Immunoglobulin Compositions of the Feline Body Fluids

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ABSTRACT. Immunoglobulin concentrations were determined in feline serum, colostrum, milk, bile, intestinal fluid, saliva, tear, nasal secretions, and tracheal secretions by single radial immunodiffusion. The results were as follows. In the serum, colostrum and milk, IgG was predominant (78.1% to 90.7% of the total immunoglobulin contents); IgA and IgM were minor (5.9% to 11.7% and 2.8% to 10.2%). In other secretions, IgA was predominant (41.4% to 70.7%). However, IgG ratio was higher than IgM ratio in the nasal and tracheal secretions. In contrast, IgM ratio was superior to IgG ratio in the bile and intestinal fluids.—KEY WORDS: body fluid, cats, immunoglobulin.


The immunoglobulin compositions of various body fluids have been determined in many species, and it has been thought that IgG is predominant in the serum and internal secretions, and IgA is in the external secretions [28]. There, however, are some indications that the immunoglobulin compositions of body fluids depend on species, age and type of fluids [35]. Immunoglobulins in several secretions have been thought to play a very important role in the local immunity of mucosa [2, 33]. In cats, there are a few reports about the occurrence of immunoglobulins in the serum and secretions [25] and about immunological characteristics of germfree and specific pathogen-free cats [10]. However, the immunoglobulin compositions of feline body fluids are not clear. It is necessary to estimate immunoglobulin levels and immunoglobulin compositions of feline respiratory and gut associated secretions for understanding the local immunity of respiratory and digestive organs in cats. In this study, the immunoglobulin levels and compositions of feline body fluids are described.

MATERIALS AND METHODS

Immunoglobulins: IgG was purified by the method of Vaerman [29] with a last addition of Sephadex G-200 gel filtration. IgA was purified by many modifications to the method of Vaerman [29]. The whole globulin fraction of cat serum was obtained by precipitation with 50% ammonium sulfate saturation. The precipitate (4.5 g/dl, 10 ml) was dialyzed against 0.01 M Tris-HCl buffer pH 8.0, and applied to a DEAE-cellulose column (3.0×40 cm) equilibrated with the same buffer. Stepwise elution was carried out with 0.01 M, 0.08 M, 0.15 M, 0.20 M, 0.30 M and 0.55 M buffer (0.01 M Tris-HCl+NaCl). The fractions obtained with the 0.15 M buffer were pooled and concentrated by negative pressure dialysis against 0.15 M NaCl solution with Colodion bags Sm-13200 (Sartorius Membranfilter, Göttingen), then the zinc sulfate precipitating method was performed as described by Vaerman et al. [31]. The protein of the supernatant was precipitated with 50% ammonium sulfate saturation and dialyzed against 0.05 M sodium barbital buffer pH 8.6. The protein solution was submitted to
starch block (15×1×50 cm) preparative electrophoresis, which was performed with some modifications of the method proposed by Kunkel [13], using 0.05 M sodium barbital buffer pH 8.6, for 24 hours at 4°C. The areas of the block which contained IgA immunophoretically were removed and the protein was recovered by extraction with 0.15 M phosphate buffered saline (PBS) pH 7.2. The concentrate of the extract was gelfiltrated twice by Sephadex G-200. IgM was purified from the fraction eluted with 0.3 M buffer on DEAE-cellulose chromatography at the purification of IgA, the fraction was submitted to preparative electrophoresis. The areas containing IgM were extracted and concentrated. The concentrate was fractionated twice by Sephadex G-200 gel filtration. The patterns of immunoelectrophoresis and double diffusion of purified immunoglobulins are shown in Figs. 1 and 3.

Anti sera: Anti-cat whole serum was prepared in a rabbit as described by Okoshi et al. [21]. Anti-cat γ-, α- and μ-chain sera were prepared in rabbits. Each rabbit received four injections of approximately 0.2 mg protein-quantity of each purified immunoglobulin, to which was added Freund’s adjuvant (complete in initial time and incomplete in other times) every other time; one injection every 10 days. Anti-cat IgG-serum was absorbed with IgA and IgM coupled to CNBr-activated Sepharose 4B by the immunoadsorbent method described by Axen et al. [1]. Anti-cat IgA-serum was absorbed with IgG and IgM. Anti-cat IgM-serum was absorbed with IgG and IgA. The immunophoretic patterns of specific antisera are shown in Fig. 2.
Animal: Healthy adult cats, which had been maintained in a research colony, were used. The health status of the cat was evaluated by physical examination, electrophoretical analysis of serum proteins and fecal examination.

