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Kinetics of Immunoglobulin Transport into Canine Bronchial Secretions (39425)

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The established use of periodic gamma globulin injections to help control sino-pulmonary infections in patients with hypo- or agammaglobulinemia (1-3) suggests that antibody from the intravascular space gains access to lung tissue and respiratory secretions. These commercial preparations of gamma globulin contain primarily immunoglobulin G (4). It has been established that IgG is a major constituent of the protein milieu of the lower respiratory tract in humans (5-7) and a variety of animals including the rabbit (8), dog (9-11), and rat (12). Other immunoglobulins such as IgA and IgE are present, but IgM is rarely found (7) or, if so, in very small amounts (11). *In vitro* studies of bacterial phagocytosis by alveolar macrophages obtained from humans (13) and rabbits (14) have shown opsonic IgG antibody to enhance bacterial uptake by these cells and thus help explain the apparent usefulness of gamma globulin therapy (3).

This present study examines the transfer of intravascular IgG into broncho-alveolar secretions, the kinetics of this protein in the lower respiratory tract, and the effect of altering the IgG molecule on this transfer. Normal dogs have been used.

Materials and methods. Animals. One-year-old male beagle dogs weighing approximately 10-15 kg were used. Animals were housed individually in cages which were designed to collect urine specimens.

Experimental design. To study the plasma to broncho-alveolar fluid transfer of parenterally administered immunoglobulins, groups of dogs were injected with ^{125}I-labeled purified proteins and the distributions of radioactivity evaluated. After intramuscular injection, serum samples were taken at 2-hr intervals for the first 8 hr, then daily (9 AM) for the first week and twice weekly thereafter. The urine was monitored daily for ^{125}I activity. Pulmonary lavage was performed 8 hr after injection of labeled protein, daily for the first week, and twice weekly thereafter.

Preparation of immunoglobulin G and M for injection. Methods used to obtain canine serum IgG and IgM have been described (15, 16). In brief, pooled dog serum was precipitated with 30% dry ammonium sulfate; the precipitate was redissolved and dialyzed extensively against borate-saline buffer to remove excess ammonium sulfate. After a final dialysis in 0.02 *M* tris(2-aminohydroxymethyl-1,3-propanediol) HCl buffer, the preparation was chromatographed on DEAE-cellulose and eluted with a 0.3 *M* NaCl-Tris gradient. IgG containing fractions of low molarity (conductivity 1.0-9.0 mmho) and pH 8.0-8.5 were collected and gel filtered through Sephadex G-200. Effluent protein was identified as IgG by the criteria of cathodal electrophoretic mobility on immunoelectrophoresis, immunoprecipitation with specific anti-Fc gamma (γ) chain antiserum (15), and a sedimentation coefficient of 6.7S found with ultracentrifugation (16). IgG was principally of the subclass γa,b (15).

IgM was isolated from the same serum preparations except that protein fractions of conductivity 12-16 mmho and pH 9.0 after DEAE chromatography were pooled. This material was passed over a Sepharose 2B rabbit anticanine IgM immunoadsorbent (17) and IgM was eluted with 0.1 *M* citrate, pH 2.2. Finally, IgM was gel filtered through Sepharose 6B and concentrated with positive pressure ultrafiltration. Immunoglobulin preparations were stored at 4° until iodinated.

$F(ab')_2$ fragments of IgG were made by pepsin digestion (14). Purified IgG was digested with pepsin (Worthington Biochemical Co., Freehold, NJ) at pH 4.3 for 8 hr

and gel filtered on Sephadex G-150. Sedimentation coefficients for the fragments were determined by ultracentrifugation (Beckman model E).

Protein iodination. Purified serum immunoglobulins were labeled with carrier-free ^{125}I (New England Nuclear, Boston, Mass.) by the method of McConahey and Dixon (18) using choloramine-T and sodium metabisulfate. The excess ^{125}I was removed by extensive dialysis against 0.9% saline buffer. The specific activity of the preparations used ranged from 7.85×10^7 to 1.73×10^8 cpm/mg. Each animal received a 3-ml intramuscular injection containing 2.5 mg of radiolabeled purified immunoglobulin.

Collection and processing of specimens. Selective lavage of lower lung lobe was done through a fiberoptic bronchoscope as described previously (19). The bronchoscope was wedged in a lower lobe bronchus and two 50-ml aliquots of sterile 0.9% saline were infused and aspirated into a sterile syringe. Sequential washes on successive days were done in alternate lower lobes. All animals tolerated the procedure well and had no noticeable adverse effects.

