

Induced homocytotropic antibody to bovine serum albumin in nonatopic dogs

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Six dogs with no skin reactivity to bovine serum albumin (BSA) were used to determine the antibody response to that antigen. The animals were divided into 3 equal groups given 5, 10, or 25 mg. BSA in Freund's incomplete adjuvant weekly, until a definite skin reaction could be detected. All sera were studied by passive transfer, double-gel diffusion, tanned sheep red blood cell hemagglutination, coprecipitation, and passive cutaneous anaphylaxis (PCA). Serum from one dog contained passively transferable antibody and was studied further by autoradiography and fractionation on Sephadex G-200. Skin reactivity to BSA could be detected up to 27 weeks in 5 of the 6 dogs. Hemagglutinating antibody was inhibited by the addition of 2-mercaptoethanol, and coprecipitation demonstrated that the antigen-binding capacity could be correlated with hemagglutinating antibody. Serum from the dog with passive transfer activity also contained precipitating antibody, and PCA activity could be demonstrated in guinea pigs. Autoradiography of the serum demonstrated 2 distinct arcs of anti-BSA activity. Homocytotropic antibody activity could be detected on the ascending slope of the second peak by Sephadex G-200 filtration, while hemagglutination antibody was localized to the first peak. These results indicate that antibodies with different activities can be induced in the dog following injection of BSA. The induced homocytotropic antibody has characteristics similar to spontaneous canine homocytotropic antibody.

The term *homocytotropic antibody* has been applied to the reaginic antibody that sensitizes homologous skin.¹ We have previously characterized induced homocytotropic antibody to ragweed and timothy pollen in nonatopic dogs,² and have shown it to be similar to the spontaneous homocytotropic antibody in the dog, as described by Patterson, Roberts, and Pruzansky.³ The biologic activities and immunochemical characteristics of these homocytotropic antibodies suggest that they belong to the class of immunoglobulins analogous to human IgE.

The biologic activity of other canine antibodies, such as nonprecipitating and precipitating antibody to bovine serum albumin (BSA), has been described by

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TABLE I. Amount of BSA administered and duration of skin reactivity of animals

Dog	Mg. of BSA in Freund's incomplete adjuvant weekly	Total dose of BSA in mg.	Skin reactivity weeks inclusive
Group 1	5	20	4-23
I 2		20	4-7
Group 3	10	40	Nonreactive
II 4		30	3-5
Group 5	25	75	3-17
III 6		75	3-30

Patterson, Pruzansky, and Janis,⁴ but canine skin reactivity to this antigen has not been described. The present study was, therefore, carried out to determine if homocytotropic antibody to bovine serum albumin could be induced and detected in the dog.

MATERIALS AND METHODS

Antigen

Crystallized BSA (Miles Labs., Inc., Kankakee, Illinois) was dissolved in 0.08M NaCl phosphate buffer, pH 8.0, and used as the antigen in all studies. BSA was labeled with I^{125} by the method of Bale and associates,⁵ and the radioactivity was measured in a NaI scintillation counter. Following dialysis against distilled water, over 98 per cent of the I^{125} was bound to BSA, as determined by precipitation with 10 per cent trichloroacetic acid.

Immunization procedure

Six outbred dogs with no demonstrable wheal and flare skin reactions after the intracutaneous injection of 50 μ g of BSA, followed by 5.0 ml. of 0.5 per cent Evans blue dye given intravenously, were used in the study.

The animals were divided into 3 groups of 2 dogs each, based on the total amount of BSA to be given (Table I). All dogs were given intramuscular injections of BSA in incomplete Freund's adjuvant until a definite positive wheal and flare reaction could be detected by weekly intracutaneous testing, or until a total of 4 injections was administered. Two dogs (Group I) received 5 mg. of BSA per week, 2 (Group II) were given 10 mg. BSA per week, and the remaining 2 animals (Group III) were given 25 mg. of BSA per week. In Group I, each dog received a total of 20 mg. of BSA; in Group II, one dog received a total of 40 mg. BSA and the other received 30 mg.; in Group III, each dog received a total of 75 mg. of BSA. The induced dog sera (IDS) were collected prior to the first injection of antigen and at least every 2 weeks thereafter, until the study was completed, and were stored at -70° C. in aliquots until used.

Skin testing

Direct skin tests were performed weekly using 0.02 ml. of antigen injected intracutaneously in serial tenfold dilutions. The concentrations used ranged from 1.0 to 0.0001 mg. per milliliter BSA, and a similar volume of buffered saline was used as a control. Five milliliters of 0.5 per cent Evans blue was administered intravenously prior to the test procedure to make the reactions more apparent. The animals were tested by this method up to 27 weeks after completion of the immunization.