Body fluids: Most samples, except the serum, colostrum, and milk, were obtained while the cats were anesthetized with ketamine hydrochloride. Colostrum and milk samples had sodium azide in 0.1 to 0.5% added and were stored at −20°C until use. Serum total protein was estimated by a manul protein refractometer. Colostrum was obtained by manually expressing the teats of dams from parturition to 5 days later. Milk was obtained by stimulating with injection of oxytocin (1 unit/head) dams of 2 to 5 weeks post-parturition. Bile was aspirated from the gallbladder with an injection syringe and needle under laparotomy. Saliva, nasal secretion, and tear were collected following stimulation by subcutaneous injection with 1% pilocarpine hydrochloride solution (0.5 ml/head) as described by Kaltreider and Chan [11], and clarified by a centrifugation at 3,000 rpm for 10 minutes. Tracheal secretion was obtained by washing of tracheal cavity with 3 ml of PBS as described by Ewert et al. [6] after euthanising by drawing blood from heart under anesthesia, and lyophilized after clarification by centrifugation. That was reconstituted with 0.5 ml of sterile distilled water just before analysis. Intestinal fluid was extracted from feces by centrifugation (3,000 rpm, 20 min.). The feces were aspirated from the rectum by a polyethylene tube attached to an injection syringe after stimulation with pilocarpine hydrochloride and added an equal weight of PBS.

Analytical procedure: Immunoelectrophoresis was carried out by modifications of the procedure described by Scheidegger [24]. Quantitative estimation of immunoglobulins was performed by single radial immunodiffusion (SRID) [17]. The protein concentration of standard immunoglobulins was estimated by the method of Lowry et al. [15]. Double diffusion was performed by the method of Ouchterlony [22].

RESULTS

IgG was detectable in all of the serum, colostrum, milk and bile; however, it was detected in only one-third samples of the intestinal fluid examined by SRID (Table 1). IgA was detected in all the samples except the tracheal secretion where it was detectable in 90%. IgM was seen in all the samples of serum, colostrum, milk and bile, and detected in only one-half samples of the saliva, nasal secretion and intestinal fluid examined.

IgG was predominant and occupied more than 70% of the total immunoglobulin content in the serum, colostrum and milk (Table 2). In the colostrum, IgG level was high right after parturition, but it decreased rapidly from one-half to one-fourth of the initial-level by nursing (Fig. 4). In contrast, it was minor component in bile, tear and intestinal fluid, and was lower than 10% of the total immunoglobulin content (Table 2). IgG level was variable in the nasal secretion, tear, tracheal secretion and intestinal fluid.
Table 2. The immunoglobulin levels of feline body fluids

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Number of animals examined</th>
<th>Mean values and range of immunoglobulin levels (mg/ml)</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>24</td>
<td>18.94(11.71-22.58)</td>
<td>2.85(1.02-5.82)</td>
<td>2.47(0.60-3.90)</td>
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<tr>
<td>Colostrum</td>
<td>6</td>
<td>35.70(27.50-46.74)</td>
<td>2.54(0.50-4.88)</td>
<td>1.10(0.31-3.00)</td>
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</tr>
<tr>
<td>Milk&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>4</td>
<td>1.89(0.95-2.55)</td>
<td>0.13(0.09-0.20)</td>
<td>0.20(0.10-0.40)</td>
<td></td>
</tr>
<tr>
<td>Saliva&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>24</td>
<td>0.02(0.0-0.06)</td>
<td>0.07(0.03-0.20)</td>
<td>0.03(0-0.12)</td>
<td></td>
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<tr>
<td>Nasal secretion&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>23</td>
<td>0.05(0.0-0.39)</td>
<td>0.07(0.03-0.29)</td>
<td>0.02(0-0.15)</td>
<td></td>
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<tr>
<td>Tracheal secretion&lt;sup&gt;c)&lt;/sup&gt;</td>
<td>10</td>
<td>0.20(0.0-1.46)</td>
<td>0.42(0-1.56)</td>
<td>0.11(0-0.34)</td>
<td></td>
</tr>
<tr>
<td>Tear&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>24</td>
<td>0.02(0.0-0.17)</td>
<td>0.14(0.05-0.33)</td>
<td>0.04(0-0.12)</td>
<td></td>
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<tr>
<td>Bile</td>
<td>14</td>
<td>0.11(0.03-0.41)</td>
<td>0.46(0.18-0.89)</td>
<td>0.54(0.06-0.96)</td>
<td></td>
</tr>
<tr>
<td>Intestinal fluids&lt;sup&gt;d)&lt;/sup&gt;</td>
<td>20</td>
<td>0.03(0.0-0.20)</td>
<td>0.35(0.10-0.84)</td>
<td>0.34(0-2.34)</td>
<td></td>
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</table>

