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Genetics

Genetic characterization of *FLA*, the cat major histocompatibility complex

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ABSTRACT **The major histocompatibility complex (MHC) of the domestic cat (termed *FLA*) has been refractile to genetic and serological definition largely because of repeated failure to detect cytotoxic antibodies in multiparous cats or to elicit antibody following allogeneic lymphocyte immunization. We have developed a protocol for producing cytotoxic alloantisera in the cat following rejection of multiple surgical skin grafts. Of 59 cats subjected to grafting, 13 produced lymphocytotoxic antisera which had varying specificities among a panel of outbred cat cells. A population cluster analysis of the 13 alloantisera permitted the identification of six clusters of overlapping *FLA* specificities. Serological analysis of cells from 12 cat kindreds led to the definition of 24 allogeneic haplotypes, which segregate as a single Mendelian complex. Feline *FLA* antisera were characterized as class I or class II specific by immunoprecipitation of *FLA* gene products on lymphocyte cell surfaces. Abundant antigenic polymorphisms for both class I and class II MHC determinants were discovered, a result consistent with precedence in other species and the common expectation of the adaptive value of MHC variation. Development of feline MHC typing reagents and the definition of haplotypes for the cat hold promise for experimental analysis of valuable feline models for virus-induced immune deficiencies.**

The major histocompatibility complex (MHC) is a multigene cluster in mammals encoding two distinct classes of molecules involved in the presentation of foreign antigen to the T-cell receptor (1–5). Class I genes encode a 45-kDa glycoprotein that is noncovalently linked with an invariant light chain, β_2-microglobulin (β_2m), on the surfaces of virtually all nucleated cells (4, 6). Class II genes encode the heavy (32–35 kDa) and light (25–28 kDa) subunits of the noncovalently associated $\alpha\beta$ heterodimeric glycoproteins expressed on the surfaces of antigen-presenting cells and activated T cells (5). By serological analysis, both class I and class II antigens are abundantly polymorphic in most outbred species. DNA restriction analysis has also revealed extensive polymorphism of the MHC of mice, humans, and several other species (7, 8). The function of both class I and class II antigens is to present foreign, often viral, antigen to the T cell receptor. In this way, the cellular immune response is directed to parasitized cells instead of free virus particles (9, 10).

The domestic cat is an important model for at least two viral diseases of the immune system in humans, human T cell leukemia and acquired immunodeficiency syndrome (AIDS) (11, 12). The characterization of the feline MHC as a prerequisite for the study of disease progression has been difficult because of an apparent inability to raise typing alloantisera in the cat (13). Early observations that cats show prolonged allograft survival (14–16), fail to develop lymphocytotoxic alloantibodies after pregnancy or transfusions (13), and succumb to multiple viral infections have led to speculation that cats may have limited or no polymorphism at the MHC (7, 13). However, recent reports suggest that domestic cats do have polymorphic class I genes and MHC gene products (17–20).

MATERIALS AND METHODS

Details of allograft transplantation are described elsewhere (ref. 21; C.W. and S.O., unpublished data). Briefly, split thickness skin grafts were surgically exchanged between selected cats at 21-day intervals. In some cases, intraperitoneal booster injections of peripheral blood lymphocytes (PBLs) from 15 ml of blood were performed at 4-day intervals after grafting (21). Serum was collected from graft cats weekly and used in a cat complement-dependent cytotoxicity assay. A modification of a two-stage assay using ^{51}Cr release as an indicator of cell death was used to detect alloantibodies against donor cat lymphocytes. The monoclonal antibody W6/32 (23), specific for human class I heavy chain, and the monoclonal antibodies IB5 (human DR and DQ specific) (24) and ISCR3 (mouse I-E^K specific) (25) were used as positive controls in immunoprecipitation experiments.

Interleukin 2-stimulated or fresh cat PBLs were incubated with [^{35}S]methionine at room temperature for 1 hr followed by a 2-hr chase to label cell-surface proteins. Viable target cells (10^7) were incubated with cat alloantisera and anti-class I or anti-class II monoclonal antibodies for 1 hr. Intact cells were used to adsorb antibodies from low titer alloantisera and to present cell-surface antigens in their native configuration. Cells were washed to remove unadsorbed antibody and were disrupted in a lysing buffer. The antibody-binding molecules were collected on protein A-Sepharose beads and analyzed by standard SDS/PAGE (26).

