tumor course with the exception that lamb 11A had a marked hypercalcemia which undoubtedly led to the comatose state. Unfortunately, due to the rapid tumor progression, the hypercalcemia was not detected until after death. One can speculate that there were tumor hormone secreting cells that led to a para-neoplastic syndrome. Reports have been published concerning hypercalcemia of naturally occurring tumors of man [25] and outbred animals [26] but this syndrome apparently has not previously been associated with FeSV induced tumors [25–27]. Others may wish to speculate on the relatively low alkaline phosphatase values noted at the time when tumors were largest, and the relation of these values to the paraneoplastic syndrome.

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