<sup>a)</sup> 2 to 5 weeks after parturition.  
<sup>b)</sup> stimulated with pilocarpine hydrochloride.  
<sup>c)</sup> collected by washing of tracheal cavity with PBS.  
<sup>d)</sup> extracted from feces with PBS.

IgA was predominant in the saliva, nasal secretion, tear, tracheal secretion, intestinal fluid and bile, accounting for 41.4% to 70.7% of the total immunoglobulin content (Table 2). In contrast, it accounted for 5.9% to 11.7% of the total immunoglobulin content in the colostrum, milk and serum.

IgM was predominant (approximately 47%) together with IgA in the intestinal fluid and bile, but they were a minor component (2.8% and 9.0%) in the colostrum and milk (Table 2).

**DISCUSSION**

In the colostrum, IgG was the most predominant immunoglobulin, and the concentration was higher than that of the serum. The concentration of IgA and IgM were approximately the same level as the serum. These results are the same as in cattle [16], but differ from pigs [3] and dogs [9, 23, 30], in which, the IgA level in colostrum is about 10 times that in serum. In the present study, serum-IgA was used as a standard protein for SRID. The actual IgA levels of the secre-

Fig. 4. The change of immunoglobulin levels in the colostrum according to days after parturition. Colostrum from cat A (○: IgG, △: IgA, □: IgM) and from cat B (●: IgG, ■: IgA, ▲: IgM).
tions may be slightly higher than this result since the IgA molecular size of secretions was thought to be larger than that of serum. The high IgG level of colostrum suggest the selective transfer of IgG from the maternal serum to the colostrum [8] as in other animals [20, 34]. In the milk, both IgG and IgA levels were below 10% of the colostrum, however, IgG was most predominant. This result is approximately the same as in ruminants [20], and markedly different from dogs [23, 30], horses [18] and pigs [3], in which IgA is a major immunoglobulin. Feline colostrum and milk have an immunoglobulin composition similar to ruminants.

In the nasal and tracheal secretions, IgA was predominant, however, IgG was a close second to IgA, and IgM was a minor component. This result is the same tendency as seen in pigs [19] and sheep [27], but it is not as extreme a level as in dogs where the IgG level is 2 times the IgA level in tracheal secretions [11]. This IgG has been thought to be transported from serum [28] or synthesized in the lower respiratory tract [12, 19, 35].

In the bile and intestinal fluid, IgA was most predominant, however, IgM was a close second to IgA. This result was similar to that in pigs [7]. Secretory component (SC)-mediated transfer of IgA into external secretions was shown in several early studies [4, 28, 33]. Subsequent studies showed SC-mediated transfer of IgA into bile and of IgM into intestinal fluids in rats and human [5, 32]. However, the detection ratios of IgG and IgM were lower in the feline intestinal fluid than in the bile, and the IgG and IgM levels in intestinal fluid were quite variable. These results may be due to the action of proteolytic enzymes in part [14].

In the present investigation, some facts were confirmed. IgG was predominant with IgA and IgM being minor in the serum, colostrum and milk, and IgA was predominant in other secretions. Furthermore, there was a tendency for the IgG ratio of total immunoglobulin concentration to be higher than the IgM ratio in the respiratory secretions, and the IgM ratio was higher than the IgG ratio in the intestinal fluid. The source of immunoglobulins in secretions is thought to be local production [28] or selective transport of serum-derived immunoglobulins [26]. Further investigation of immunoglobulin-positive cells in respiratory and intestinal mucosa is necessary.

REFERENCES

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