RESULTS

Domestic Cats Reject Allogeneic Skin Grafts Promptly and Produce Lymphocytotoxic Alloantibodies Following Alloimmunization. Split thickness skin grafts were exchanged between 59 cats to obtain information about graft survival times and to produce cytotoxic alloantisera. The majority of the skin grafts were immunologically rejected between 7 and 13 days after grafting (Fig. 1*A*). A few, however, and only when exchanged between siblings, rejected much later (21–31 days) after the graft procedure. None of the eight skin grafts between unrelated cats survived longer than 10 days ($\bar{x} = 8.5 \pm 0.93$) (Fig. 1*A*). Skin grafts between parents and offspring, which generally differed by a single MHC haplotype, were also rapidly rejected ($\bar{x} = 10.1 \pm 2.1$ days).

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Abbreviations: MHC, major histocompatibility complex; PBL, peripheral blood lymphocyte; β_2m, β_2-microglobulin.

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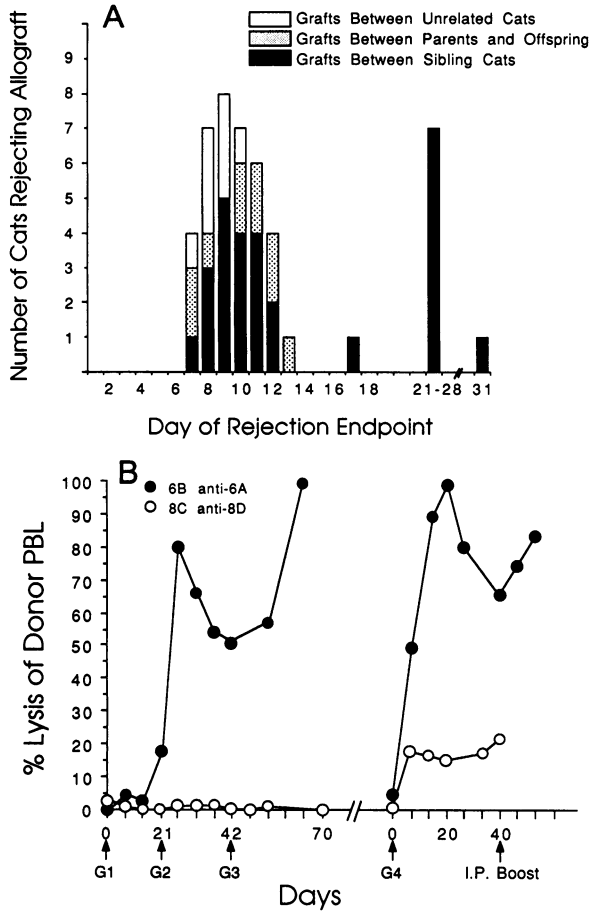

FIG. 1. (*A*) Skin allograft survival in related and unrelated cats. The allograft rejection endpoint was taken at the point at which the graft became hemorrhagic, necrotic, and indurated. Six of the allografts showing prolonged survival were edematous at day 21 and fully rejected by day 28. Fifty-nine cats received allografts; however, results are only presented for the 45 allografts that were examined daily for signs of rejection. (*B*) The lymphocytotoxic antibody response of cats following immunizations by multiple skin grafts and intraperitoneal (I.P.) blood leukocyte injections. G1, G2, and G3 are skin grafts placed 21 days apart. G4 grafts were placed 12–19 months after the third graft.

