distinguishing the mutant from HbA by either complement fixation or precipitin tests (*11*), although attempts to actually isolate a mutant-specific antibody population were only reported with goat antibodies to HbS (*12*).

All the variants eliciting monospecific antibody (Table 1) contained substitutions exposed to the exterior of the macromolecular surface, a finding consistent with observations made with other globular protein antigens (*13, 14*). Since the amino acid substitution in each of the variant hemoglobins is responsible for inducing a specific antibody response, it must be located in or near an antigenic determinant area. Therefore, either these areas exist as natural determinants on normal hemoglobin, or the substitutions transformed immunogenically silent areas into new reactive sites. To examine these alternatives we absorbed antiserum to HbA with HbG Philadelphia and obtained antibody reactive with HbA but not with HbG Philadelphia, suggesting that the asparaginyl residue at α^{68} of HbA is part of a normal antigenic determinant. In contrast, absorption of antiserum to HbA with Hb Beograd removed all antibody to HbA, suggesting that the glutamyl residue at β^{121} is not a part of a determinant in normal HbA. Reichlin has also identified the involvement of the asparaginyl residue at α^{68} in an antigenic determinant site of HbA (*15*), whereas a substitution at β^{121} had no effect on the antigenicity and was presumably not located in an antigenic area (*14*). Similarly, absorption of antiserum to HbA with HbS or HbC completely eliminated all antibody activity to HbA, indicating that the glutamyl residue at β^{6} is not generally immunogenic in rabbits and may explain the difficulty experienced by other investigators in preparing specific HbA [β^{6}(Glu)] antiserums. Another explanation advanced for the failure to obtain rabbit antibodies specific for the β^{6} (Glu) residue after absorption is that the antibody has comparable degrees of binding affinity for both HbA and HbS and is removed by immunoabsorption (*14*). Further absorption of antiserum to HbA with a variety of different variant hemoglobins should help clarify the structural basis of the immunogenicity and antigenicity of hemoglobin.

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Feline Oncornavirus–Associated Cell Membrane Antigen: Expression in Transformed Nonproducer Mink Cells

Abstract. *The feline oncornavirus–associated cell membrane antigen (FOCMA) is a target for naturally occurring immunity that protects the cat against development of fibrosarcoma and leukemia. Feline sarcoma virus–transformed "nonproducer" mink cells express high levels of FOCMA, but not of the major viral structural proteins. Transformation of the same cells by murine sarcoma virus, or infection with feline leukemia virus, which is nontransforming for epithelial or fibroblastic cells, did not induce FOCMA. Thus, FOCMA expression in mink lung cells is specifically associated with transformation by feline sarcoma virus.*

Antibody to feline oncornavirus–associated cell membrane antigen (FOCMA) is the major factor in determining whether or not an animal successfully resists tumor development after infection with feline sarcoma virus (FeSV) (*1, 2*) and feline leukemia virus (FeLV) (*3*). In vivo, FOCMA is immunologically identical whether induced by FeLV or FeSV (*3, 4*). Healthy viremic cats often have significant titers of antibody to FOCMA, but such animals never have free antibody to the major virus envelope (gp70) and core (p30) proteins (*4, 5*). By a number of criteria, FOCMA appears to be distinct and separate from the viral structural proteins (*4–6*).

The availability of nonproducer FeSV-transformed mink cells (*7, 8*) has made it possible to test for FOCMA expression in cells of a heterologous species in the absence of virus production. Three independently isolated cell lines of FeSV-transformed mink lung cells (Mvl-Lu) were used; each had been transformed with the Gardner-Arnstein strain of FeSV (*9*). Our data indicate that FOCMA expression is dependent on transformation by FeSV, and independent of the presence of viral structural proteins. In association with previous results in vivo, FOCMA, therefore, appears to be induced by transformation events associated with the expression of either FeLV or FeSV. We believe this is the first report describing a tumor virus–associated antigen on nonproducer tumor cells that is known to be immunogenically effective under natural conditions.

The FeSV-transformed nonproducer mink cell cultures used are as follows: lines 64F1 and 64F2 which were uncloned mixed cultures of transformed and untransformed cells; F1 Cl 10, F1 Cl 13, and F1 Cl 16 that were derived from transformed single cell clones of 64F1; and 64F3 Cl 7 which was a clonal culture of transformed cells derived from a third mixed culture. The parent line (Mvl-Lu) was also nonproductively transformed with the Kirsten murine sarcoma virus (Ki-MSV) and designated

64J1. The 64J1 line superinfected with heterologous helper virus, known as 64J1 M111I, was also tested (*7*, *10*). None of the nonproducer transformants release into the supernatant virus that is detectable by the reverse transcriptase assay or by radioimmunoassay for the major viral protein, p30 (*10*). Rescuable sarcoma virus was demonstrated by superinfection with a variety of helper viruses (*7*). All lines of mink origin were maintained in Dulbecco's modification of Eagle's minimal essential medium (MEM) with 15 percent fetal bovine serum.

