INFECTION AND IMMUNITY, Feb. 1974, p. 391-393
Vol. 9, No. 2

Feline Immunoglobulins

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Received for publication 7 March 1973

Immunoglobulins (Ig) in feline sera and secretions were identified by immunoelectrophoresis and immunodiffusion with rabbit antisera prepared to feline IgG, IgA, IgM, and whole serum. Adult cat sera, colostral whey, postcolostral sera, tears, and nasal secretions contained IgG, IgA, and IgM. IgG was the only Ig identified in precolostral sera and cerebrospinal fluid. Milk, intestinal contents, pooled allantoic and amniotic fluids, and saliva from adult cats and urine from suckling kittens contained IgG and IgA. Ig were not detected in urine from adult cats. Bile was unique in that IgA and IgM were the predominant Ig.

The literature on the basic immunity of cats was recently reviewed by Gorham et al. (1). It was suggested that, although cats have become a popular research animal, particularly in cancer research, the study of immune phenomena of cats is in its infancy.

Immunoelectrophoretic characterization of feline serum proteins was reported by Okoshi et al. (6). These workers reported that electrophoretic precipitin arcs corresponding to human immunoglobulin (Ig) A, IgM, and IgG were present in cat serum. More recently Vaerman has provided definitive evidence, by immunological homologies with their human counterparts, for the presence of cat IgA and IgM. Vaerman described subclasses of IgG as $IgG_{1,2}$, similar to the subclass identification assigned to pig IgG (J. P. Vaerman, Ph.D. thesis, Universite Catholique de Louvain, Louvain, Belgium, 1970).

The present study was designed to identify, by immunoelectrophoresis and immunodiffusion, IgG, IgA, and IgM in serum and secretions of cats. The information will provide parameters of immunoglobulins for future comparison of samples from viral-infected and leukemic cats.

MATERIALS AND METHODS

Immunoelectrophoresis and immunodiffusion. The method of immunoelectrophoresis was the micro method of Scheidegger (8) with minor modifications. A 0.02 M barbital buffer was used to make the 1.5% agar solution, and the chamber (Gelman Instrument Co., Ann Arbor, Mich.) contained 0.03 M barbital buffer at pH 8.6. Electrophoresis was for 1 h at 10 mA/slide tray. (For complete details, the reader is referred to the technical manual available from Gelman Instrument Co., Ann Arbor, Mich.) The method of immunodiffusion was that of Ouchterlony, using the same agar solution as above (7).

Antisera. Rabbit antisera to whole cat serum and cat serum precipitated with a 33% saturated $(NH_4)_2SO_4$ solution were prepared by inoculating rabbits intramuscularly once a week for 3 weeks with 20 to 30 mg of biuret protein incorporated in Freund complete adjuvant. One final sample of 15 mg of either whole cat or $(NH_4)_2SO_4$-precipitated serum was injected intravenously, and the rabbits were test bled 7 days later. Rabbit antisera to cat IgG, IgA, and IgM were prepared by the immunoprecipitation procedure (5). In brief, the procedure involves separating the protein by immunoelectrophoresis, identifying the appropriate precipitin line (antigen-antibody complex) with the reference antisera, and cutting the agar which contains the antigen-antibody complex. The agar is washed, homogenized with complete Freund adjuvant, and inoculated into rabbits. In the immunoprecipitation procedure, cat IgG and IgM were prepared for injection by precipitation of cat serum with anti-whole cat serum or antisera to $(NH_4)_2SO_4$-precipitated cat serum. IgA was prepared by precipitation of bile with anti-cat serum. Identification of the immunoglobulins and specificity of the antisera were determined with monospecific antisera kindly supplied by J. P. Vaerman. The monospecific anti-IgA and -IgM sera were prepared by precipitation of cat serum proteins with antisera monospecific for human IgA and IgM, respectively, by the immunoprecipitation procedure (10; J. P. Vaerman, Ph.D. thesis). Therefore, first order criteria have been met for classifying these cat immunoglobulins as analogous to human IgA and IgM.