In a typical outbred species with a polymorphic MHC, the usual mating is between two individuals that differ maximally at their MHC (i.e., A/B × C/D). In this case, four classes of offspring are expected (AC, AD, BC, and BD). Grafts exchanged between any two siblings will, therefore, fall into one of three genetic classes: (*i*) the two siblings will be haploidentical and differ at one haplotype ($P = 0.5$), (*ii*) the siblings will be haplodistinct and differ at two haplotypes ($P = 0.25$), or (*iii*) the siblings will be MHC identical and share both haplotypes ($P = 0.25$). Our results showed that 70.4% (19/27) of the sib-to-sib grafts were rapidly rejected (9.7 ± 1.4 days), while 29.6% (8/27) of the sib-to-sib grafts showed a delayed rejection time course (17–31 days) (Fig. 1*A*). These results are consistent with the hypothesis that the genes controlling the cat MHC alloantigens are polymorphic and that the MHC haplotypes are codominantly expressed and segregated in cat families as a single gene or a linked multigene complex.

Sera from 59 cats, each receiving one or more skin grafts, were monitored weekly for lymphocytotoxic antibodies against donor cells, using a ^{51}Cr release two-stage cytotoxicity assay. A serum sample was considered positive if it lysed at least 17% of the target cells in two separate experiments. Thirteen of the 59 cats (22.0%) produced detectable levels of antibodies. The kinetics of seroconversion differed between cats, and two representative examples are presented in Fig. 1*B*. Although initial immunizations with allogeneic PBLs or by pregnancy failed to produce cytotoxic antibodies (unpublished observations), we were successful in boosting production in grafted cats by immunization with PBLs.

Immunoprecipitation of FLA Class I and Class II Antigens with Cat Lymphocytotoxic Antisera. The molecular specificity of 12 of the 13 derived feline antisera was determined by immunoprecipitation of [^{35}S]methionine-labeled cell-membrane proteins from cat cells lysed by each serum. Feline *FLA* gene products were visualized on SDS/polyacrylamide gels following immunoprecipitation with cross-reacting monoclonal antibodies W6/32 specific for human HLA class I heavy chain, IB5 specific for class II *HLA-DR* and *HLA-DQ* gene products, or ISCR3 specific for the murine *H-2E*^{k} (class II) molecule. The class I-specific monoclonal antibody W6/32 immunoprecipitated a single 46-kDa cell-surface protein on several cells (Fig. 2*A*). No β_2m was precipitated from any of the cats' cells either with the monoclonal antibody or with feline alloantisera that reacted with class I molecules, possibly because cat β_2m, like bovine β_2m, does not contain a methionine residue (27). In a control experiment with human cells, both heavy (46 kDa) and light β_2m (12 kDa) chains were immunoprecipitated (Fig. 2*B*, lane 2). The class II monoclonal antibodies IB5 and ISCR3 immunoprecipitated three major chains of 30, 40, and 12 kDa (Fig. 2*A*, lanes 4 and 8).

The pattern of immunoprecipitation in eight of the antisera was identical to the class I monoclonal antibody (46 kDa) and was designated as class I specific (see Table 2; Fig. 2*A*). Two of these antisera gave a pattern identical to the class II