Detection of antibody

The passive transfer technique used to detect homocytotropic antibody was done on all dogs with demonstrable wheal and flare skin reactivity to BSA. Two nonimmunized dogs with

TABLE II. Summary of immunologic reaction in 6 dogs sensitized with 3 different doses of BSA

Dog	Week post-immunization	Skin reactivity (mg. BSA)	Hemagglutination*	Coprecipitation†	Precipitins*	PCA*	P-K
1	Control	0	1:40	0	0	0	0
	9	0	1:520	11.25	0	0	0
	17	0.001	1:320	0	0	0	0
2	Control	0	1:80	—	0	0	0
	6	0.1	1:2,560	1.75	0	0	0
3	Control	0	1:80	—	0	0	0
	4	0	1:1,280	0	0	0	0
	8	0	1:5,120	6.25	0	0	0
4	Control	0	1:80	0	0	0	0
	3	0.1	1:320	0	0	0	0
	7	0	1:2,560	3.12	0	0	0
5	Control	0	1:40	—	0	0	0
	6	0.01	1:80	0	0	0	0
	14	0.001	1:5,120	6.31	0	0	0
6	Control	0	1:160	1.25	0	0	0
	4	0.1	1:640	12.5	+	+	+
	8	0.001	1:10,240	12.5	0	0	+
	10	0.01	1:327,640	25.6	0	0	0
	25	0.01	1:10,240	6.25	0	0	0

*Using BSA as antigen.

† μ g BSA N added at a point where 80 per cent of antigen was in precipitate.

no wheal and flare reactivity to BSA were used as recipients for each test. No dog was used for more than one set of passive transfer tests. One-tenth milliliter aliquots of IDS were injected intracutaneously in twofold dilutions into the shaved skin of the recipients. After 24 hours the sites were challenged by intracutaneous injections of 50 μ g of BSA, followed by the intravenous injection of 5 ml. of 0.5 per cent Evans blue dye. Control sites consisted of aliquots of undiluted serum from dogs spontaneously sensitive to ragweed, serum from nonimmunized dogs, and buffered saline. The sites were read at 20 minutes, and the titer of each serum was considered to be the highest dilution transferring skin reactivity.

Aliquots of IDS with passively transferable wheal and flare activity were then heated at 56° C. for 4 hours and injected into the recipients. Appropriate unheated control sera were also used. The intracutaneous sites were challenged 24 hours later with BSA as above.

Immunodiffusion studies

Agar plates were prepared in 1 per cent agar (Agarose, Bausch and Lomb, Rochester, New York) in 0.08M NaCl phosphate buffer, pH 8.0. Double-gel diffusion was performed with IDS, using concentrations of BSA ranging from 5 to 1 mg. per milliliter.

Autoradiography was done by subjecting canine serum to electrophoresis in 1 per cent agar gel on glass slides, 10 cm. by 8.75 cm., using 180 V. at 8 ma. for 3 to 4 hours. Precipitin bands were developed with rabbit anti-canine serum. After washing with 0.15M NaCl for 72 hours, the slides were incubated for 48 hours with I^{125} -labeled BSA, rewashed, dried, and placed in contact with x-ray film for variable lengths of time until bands of radioactivity could be detected.

Passive cutaneous anaphylaxis

The method of Ovary⁶ was used to determine if antibodies against BSA could be detected by passive cutaneous anaphylaxis (PCA) in guinea pigs. One-tenth milliliter samples of undiluted IDS collected at various dates and control sera obtained prior to immunization were injected intracutaneously into 2 guinea pigs. Four hours later 1 mg. of BSA and 1.0 ml. of