The lavage fluid was centrifuged at 500*g* for 15 min at 25° and the supernatant was decanted from the cell pellet. The percentage of red blood cells in the pellet was consistently less than 3% in analyzed specimens. A 1-ml aliquot of the lavage fluid was analyzed for radioactivity in an automatic gamma counter (Series 1185, Nuclear Chicago Corp., Des Plaines, IL). In certain experiments the entire supernatant fluid was concentrated to a 2–3 ml vol with positive pressure ultrafiltration (UM-10 filter, Amicon Corp., Lexington, MS) at 4°.

Serum was extracted from clotted blood. Twenty-four-hour urine samples were collected from animal cages, and after the volume was measured, 1 ml was examined for radioactivity.

Sucrose density ultracentrifugation (8, 16) was used to separate serum and bronchial proteins. Samples (0.25 ml) were layered on 5-ml linear sucrose-density gradients (10–40%) and centrifuged at 35,000 rpm for 18 hr at 4°. Fractions of 0.2 ml were collected and counted in a gamma counter to identify the position of the labeled protein in the serum and bronchial secretions. Immunoglobulins were identified in gradient fractions by gel diffusion, using antisera specific for each protein. Rabbit anti-IgG Fc and Fab antisera were used (15). It was felt that ultracentrifugation provided a more accurate identification of labeled immunoglobulins than nonspecific precipitation of protein.

Calculations. Data were plotted semilogarithmically and the slopes of curves determined by the method of least squares. Student's *t*-test was used to compare the decay curve slopes. The half life was derived from the equation for exponential decay (20), with corrections made for ^{125}I decay during the time of observation. The plasma volume was assumed to be 7% of each animal's body weight (21). The urine activity was corrected to total urine volume on the basis of 24 hr urine collection.

Radioactivity in broncho-alveolar washings was corrected to total bronchial surface area. It was determined in a group of control animals that the 100 ml lower lobe lavage consistently sampled about 25% of the bronchial surface area. When an equivalent amount of radiopaque dye was infused and aspirated through the bronchoscope, chest X-rays taken immediately after removal of the dye showed selective opacification of the lower lobe area. Autopsy and *in vitro* lung infusions were used to verify this estimate.

Results. Reproducibility of broncho-alveolar lavage. Seventy-five 100-ml lavages were performed in these studies, and the mean recovery of fluid was 78 ± 0.77 ml (range, 61–90 ml). To document the consistency of protein values obtained by serial bronchial lavage, two dogs were lavaged daily for 5 successive days and the albumin and IgG values in concentrated lavage fluid specimens (mean final volumes 2.8 ml ± 0.2 SEM) measured. There was a variation of 5–8% in the daily values of these two proteins with repeated lavage. The mean ratio of IgG (mg/ml) to albumin (mg/ml) was 0.06 ± 0.003 for the 10 lavages and was similar in both animals. Serum values for IgG and albumin showed no fluctuation during the 5 days of lavage.

Distribution of radiolabeled immunoglobulin. The pattern of distribution of IgG is

shown in Fig. 1. Each point represents the arithmetic mean and standard error of determinations from four dogs. Rapid absorption of intramuscularly administered IgG was noted by 2–4 hr with maximal plasma activity evident at 24 hr, followed by gradual diminution over the next 25 days. At 8 and 24 hr there was evidence of some radioactivity in the bronchial secretions but maximal activity was seen at 48 hr, followed by an elimination rate during the next 3 weeks that was not significantly different ($p > 0.20$) from that in serum. Figure 2 shows a representative separation in sucrose density gradients of serum and concentrated bronchial secretions obtained 48 hr after injection of labeled IgG. Greater than 95% of the radioactivity is associated with gradient

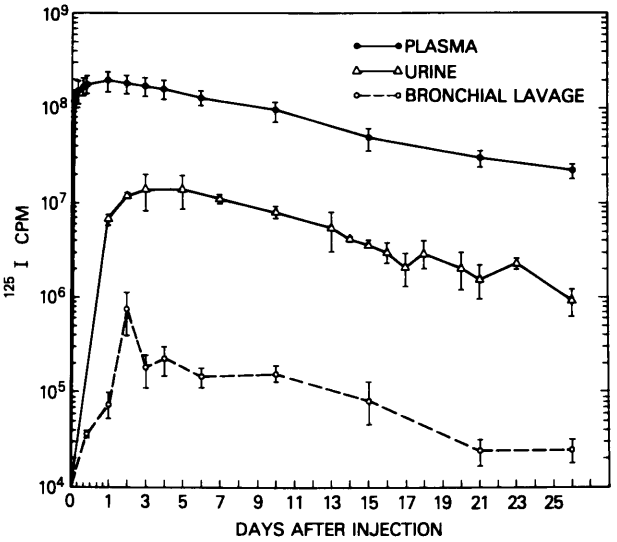

FIG. 1. Distribution of radioactivity after intramuscular administration of ^{125}I-labeled IgG in plasma, urine, and bronchial lavage fluid is shown for 26 days of observation. ^{125}I activity depicted on the ordinate has been corrected to total plasma volume, urine volume, and bronchial surface area. Each point represents the mean and standard error of the activity found in four dogs.