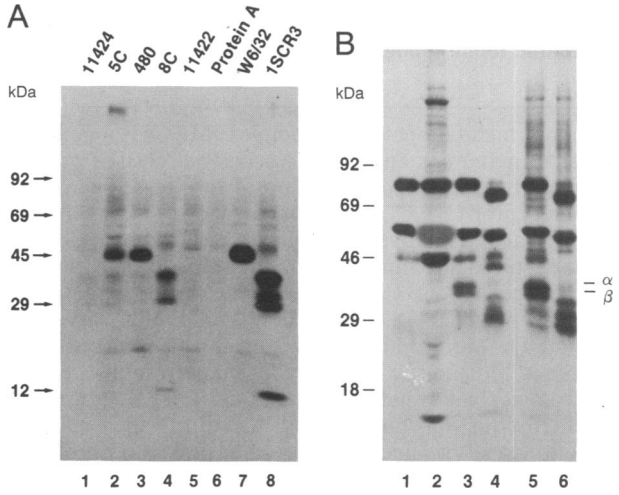

FIG. 2. (*A*) SDS/PAGE of immunoprecipitated cat class I and class II proteins. Interleukin 2-stimulated cat PBLs from cat 11422 were metabolically labeled with [^{35}S]methionine and immunoprecipitated with either cat lymphocytotoxic antibodies or with monoclonal antibodies directed at human class I or class II proteins. Immunoprecipitations with cat antisera 11424 (lane 1), 5C (lane 2), 480 (lane 3), 8C (lane 4), and 11422 (lane 5). Lane 6 is a control (no antibody). Lanes 7 and 8, immunoprecipitations with monoclonal antibodies W632 (lane 7) or ISCR3 (lane 8). (*B*) Effects of endoglycosidase F digestion of human and cat class II antigens. Human B cells and interleukin 2-grown lymphocytes from cat 248 were labeled with [^{35}S]methionine and immunoprecipitated with antibody and protein A-Sepharose, and then treated eluates were analyzed by SDS/PAGE after a 2-hr digestion with 0.3 unit of endoglycosidase F. Lanes: 1–4, human B cells; 5 and 6, cat 248 cells. Lane 1, protein A-Sepharose alone, no antibody; lane 2, W632 antibody (anti-class I); lane 3, ISCR3 antibody (anti-class II); lane 4, ISCR3 antibody, endoglycosidase F digestion; lane 5, ISCR3 antibody; lane 6, ISCR3 antibody, endoglycosidase F digestion.

Table 1. Summary of FLA lymphocytotoxic alloantisera

Specificity	Serum	% lysis of donor PBL	Titer	Class	Gene frequency
FLA1.1	415	85 ± 2	8	I	0.2189
FLA1.2	5B	24 ± 5	2	I	0.0871
FLA1.3	6B	86 ± 17	32	I	0.0518
FLA2.1	8D	18 ± 6	32	II	0.0582
FLA2.2	480	100 ± 0	16	I	0.2822
FLA3.1	6A	46 ± 4	8	I	0.0793
FLA3.2	11422	52 ± 3	16	I	0.1877
FLA3.3	7C	78 ± 26	16	I	0.3131
FLA3.4	5C	59 ± 3	32	I, II	0.2749
FLA4.1	5A	82 ± 11	8	I, II	0.3557
FLA4.2	519	67 ± 24	32	I	0.3675
FLA5.1	8C	24 ± 3	8	II	0.1261
FLA6.1	3842	23 ± 3	1	NT	0.0264

Specificities were determined by association analysis. All preimmune sera tested negative for cytotoxicity with the exception of serum 6A, which lysed <10% of the donor cells. Results for unpooled sera only are reported. A two-stage complement-dependent cytotoxicity ^{51}Cr release assay was used and the % lysis is reported as the mean of three separate experiments. Individual assays were run in triplicate. Class of antigen recognized by the serum was determined by immunoprecipitation SDS/PAGE analysis. Gene frequencies (gf) were calculated by the formula $gf = 1 - \sqrt{1 - f}$, where f is the phenotype frequency in the population. NT, not tested.

monoclonal antibodies (30, 40, and 12 kDa), and two antisera had specificities for both classes of molecules. These results define the molecular mass of feline class I (46 kDa)- and class II (30, 40, and 12 kDa)-related cell-surface proteins and indicate the specificities of the allotypic serological reagents (Table 1). To determine whether native antigens immunoprecipitated by the various feline antibodies contained carbohydrate residues, a diagnostic feature for mammalian MHC molecules, the immune complexes were enzyme digested with endoglycosidase F. The reduction of molecular mass following endoglycosidase F digestion is consistent with the interpretation that the feline immunoprecipitates are MHC class II molecules (Fig. 2*B*).

Differences in the timing of antibody production and in the quantity of lymphocytes lysed between alloantisera were consistent with the MHC class recognized in the immunoprecipitation experiments. Class II antigens are present on B cells and monocytes but not on T cells, which constitute the majority cell type in preparations of mononuclear cells from whole blood (28). The class II-specific antibodies (detected in sera 8D and 8C) were detected in cats only after each cat rejected four skin grafts and the alloantisera lysed only 18–25% of the cells (Fig. 1*B*).