The standard reference cat lymphoma cell line, F1-74, induced in vivo by FeLV, was the positive control target cell for all antigens induced by FeLV-FeSV (*1*, *6*, *11*). To establish FOCMA induction in vitro in cat cells, several cultures were used. A normal feline fibroblast cell line, FLF-3 (*12*) was used as an uninfected control, and also used after infection with FeLV or infection and transformation with FeSV (FeLV). CCC-clone 81, a transformed nonproducer feline kidney line containing the Moloney sarcoma virus (MSV) genome was also used before and after superinfection with FeLV (*13*). The cat cell cultures maintained in McCoy's 5a medium were F1-74, with 20 percent fetal bovine serum, and CCC-81, with 15 percent fetal bovine serum. FLF-3 was maintained on MEM with 20 percent fetal bovine serum.

For the demonstration of FOCMA, FeLV gp70, and FeLV p30 on cell membranes, indirect immunofluorescence was used. This test has been described previously for detection of the antigens on the reference cultured lymphoma cells (F1-74) which grew free in suspension (*1*, *6*, *11*). For the other lines, which grow in an adherent or partially adherent manner, the cells were trypsinized, washed, and resuspended in rapidly agitated fresh growth medium at 37°C for at least 3 hours before examination.

The positive reference antiserum to FOCMA was obtained from a healthy nonviremic cat (I-12) from a breeding cattery where FeLV is known to be present and where constant exposure to the virus occurs (*14*). This positive reference antiserum was exhaustively absorbed with FeLV until antibodies to the FeLV gp70 and p30 proteins were no longer detectable by radioimmunoprecipitation (*4*, *5*). For detection of membrane FeLV proteins, standard high-titered goat antiserum to FeLV p30 (a gift from F. de Noronha) and rabbit antiserum to FeLV gp70 (a gift from Dr. W. Hardy) were used. Fluorescein-conjugated goat antiserum to rabbit immunoglobulin G (IgG) was used at a dilution of 1 : 30 and fluorescein-conjugated rabbit antiserum to sheep IgG was used at a dilution of 1 : 10. Details on the serum reagents and testing procedures have been described (*6*, *11*).

For titration of infectious FeLV, the CCC-81 line was used in an infectious center assay. This technique substitutes tenfold dilutions of cells for culture supernatant as described by Fischinger *et al.* (*13*).

The morphologic appearance of the mink cells is shown in Fig. 1. The control line, Mvl-Lu, appears as an epithelial-like flat monolayer, whereas the transformed phenotype of the others range from mild for 64F1 to extreme for 64F3 Cl 7 and 64J1. Of the antigens tested for, only levels of FOCMA corresponded to the state of transformation induced by FeSV of the cells (Table 1). Mvl-Lu displayed no evidence of FOCMA on the plasma membrane and no morphologic alteration. The percentage of the cells in the 64F1 line that are rounded (35 to 45 percent) parallels the percent positive for FOCMA (34 percent). More than 80 percent of the cells in F1 Cl 10, 13, 16, and F3 Cl 7 appear