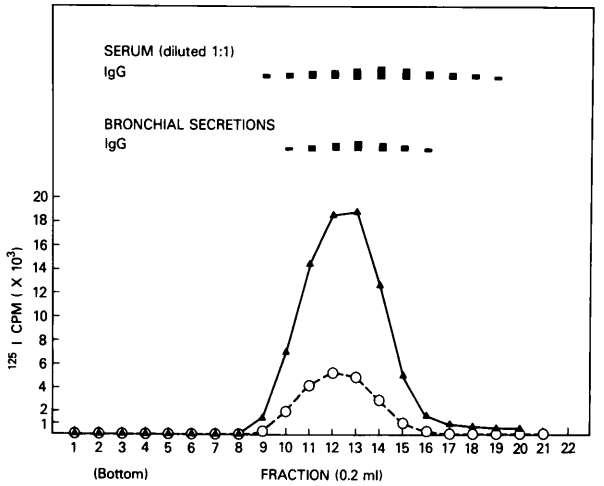

FIG. 2. Separation is shown for serum and concentrated bronchial secretions obtained 48 hr after intramuscular injection of radiolabeled IgG on identical 10 to 40% linear sucrose density gradients. Vertical bars give relative IgG concentrations in the gradient fractions. ^{125}I activity measured on the ordinate coincides with the sedimenting position of IgG in both the serum (▲– –▲) and bronchial secretion (○– –○) gradients.

fractions containing IgG in both specimens, confirming that nonspecific radioactivity or protein degradation products are not responsible for the measured ^{125}I activity. However, in urine samples radioactivity was nonprecipitable and not attached to identified protein moieties.

The elimination of radiolabeled IgG from the serum of this group of dogs was 9 days ($T_{1/2}$, Table I). In the lavage specimens, the $T_{1/2}$ of 7.5 days was not statistically different from serum ($p > 0.20$). Cumulative results from the 26 days of study indicated that 0.15% of the initial IgG dose was recovered in the lung secretions.

Pepsin-digestion of IgG produced $F(ab')_2$ fragments with a sedimentation coefficient of 5.5 $s_{20,w}$. After labeling with ^{125}I, these fragments were injected into a pair of dogs and the distribution monitored (Fig. 3). Again, there is rapid absorption of the intramuscularly administered protein and maximal radioactivity in bronchial secretions is seen 24 hr after injection, although some activity is evident at 8 hr. Most of the radioactivity in the bronchial secretions was gone by 10 days after injection of labeled protein. Repeated sucrose density gradient separations of serum and concentrated bronchial secretions from these dogs indentified radioactivity in the $F(ab')_2$ fractions of the gradients. Cumulatively, only 0.03% of initial dose of $F(ab')_2$ fragments were recovered in lung lavage specimens. As given in Table I, the elimination rates in serum and lavage were much less for the fragments than intact IgG.

Similar studies in a pair of dogs were done with IgM. The kinetics of IgM absorption were similar to those found for IgG except the elimination rate from serum was less. No IgM associated radioactivity was detected in broncho-alveolar lavage specimens.

TABLE I. HALF-LIFE OF ^{125}I-LABELED PROTEINS IN SERUM AND LUNG LAVAGE.

	Serum	Broncho-alveolar lavage
IgG ($n = 4$)	9.0 ± 0.08[a] (8.8-9.2)	7.5 ± 0.38 (6.5-8.3)
F(ab') ($n = 2$)[2]	4.3[b]	1.8
IgM ($n = 2$)	5.2	

[a] Mean $T_{1/2}$ in days corrected for ^{125}I decay ± SEM.
[b] Average value.

Discussion. The objective of this study was to determine the kinetics of immunoglobulins entering the lower respiratory tract secretions from the intravascular space in normal animals. In this respect a very small percentage (0.15%) of parenterally administered IgG was recovered in broncho-alveolar lavage specimens during approximately 4 weeks of observation. Alteration of the IgG molecule to create an $F(ab')_2$ fragment, devoid of the Fc portion of the gamma chain, effectively reduced the transudation of IgG into respiratory secretions to an almost negligible amount. Moreover, intact IgM did not appear in the broncho-alveolar lavage.