FLA Cluster Analysis of a Feral Cat Population. The 13 lymphocytotoxic alloantisera were tested against PBLs from a feral cat population of 115 collected in western Maryland. Phenotypic incidence of each serum specificity was measured and used to compute the allelic frequency (29) (Table 1). Two-by-two contingency/association tables were generated for all serum combinations based on their reactivities in the population survey (see refs. 30 and 31). These results were used to group the 13 alloantisera into six clusters, each with a statistically significant tendency toward concordant reactions (Table 2). Four of the clusters contained sera that had a significant χ^2 value for independence ($\chi^2 \geq 10.8$; $P \leq 0.001$) and coefficient of correlation ($r \times 100$) values greater than 40. The antigenic specificities recognized by the four multisera clusters and the independent sera 8C and 3842 have been designated FLA-1 through FLA-6 for feline leukocyte antigen (Tables 1–3).

Pedigree Analysis Defines Haplotype Specificities and Produces an FLA Chart. Mendelian transmission of each of the serological specificities was demonstrated in 12 family groups of 5–17 individuals in the NIH cat colony (Fig. 3). Twenty-four haplotypes were defined as combinations of specificities, which were inherited *en bloc* as a single complex. An *FLA* chart was constructed (Table 3), which lists the haplotype specificities defined by the alloantisera. The extent of serological polymorphism exhibited is actually quite high because every haplotype we followed had a unique combination of immunologic specificities.

Once the haplotype specificities had been defined in the NIH colony (Table 3), it was possible to retrospectively examine the timing of graft rejection as a function of MHC genotype relationships between donor and recipient cats. The mean survival time for allografts exchanged between haplodistinct (i.e., sharing no haplotype) individuals was 9.3 ± 1.1 days vs. 10.3 ± 2.7 days for haploidentical (sharing one haplotype) cats ($P \geq 0.50$). The mean survival time of *FLA* identical cats was 19.2 ± 4.5 days. The difference in mean survival times of skin grafts exchanged between haplodistinct and haploidentical cats and between *FLA* identical cats was significant ($P < 0.05$). These results affirm the genetic inference that the alloantisera recognize MHC-specific determinants, which mediate the time of graft rejection.

DISCUSSION

Evidence for a polymorphic system of functional class I and class II genes in the domestic cat homologous to the MHC

Table 2. Correlation coefficients ($r \times 100$) (upper matrix) and χ^2 values for independence (lower matrix) between alloantisera tested on a leukocyte panel of 115 unrelated cats

Sera (specificity, FLA-)	Sera (specificity, FLA-)												
	415 (1.1)	5B (1.2)	6B (1.3)	8D (2.1)	480 (2.2)	6A (3.1)	11422 (3.2)	7C (3.3)	5C (3.4)	5A (4.1)	519 (4.2)	8C (5.1)	3824 (6.1)
415 (1.1)	—	59.6	49.4	20.6	33.8	40.7	64.8	32.0	37.2	20.8	6.0	19.5	34.3
5B (1.2)	23.8	—	49.1	29.2	34.9	−10.4	28.8	2.4	7.4	9.2	3.7	14.4	−4.0
6B (1.3)	14.8	15.2	—	3.8	24.9	19.7	35.1	30.1	14.6	−13.6	−1.5	4.7	−4.1
8D (2.1)	2.3	1.3	0.1	—	51.6	25.4	18.0	7.1	7.1	36.3	21.2	12.6	41.2
480 (2.2)	6.8	6.9	3.4	18.7	—	18.0	11.9	−13.3	15.4	39.5	37.7	14.1	27.5
6A (3.1)	9.9	0.1	1.3	2.6	1.4	—	66.4	45.6	50.0	39.7	30.7	39.7	40.8
11422 (3.2)	26.6	4.1	6.9	1.4	0.5	28.6	—	57.4	58.1	42.9	29.8	2.1	26.9
7C (3.3)	6.5	0.01	5.4	0.1	0.9	14.6	21.4	—	71.7	18.1	3.4	−0.1	8.1
5C (3.4)	7.9	0.1	0.7	0.1	1.1	14.7	20.6	39.2	—	58.2	42.3	−11.5	12.1
5A (4.1)	2.6	0.3	0.6	9.2	10.0	10.3	12.4	1.9	24.1	—	83.3	0.4	26.4
519 (4.2)	0.1	0.001	0.1	2.8	10.2	6.1	5.5	0.0	11.7	58.8	—	13.2	23.0
8C (5.1)	2.0	0.7	0.1	0.6	0.9	9.6	0.0	0.1	0.4	0.1	0.9	—	24.0
3842 (6.1)	6.6	1.3	0.9	9.1	3.8	7.7	2.6	0.02	0.02	4.2	3.2	2.4	—