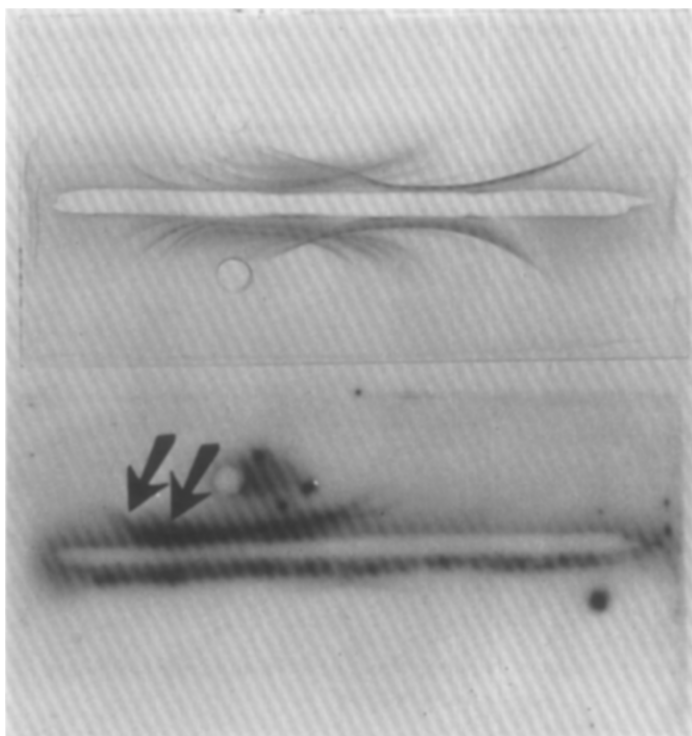


FIG. 1. Upper pattern: Immunoelectrophoretic pattern of electrophoresed dog serum-reacted with rabbit anti-canine serum. **Lower pattern:** Autoradiographic pattern indicating the presence of anti-BSA activity in the immunoglobulin region. The arrows indicate arcs of activity migrating in the region of IgG and IgA. The fast-moving arc, which also appears in the control, is an artefact.

0.5 per cent Evans blue dye were injected intravenously, and the sites were examined in 20 minutes for extravasation of dye.

Tanned sheep red blood cell hemagglutination

The method of Stavitsky⁷ was used to determine hemagglutinating titers of the IDS. Sheep cells in modified Alsever solution (Gibco Microbio Laboratory, Madison, Wisconsin) were used. Duplicate serial twofold dilutions of control sera and the various samples of IDS from each dog were incubated with tanned sheep red blood cells previously sensitized with 1 mg. per milliliter of BSA. Titers of antibody were expressed as the reciprocal of the highest positive dilution. A 0.6M solution of 2-mercaptoethanol (2-ME) (Eastman Organic Chemical, Rochester, New York) was added in an amount of 0.05 ml. to aliquots of IDS and incubated at 4° C. overnight, and the hemagglutination technique was repeated as above.

Coprecipitation of bound I¹²⁵ BSA

The amount of BSA bound by the IDS was determined by the coprecipitation technique of Pruzansky and Patterson.⁸ Sera were selected from all dogs on dates of high hemagglutinating and low SSA titers, and at times when there were high SSA and low hemagglutinating titers. Preimmunization sera were used as controls. Increasing amounts of BSA, mixed with constant amounts of I¹²⁵-labeled BSA, were added to 0.05 ml. of the dog sera and incubated for one hour. The canine immunoglobulins were then precipitated by the addition of 1.5 ml. of rabbit anticanine Fraction II. This amount of antiserum was determined previously to be sufficient to precipitate all of the immunoglobulins in 0.05 ml. of the dog serum. The percentage of

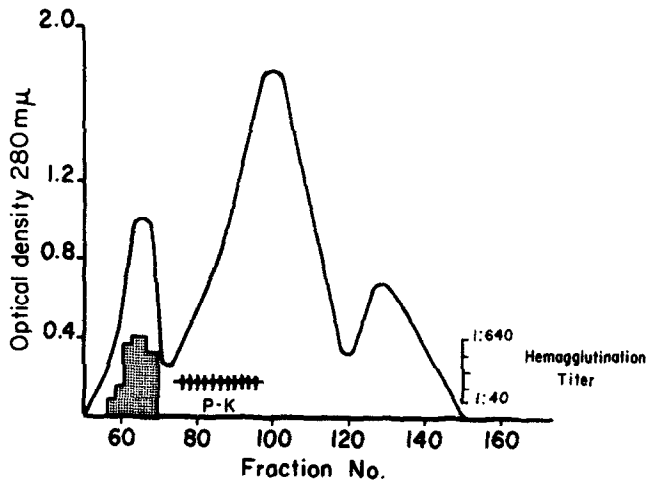


FIG. 2. Results of column chromatography of canine anti-BSA serum on Sephadex G-200. Stippled area indicates the presence of hemagglutinating antibodies. PK denotes the presence of transferable skin-sensitizing antibody.

counts present in the precipitates were determined and were plotted against the total amount of antigen nitrogen added to each sample. For comparison purposes the relative binding capacity of each serum was considered to be the amount of antigen bound at the point of 80 per cent coprecipitation.

Fractionation of sera by Sephadex G-200 filtration

Sephadex G-200 (Pharmacia Fine Chemical, Inc., Piscataway, New Jersey) was stirred with 4 times the estimated bed volume of buffer containing 1.0M NaCl in 0.1M Tris, pH 8.0. The gel was allowed to swell for 3 days before use at room temperature, and a 2.5×100 cm. column (Pharmacia Fine Chemical, Inc., Piscataway, New Jersey) was prepared.

Three milliliters of IDS were applied to the Sephadex G-200, and the same buffer was used as the eluent. Flow rates were adjusted to 8 to 9 ml. per hour. Two-milliliter fractions were collected, and the optical densities of each fraction were determined at 280μ . Fractions from various points of the protein peaks obtained were tested for anti-BSA activity by passive transfer, gel diffusion, tanned red cell hemagglutination, and 2-ME sensitivity.