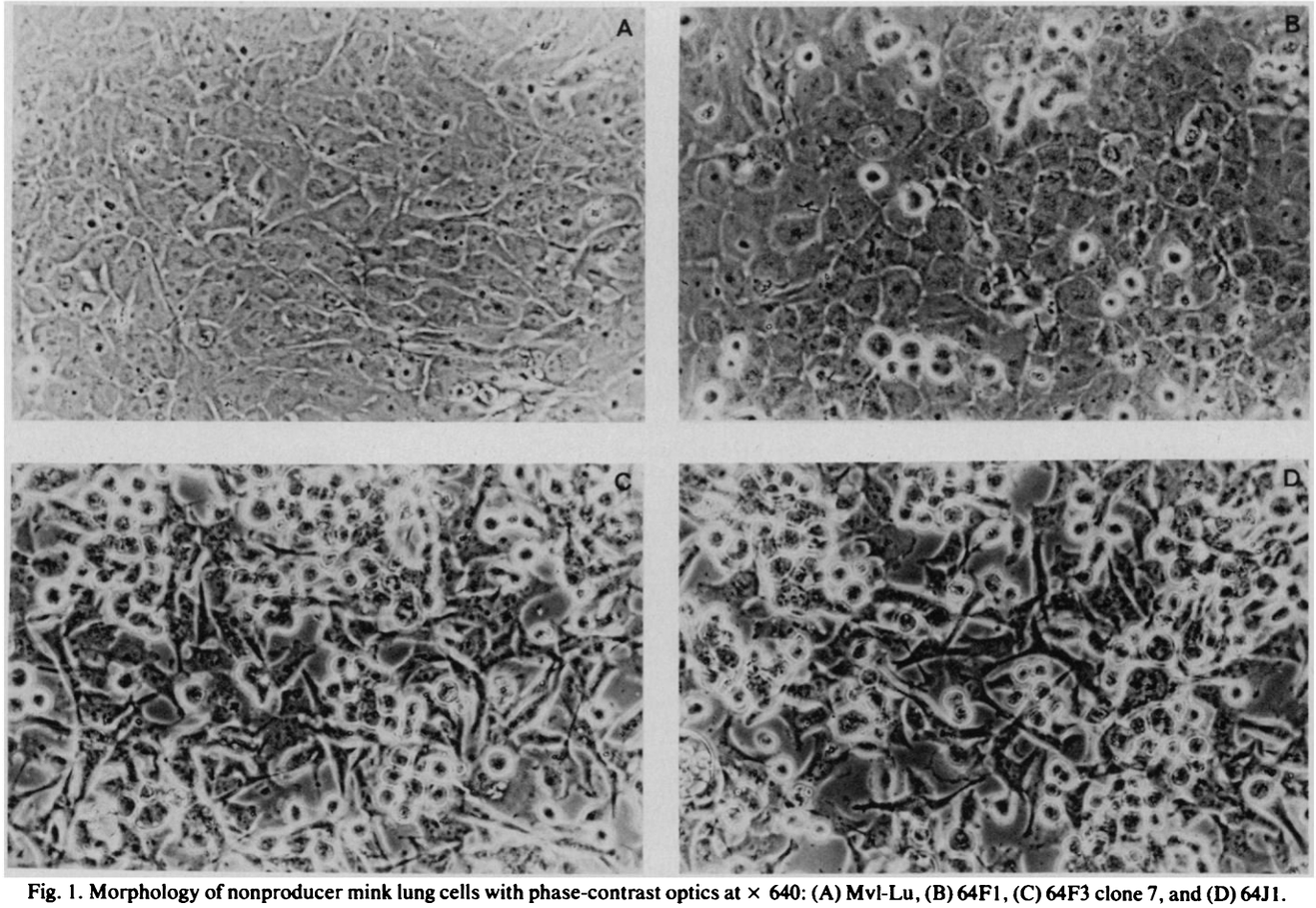

Fig. 1. Morphology of nonproducer mink lung cells with phase-contrast optics at × 640: (A) Mvl-Lu, (B) 64F1, (C) 64F3 clone 7, and (D) 64J1.

Table 1. Antigen expression and transformed phenotype in mink lung cells and cat cells. Abbreviations: NP, nonproducer cells; Ki-MSV, Kirsten murine sarcoma virus; ND, not done.

Class	Designation	Type of line	Percent transformed	Antigens present on cell surface*			FeLV cytoplasmic p30	Virus release†	Rescuable sarcoma genome
				FOCMA	FeLV gp70	FeLV p30			
Control	F1-74	Feline lymphoma	(100)	100	100	100	+	+	−
	Mvl-Lu	Mink control	< 5	< 5	< 5	< 5	−	−	−
	Mvl-Lu + FeLV	Above infected with FeLV	< 5	< 5	100	100	+	+	−
Mink	64F1	FeSV-transformed NP cell	35–45	34	< 5	< 5	−	−	+
	64F1 Cl 10, 13, 16	Clones of above	> 80	100	< 5	< 5	−	−	+
	64F2	FeSV-transformed NP cell	50–60	80	< 5	< 5	−	−	+
	64F2 + FeLV	Above infected with FeLV	50–60	80	100	100	+	+	+
	64F3 Cl 7	FeSV-transformed cloned NP cell	> 80	100	< 5	< 5	−	−	+
	64F3 Cl 7 + FeLV	Above infected with FeLV	> 80	100	100	100	+	+	+
	64J1	Ki-MSV–transformed NP cell	> 80	< 10	< 5	< 5	−	−	+
	64J1 + PP-1R	Above infected with heterologous helper	> 80	< 10	< 5	< 5	+	+	+
Cat	CCC-81	Feline kidney, S+L− with MSV	< 5	< 5	< 5	< 5	ND	−	+
	CCC-81 + FeLV	Above infected with FeLV	> 80	< 5	100	100	ND	+	+
	FLF-3	Normal feline fibroblast	< 5	< 5	< 5	< 5	ND	−	ND
	FLF-3 + FeLV	Above infected with FeLV	< 5	< 5	100	100	ND	+	ND
	FLF-3 + FeSV	Above transformed with FeSV	> 80	100	100	100	ND	+	ND