Statistically significant values ($P \leq 0.001$; 1 df) are boxed.

Table 3. Antigenic determinants controlled by the *FLA* complex in the domestic cat

FLA haplotype	Antiserum specificity, FLA-												
	1.1	1.2	1.3	2.1	2.2	3.1	3.2	3.3	3.4	4.1	4.2	5.1	6.1
1a	−	−	−	+	+	−	−	−	−	0	+	−	−
1b	−	−	−	−	−	−	−	−	−	0	−	−	−
1c	+	−	−	−	+	−	−	−	−	0	−	−	−
2a	−	−	−	−	−	−	−	−	−	0	−	−	−
2c	+	−	−	−	0	−	−	−	−	−	−	+	−
2d	−	+	+	−	0	−	−	−	−	−	−	−	−
3a	+	−	+	−	+	−	−	0	−	−	−	−	−
3b	+	−	−	−	−	−	−	0	0	−	−	−	−
3c	−	−	−	−	−	+	−	0	0	−	−	+	−
4a	−	−	−	−	−	−	−	−	−	−	−	+	−
4c	+	−	−	−	+	−	−	−	−	−	−	−	−
4d	+	−	−	−	−	−	−	−	−	+	+	−	−
5a	+	−	−	+	0	−	−	−	0	0	−	−	−
5b	−	−	−	−	−	−	−	−	0	−	−	+	−
5c	−	−	−	−	+	−	−	−	+	+	−	−	−
6c	−	−	−	−	−	0	+	+	0	+	+	0	−
7a	−	−	−	−	0	+	+	+	+	0	0	0	−
8a	−	−	−	−	−	−	−	−	+	−	−	+	−
8c	−	−	−	−	−	−	−	−	+	−	−	−	−
8d	−	−	−	−	−	−	−	−	+	+	−	+	−
9e	−	−	−	−	−	−	−	+	−	+	−	−	−
9g	−	−	−	−	−	−	+	+	+	+	+	−	−
10i	−	−	−	−	+	−	−	−	−	−	−	−	−
12k	−	−	−	+	−	−	−	+	−	−	−	−	−

+, Serum lysed ≥17% of target lymphocytes; −, serum lysed ≤10% of target lymphocytes; 0, specificity was present in both parents; therefore, the haplotype origin cannot be determined. Each family was given a number, and the offspring were assigned a letter designation.

loci of most other mammalian species has been hampered by difficulties in raising serological reagents that define the cat MHC gene products (7, 13–16, 19, 20). The inability of cats to produce lymphocytotoxic antibodies following immunization by pregnancy, blood, or PBLs; their weak mixed lymphocyte reactions; and an apparent prolonged survival of allografts have led to the conclusion that the cat *FLA* loci are largely monomorphic. The present results indicate that cats possess a polymorphic MHC whose products and haplotypes were resolved after multiple skin graft eliciting of lymphocytic antisera.

In spite of the polymorphism we and others (17–19) have described for the cat MHC, less than a third of the cats acutely rejecting three or more skin grafts produced detectable levels of lymphocytotoxic alloantibodies. In most species, the rejection of grafts is almost always followed by the appearance of lymphocytotoxic alloantibodies in the host's serum (7, 32, 33). We also failed to detect lymphocytotoxic alloantibodies in multiparous or transfused cats, as have others (13), but most mammalian species readily develop alloantibodies after similar challenges (7, 32, 33). Why cats do not make antibodies after these allogeneic challenges is not clear to us.