Samples. Serum samples were collected from cats in a breeding colony maintained by the Department of Veterinary Microbiology. Precolostral serum samples (sera collected prior to suckling) were collected from kittens born naturally and from kittens derived by caesarean section of the queen. Allantoic and amniotic fluids were collected from fetuses derived by caesarean section. Saliva samples were collected from

cats during anesthesia, from cats induced to salivate with Lentin (Pitman-Moore Co., Indianapolis, Ind.), or from cats during natural conditions of salivation. Tears and nasal secretions were collected during anesthesia with ketamine hydrochloride (Bristol Laboratories, Syracuse, N.Y.) or from cats with respiratory infections. Bile, urine, and intestinal contents samples were collected immediately from cats euthanatized for various reasons. The intestinal samples were collected by removing the small intestine, stripping the intestinal contents into a tube, and centrifuging the sample in the cold at 3,000 $\times$ *g*. The supernatant fluid was frozen until all the samples were collected for analysis. Colostrum was collected from the queen or, in most cases, from the stomach of kittens euthanatized shortly after suckling. Milk samples were obtained directly from the queen by manually expressing milk from the teats. Cerebrospinal fluid was obtained via a spinal tap. Postcolostral sera were sera collected from suckling kittens during the first 7 days after birth.

RESULTS

Immunoelectrophoretic patterns of an adult cat serum reacted with rabbit anti-cat IgG, IgA, IgM, and serum are shown in Fig. 1. The analysis of serum and secretions for the identification of IgG, IgA, and IgM are presented in Table 1.

In adult serum, IgG was easily identified in all samples. Electrophoretic precipitin arcs suggestive of two subclasses of IgG are shown in Fig. 1. IgA was present in most adult sera, and IgM, although more difficult to detect, was identified in all except six samples. In contrast to adult serum samples, IgG was the only Ig detected in the precolostral serum samples and, with one exception, in the fetal fluids (pooled allantoic, and amniotic). The three major Ig classes were detected in the majority of the colostral whey samples and postcolostral serum samples. IgG persisted as the major immunoglobulin class in milk samples collected from 1 to 8 weeks postparturition.

Among the other secretions examined, bile was unique in that IgA and IgM were the predominant immunoglobulins.

Immunoglobulins were not detected in urine from adult cats; however, three samples from suckling kittens contained only IgG and two contained IgA and IgG. IgG and IgA were detected in the saliva of two cats not chemically stimulated to salivate.

Ouchterlony immunodiffusion analysis of two adult sera, precolostral serum, milk, bile, and intestinal contents with antisera to IgG, IgA, and IgM, as well as anti-whole cat serum, are shown in Fig. 2. It is apparent from the immunodiffusion results that IgG is readily detectable in serum, milk, and intestinal contents; IgA is readily detectable in bile and is less apparent in intestinal contents and serum; IgM is readily detected in serum.

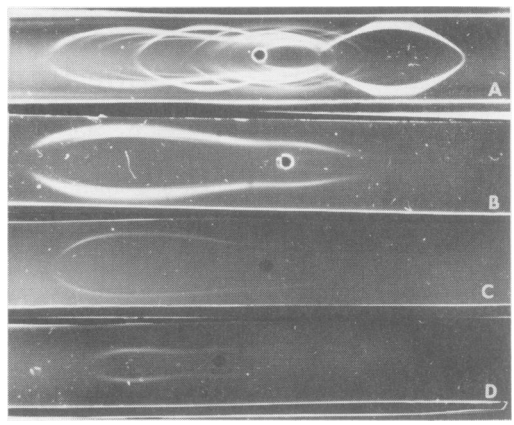

FIG. 1. *Immunoelectrophoretic pattern of cat serum developed with rabbit anti-cat serum (A), anti-cat IgG$_{1,2}$ (B), anti-cat IgA (C), and anti-cat IgM (D). Cathode at the left.*

TABLE 1. *Occurrence of immunoglobulins in sera and secretions of cats*

Sample	Immunoglobulin detected by immunoelectrophoresis		
	IgG	IgA	IgM
Adult sera	100[a] (75/75)[b]	97 (73/75)	91 (69/75)
Postcolostral sera (1 to 7 days)	100 (60/60)	100 (60/60)	83 (50/60)
Colostral whey	100 (10/10)	90 (9/10)	70 (7/10)
Precolostral sera	33 (15/45)	0 (0/45)	0 (0/45)
Fetal fluids (allantoic and amniotic)	75 (15/20)	5 (1/20)	0 (0/20)
Bile	1 (1/65)	60 (39/65)	26 (17/65)
Intestinal contents	55 (22/40)	50 (20/40)	0 (0/40)
Saliva[c]	10 (2/20)	10 (2/20)	0 (0/20)
Tears	80 (8/10)	80 (8/10)	50 (5/10)
Nasal secretions	80 (4/5)	80 (4/5)	60 (3/5)
Milk (1 to 8 weeks postparturition)	100 (5/5)	80 (4/5)	0 (0/5)
Cerebrospinal fluid	50 (2/4)	0 (0/4)	0 (0/4)
Urine[d]	10 (5/50)	4 (2/50)	0 (0/50)

[a] Percentage of samples in which the respective immunoglobulin was detected.