*Percent of cell population. †Tested by infectious center assay on CCC-81 cells.

altered, and likewise these lines express the extreme of FOCMA expression, with all the cells positive. Those cells which are positive give reactions with the positive reference serum at dilutions ranging from 1 : 16 to 1 : 128, while the negative cells give no reaction at 1 : 2. In contrast to this trend, no viral structural proteins were detected on the surfaces of any of these cell lines.

To determine whether viral antigens could be induced independent of FOCMA, the FeSV nonproducer as well as the untransformed mink cells were infected with subgroups A and C FeLV. After 1 month, with passages three times per week subsequent to superinfection, virus replication was present regardless of the state of morphologic transformation and the presence of FOCMA. The control line remained negative for FOCMA, but became positive for gp70 and p30. All the transformed nonproducer lines converted to positive for the structural antigens but were otherwise unchanged. The growth pattern of all the cell lines was unaltered by infection with FeLV. Viral structural proteins on the cell membrane, as detected by immunofluorescence, correlated with virus release when tested by the infectious center assay. These results suggest that FOCMA expression is associated with transformation and is not associated simply with virus replication. To establish that FOCMA is specifically dependent on transformation by FeSV, rather than being an indirect effect of the transformed state itself of the mink cells, a mink cell line (64J1, which had been transformed by Ki-MSV) of equal morphologic alteration as 64F3 was tested. Transformation by Ki-MSV did not induce FOCMA expression. The same line infected with a heterologous helper virus did not react with antiserum to feline gp70 or p30 although it did react with an antiserum to murine leukemia virus.

Of the feline cell monolayer cultures, only those infected and transformed with FeSV expressed FOCMA. The same line infected with FeLV, which is nontransforming for adherent cells, became positive for the structural proteins of the virus (gp70 and p30) but did not become positive for FOCMA. The presence of the Moloney MSV genome in CCC-81 did not induce FOCMA, nor did the phenotypic alteration induced in this line after superinfection with FeLV. The results from both the mink and cat cells imply that FOCMA expression is specific for transformation by FeSV.

Under field conditions, FOCMA has relevance as the target for a naturally occurring immunosurveillance response against leukemia and sarcoma development (*3, 14, 15*). The amounts of humoral antibody present in cats to FOCMA are inversely correlated with tumor progression (*3, 14, 16*). Antibodies directed to FOCMA are cytotoxic in the presence of complement (*4, 17*). Cells that contain FOCMA have been used as a vaccine to protect against development of leukemia or sarcoma subsequent to challenge with FeLV or FeSV (*18, 19*). Successful vaccination was not associated with antiviral immunity, an observation which is compatible with a distinction between FOCMA and the FeLV structural proteins (*19*). Another line of evidence suggesting that FOCMA is discrete from the known viral structural proteins was obtained from observations of discordance between antibody to FOCMA and to various virus antibody titers, and a failure to remove FOCMA by absorption either with whole FeLV or with purified FeLV proteins (*4–6*).

The FeSV-transformed nonproducer cells described should represent a valuable resource for further characterization of FOCMA. The data from these mink cell lines have demonstrated that FOCMA production correlates with morphologic transformation specifically induced by FeSV, and is independent of the presence of viral structural proteins on the plasma membrane. Previous in vivo data have indicated that FOCMA occurs on the plasma membranes of feline lymphoid cells transformed by FeLV. Together, these results demonstrate that the expression of FOCMA parallels transformation induced by either FeLV or FeSV. Therefore, FOCMA appears to be the first described nonvirion tumor-specific surface antigen that is induced by a naturally occurring oncornavirus of an outbred mammalian species.

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Potential Operating Region for Ultrasoft X-ray Microscopy of Biological Materials

Abstract. *Calculations are presented which indicate an extensive suboptical region in the microscopy of biological materials in their natural state which is accessible to ultrasoft x-ray transmission microscopy. Throughout most of the region, radiation dosage levels to the specimen are lower than in electron microscopy.*

It has been shown in recent work (*1*) that contact microradiography with the use of ultrasoft x-rays and high-resolution polymer resist detectors is capable of resolutions of the order of 100 Å with unstained biological materials. Similarly, the feasibility of ultrasoft x-ray microscopy with the use of Fresnel zone-plates and of scanning microscopy with synchrotron x-rays has been shown (*2*, *3*), although at lower resolutions than the above. These accomplishments suggest the desirability of ascertaining just what the potential operating region for ultrasoft x-ray transmission microscopy may be.