A molecular analysis of the feline MHC revealed that class I genes exhibited restriction fragment length polymorphism variation comparable to the extent seen in humans but more than is seen in Syrian hamster or the cheetah, species with minimal MHC variation (17). Both class I and class II were mapped to chromosome B2 in a linkage group homologous to mouse chromosome 17 and human chromosome 6, where

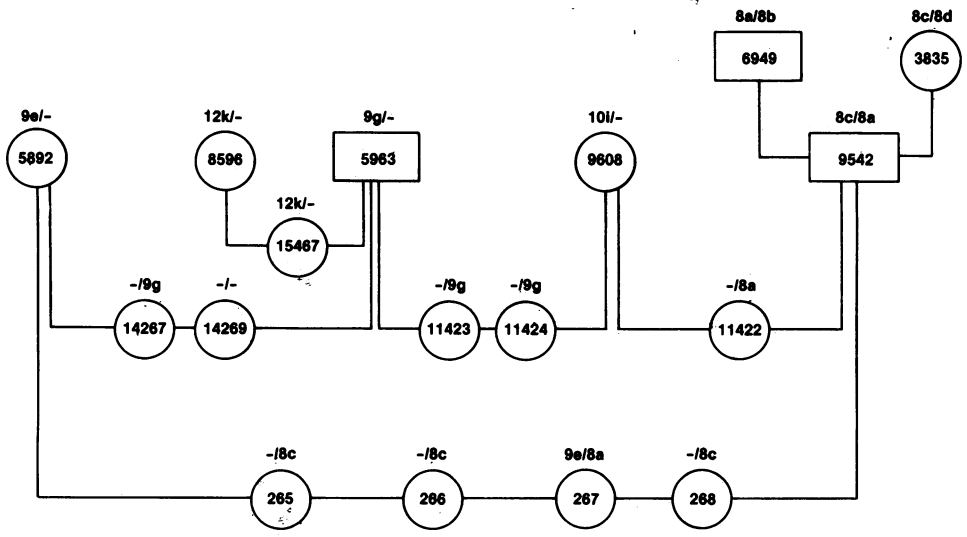

FIG. 3. Segregation of *FLA* haplotypes in an NIH cat family. Haplotype code: 8a-FLA5.1, 3.4; 8c-FLA3.4; 8d-FLA3.4, 4.1, 5.1; 9e-FLA3.3, 4.1; 9g-FLA3.2, 3.3, 3.4, 4.1, 4.2; 10i-FLA2.2; 12k-FLA2.1, 3.3; −, none (see Table 3).

these species' MHC are located. N. Yuhki, G. F. Heidecker, and S.O. (unpublished data) have recently analyzed eight class I cDNA clones from a feline T-cell lymphoma and have uncovered only two RNA transcripts. These data were interpreted to suggest that in the studied cell line only a single class I locus (but with two alleles) was transcriptionally active. If cats in general have a single functional MHC locus, this situation might help explain the limitation of antibody production reported in our grafting experiments.

The development of serological reagents specific for *FLA* has a potential utility in certain feline models of human disease. The clinical emphasis on pet cats has resulted in the identification of >20 heritable genetic disorders that are homologous to human inborn errors of metabolism (34, 35). The development of *FLA* typing reagents should prove useful for therapeutic protocols involving bone marrow transplantation and gene therapy of these diseases (35–37). In addition, *FLA* typing reagents will be of value in development of *in vitro* cellular immunological procedures. The lack of such reagents has slowed progress in functional studies of feline retroviral leukemia, lymphoma, and immune deficiency (11, 38). We have previously suggested that an abrogation of MHC restriction or possibly interference with MHC viral presentation are potential explanations for the extreme susceptibility of the African cheetah to the feline infectious peritonitis coronavirus (39). The availability of *FLA* typing reagents that could recognize homologous antigens on cheetah lymphocytes may be useful in empirical testing of various aspects of these hypotheses. Finally, the serological definition of the feline MHC confirm in an important laboratory species the evolutionary conservation of the gene complex involved in self versus nonself recognition.

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