[b] Numerator, number of positive samples; denominator, total number of samples.

[c] Positive samples from cats not stimulated with chemicals.

[d] The positive samples are from newborn kittens. All adult cats were negative.

DISCUSSION

It has been amply demonstrated in the present study that most adult cat serum samples contain IgG, IgA, and IgM. Two subclasses of IgG are generally present. This is in contrast to dogs, in which at least four subclasses of IgG

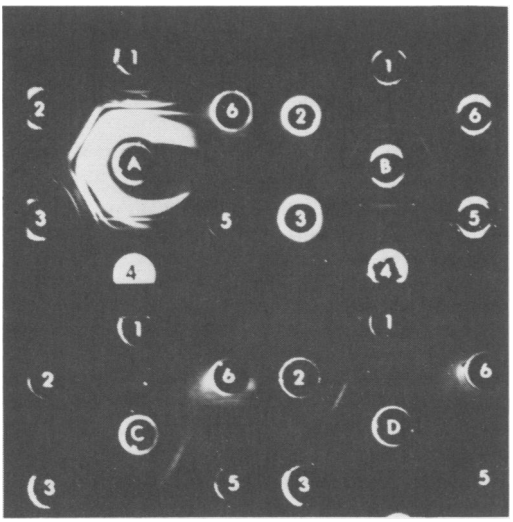

FIG. 2. *Immunodiffusion analysis of cat serum and secretion samples. A, Rabbit anti-cat serum; B, rabbit anti-cat* $IgG_{1,2}$*; C, rabbit anti-cat IgA; D, rabbit anti-cat IgM; 1, 2, adult serum samples; 3, precolostral serum; 4, milk; 5, bile; 6, intestinal contents.*

were demonstrated (J. P. Vaerman, Ph.D. thesis). The intensity of the IgA precipitin line in many serum samples suggested that IgA was present in relatively high concentrations; however, radial immunodiffusion studies are necessary to substantiate this point. IgM was more difficult to detect in serum than either IgG or IgA, suggesting a lower concentration for IgM.

Although Okoshi et al. were unable to detect immunoglobulins in fetal or precolostral sera by immunoelectrophoresis, approximately 33% of the precolostral serum samples in the present study had a weak reaction with diluted anti-IgG sera (6). This is in agreement with the findings of others, suggesting that maternal antibodies were present in precolostral kitten sera (2, 9). In addition, IgG was detected in fetal fluids, suggesting that the route of transmission of Ig to the fetus may be via the yolk sac.

Electrophoretic patterns of postcolostral serum samples were similar to colostral whey samples, indicating a nonselective absorption of Ig by the gastrointestinal tract of kittens. All milk samples had a strong IgG precipitin line. One sample collected 8 weeks postparturition did not have an IgA precipitin line, and a second sample had, in addition to a strong IgG reaction, only a weak reaction for IgA. This is unlike what was reported for dogs, where IgA was the predominant Ig (J. P. Vaerman, Ph.D. thesis).

Bile samples often contained IgA, with 43% of the samples containing IgM in addition to IgA. Vaerman examined one cat bile sample and found approximately equal amounts of IgA and IgM (J. P. Vaerman, Ph.D. thesis). Since tears and nasal secretions were obtained from calicivirus (picornavirus)-infected cats (unpublished data), the distribution of Ig may not be representative of normal secretions, since a previous report found IgA to be the predominant Ig in a single tear sample (J. P. Vaerman, Ph.D. thesis). Radial immunodiffusion studies are necessary to establish the quantity of the individual immunoglobulins in serum and secretions of cats. These studies are being planned at this time.

The inability to detect Ig in the saliva from stimulated cats may result from the dilution effect on this secretion reported to occur in man and cattle (3, 4).

Cats, therefore, share certain similarities with other species in their distribution of Ig. However, it apparently has some distinct differences from species such as dogs.

ACKNOWLEDGMENTS

This work was supported by Public Health Service research grant AI-10042 from the National Institute of Allergy and Infectious Diseases.

We thank A. de Lahunta and D. F. Holmes for the cerebrospinal fluid samples and S. G. Campbell for certain milk samples. E. Hahn and J. Buck are acknowledged for their technical assistance during certain parts of this study.

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