As a contribution to the study of this question we have calculated the radiation dose D which an unstained biological specimen must undergo in ultrasoft x-ray microscopy. [It is known (*4*) that radiation dose and the damage resulting therefrom is the limiting factor in the resolution obtainable by electron microscopy of unstained biological systems.] The calculations cover bright-field and dark-field transmission x-ray microscopy, a wavelength range for the photons from 1.3 to 90 Å, and model systems representative of a number of different simplified two- and three-phase biological specimens. The dose D is calculated as a function of the specimen thickness t and the resolution d at which the microscopy is being carried out. It is assumed in the calculations that the instrumentation is ideal in the sense that it does not increase dosages over those calculated (for example, through losses in the detectors) or decrease resolution (for example, through diffraction effects or through aberrations in the optical elements).

For convenience in comparing with electron microscopy, D is similarly calculated for an extensive set of CTEM and STEM modes (*5*), and for electron energies in the range 10^4 to 10^7 ev. Analogous assumptions are made about the freedom of the instrumentation from signal and resolution loss.

Under the assumptions given, the minimum incident flux $n_{\min}$ of particles on the specimen necessary to distinguish reliably between two differing resolution elements of the specimen is given by

$$n_{\min} = 25(p_1 + p_2)/d^2(p_1 - p_2)^2 \quad (1)$$

where d is the edge-length of the resolution element, and p_1, p_2 are the probabilities of an incident particle giving rise to an event of the type being used to form the signal in the microscopy in question in the two resolution elements. Equation 1 is a modified form of the criterion originally introduced by Rose (*6*). For ultrasoft x-rays the event may be the transmission of the photon through the specimen (bright-field microscopy, mode X1 in our nomenclature), or the absorption of the photon in the specimen (dark-field microscopy, mode X2).

If one assumes that the resolution elements consist of a background material B of thickness t_B and a feature material, which is F1 or F2 in the two different types of resolution element, of thickness t_F, then

$$p_1^{X1} = \exp(-\mu_B t_B - \mu_{F1} t_F), \qquad p_2^{X1} = \exp(-\mu_B t_B - \mu_{F2} t_F) \quad (2a)$$

and

$$p_1^{X2} = 1 - \exp(-\mu_B t_B - \mu_{F1} t_F), \qquad p_2^{X2} = 1 - \exp(-\mu_B t_B - \mu_{F2} t_F) \quad (2b)$$

for X1 and X2 microscopy, respectively. Here μ_B, μ_{F1}, and μ_{F2} are the linear absorption coefficients for the x-rays in question in the materials in question, and may be calculated from tabulated data (*7*). Together, Eqs. 1 and 2 allow the minimum flux of photons to be calculated in terms of the thicknesses of background and features in the specimen, the wavelength of the x-rays and materials of the background and features, the mode of the microscopy, and the desired resolution d.

The mean radiation dosage (energy deposited per unit mass) in the initial layers of a specimen composed of equal numbers of elements containing F1 and F2, corresponding to the minimum incident flux $n_{\min}$, is

$$D = n_{\min}\bar{E}\,[2\mu_B t_B + (\mu_{F1} + \mu_{F2})\,t_F] \,/\, [2\rho_B t_B + (\rho_{F1} + \rho_{F2})t_F] \quad (3)$$

where $\bar{E}$ is the energy deposition per absorption $h\nu$ and ρ_B, ρ_{F1}, ρ_{F2} are the densities of the materials B, F1, F2, respectively. With the aid of Eq. 3, the least dosage to the specimen consistent with reliable imaging can be calculated in terms of the quantities noted above (thicknesses and materials of specimen, wavelength, mode, and resolution).

In electron microscopy, Eq. 2 is replaced by formulas corresponding to the eight CTEM and STEM modes considered (*5*). The formulas are similar to Eq. 2 in form, but involve the linear coefficients for elastic scattering μ_B^e, μ_{F1}^e, μ_{F2}^e and inelastic scattering μ_B^i, μ_{F1}^i μ_{F2}^i. The linear coefficients are calculable from the atomic cross sections for elastic and inelastic scattering (*8*). Finally, Eq. 3 is modified in the electron case by reducing $\bar{E}$ to approximately 48 ev [this choice yields values for the dose which are in close agreement with relativistic stopping power equation values (*4*, *9*)], and replacing μ's by μ^i's.