We conclude from these studies in dogs that circulating humoral IgG contributes only a small portion of the immunoglobulin found in pulmonary secretions. Thus, it appears that passive infusion of IgG is not an efficient method of increasing IgG in respiratory secretions of normal subjects. However, the critical level of specific antibody in respiratory secretions needed for protection against infectious agents is now known and may be quite small. Furthermore, pulmonary irritation or suppuration undoubtedly cause changes in pulmonary vascular permeability and serve to increase concentrations of immunoglobulins in respiratory secretions.

It was of interest that peptic digestion of IgG to create a smaller protein fragment (5.5S) devoid of the Fc portion reduced transudation or penetration into lung secretions. The Fc portion of the molecule may be critical for determining the extravascular distribution of IgG as well as other biological activity of the immunoglobulin. In rabbits (14), $F(ab')_2$ fragments generated from IgG having specific antibody activity for *Pseudomonas aeruginosa,* retain agglutinating activity and opsonizing potential. However, these antibody fragments are ineffective in promoting phagocytosis by alveolar macrophages because the antibody cannot attach to the Fc membrane receptor of the macrophage and initiate bacterial ingestion.

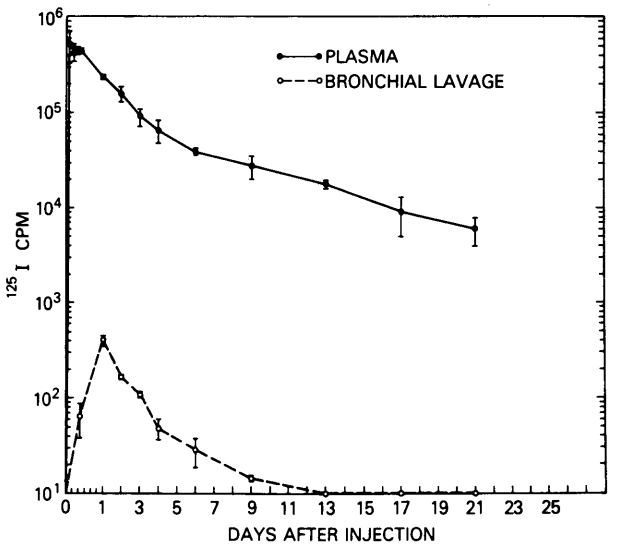

FIG. 3. Distribution of radioactivity after intramuscular administration of ^{125}I-labeled F(ab')$_2$ fragments of IgG in serum and bronchial lavage fluid is shown for 21 days of observation. ^{125}I activity on the ordinate has been corrected to total plasma volume and bronchial surface area. Each point represents an average value and the range found.

The intravascular clearance of canine IgG was similar to that previously reported for gamma globulin. The calculated $T^1/_2$ of 9.0 days for IgG in this study is comparable to that of 7 (22) and 8 (23) days found in earlier studies using dog gamma globulin. The rate of clearance of dog IgM and F(ab')$_2$ fragments have not been studied previously. Studies with mouse (24) and human (25, 26) IgG have shown that the catabolic rate of these proteins is directly proportional to the concentration of IgG in plasma, and that the Fc portion of the molecule appears to be the structural configuration critical for this concentration dependent catabolism. In contrast, this requirement does not occur in the guinea pig (27). Such factors affecting catabolism of dog IgG are not known at present. The rapid catabolic rate for human F(ab')$_2$ fragments of 5 hr (28) was not duplicated in our study, but the $T^1/_2$ value of 4.3 days (Table I) is appreciably shorter than for intact dog IgG.

Summary. Radiolabeled ^{125}I preparations of canine IgG, IgM, and F(ab')$_2$ fragments of IgG were injected intramuscularly into normal dogs to quantitate the transfer of immunoglobulins from the intravascular space into the lower respiratory tract and to observe the clearance of these proteins from lung secretions. Respiratory fluids were sampled by serial broncho-alveolar lavages during a 3–4 week interval. Only a small portion (0.15%) of the passively administered IgG was recovered in respiratory specimens indicating that minimal transfer of humoral IgG occurs under normal conditions.

Alteration of the IgG molecule by removal of the Fc portion almost eliminates its penetration into lung secretions. Furthermore, IgM, which is not a prominent component of normal lung secretions, is not detected in these secretions following passive intramuscular injection.

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