RESULTS

The results are summarized in Table II.

Skin reactivity

Five of the 6 immunized dogs developed immediate wheal and flare skin reactions to BSA. Both dogs in Group I became definitely reactive after the fourth injection of antigen. One dog in Group II remained unreactive, while the other reacted at the time of the third intracutaneous test. In Group III, both dogs reacted after the third test. Following cessation of the immunization procedure, the wheal and flare reactions could be detected by challenge after 2 to 27 weeks. Therefore, 5 of the 6 immunized dogs became skin reactive to BSA, and that reactivity, although of a variable degree, persisted in most animals for prolonged periods.

Immediate wheal and flare antibody activity could be passively transferred

from only one of the dogs—the animal that received a total of 75 mg. BSA. The reactivity could be transferred from as early as the third week to the ninth week in titers ranging from undiluted to 1:1,024. All wheal and flare reactivity was destroyed by heating at 56° C. for 4 hours.

Immunodiffusion studies

Precipitating antibody against BSA could be detected only in the dog with passive transfer activity at Weeks 2, 3, and 5. Autoradiography of serum from this dog demonstrated 2 distinct arcs of anti-BSA activity, one in the IgG region and the other in the IgA region (Fig. 1).

Passive cutaneous anaphylaxis (PCA)

Antibody activity could be demonstrated by this technique only in that immunized animal that had homocytotropic antibody, as demonstrated by passive transfer activity to the skin of other dogs. The PCA activity was detectable transiently from the ninth through the eleventh weeks postimmunization.

Tanned sheep red cell hemagglutination

Hemagglutinating antibody to BSA could be detected in low titers in control sera and prior to skin reactivity and rose to a maximum peak from the fifth to the thirteenth weeks. Elevated but declining levels of antibody could be detected in all immunized dog sera by this method throughout the study. The hemagglutinating antibody activity in all sera was inhibited by the addition of 2-ME.

Coprecipitation

Binding of BSA by the dog sera was variable, ranging from less than 1 μ g to over 25 μ g of antigen N bound per milliliter antiserum. The binding of BSA could be correlated with the level of detectable hemagglutination titer, and was highest in that animal with precipitins and skin reactivity transferable to other dogs or guinea pigs.

Fractionation of IDS by Sephadex G-200 filtration

Fractionation of the canine anti-BSA serum containing passively transferable skin reactivity resulted in 3 peaks (Fig. 2). The hemagglutination antibody was located in the first peak, with the maximal activity localized at the apex of this curve. The hemagglutinating activity of the first peak was inhibited by treatment with 2-ME. Homocytotropic antibody activity could be detected on the ascending slope of the second peak (Fig. 2). Precipitating antibody against BSA could not be detected in any of the fractions, even after concentration of the sera. No other immunologic studies were done on these fractions.

DISCUSSION

The present study indicates that, in addition to the several antibodies detectable in the dog following immunization with BSA,⁹ homocytotropic antibody to that antigen may also be detected.

Although skin reactions to BSA could be detected in all but one immunized

dog, the homocytotropic antibody activity could be passively transferred in only one animal. The heat lability and the location of the transferable skin reactivity on Sephadex fractionation were similar to features described for spontaneous or induced canine homocytotropic antibody to inhalant antigens.^{2, 3, 10} This suggests that these several antibodies, induced spontaneously or with adjuvant, were analogous to human IgE.³ The findings in the present study also suggest that the anti-BSA homocytotropic antibody was IgE.

Anti-BSA antibodies belonging to the immunoglobulin class IgG were also probably induced in the dogs. Precipitating antibodies could be detected early, and autoradiography and passive cutaneous anaphylaxis suggested that they were IgG.

IgM with anti-BSA activity was also probably induced in the dogs. All animals had detectable hemagglutinating antibody, regardless of the skin reactivity, and all control sera also had low titers of anti-BSA activity. This may have represented previous contact with BSA, as in the diet, or a nonspecific low titer hemagglutination we have previously observed.² The hemagglutinating antibody activity was inhibited with 2-ME and was localized to the first peak of the Sephadex G-200 fractionation. These properties have been shown to be those of IgM.¹¹

It is also possible that IgA was produced as a result of the immunization. Autoradiography demonstrated an arc of anti-BSA activity migrating differently than IgG or IgM.

Heterogeneity of the immune response was not seen in all dogs. Only one dog responded with detectable levels of activity in all of the immunoglobulins, and the highest titer of antibody activity was seen in that dog with the homocytotropic antibody passively transferable. This dog also demonstrated precipitating antibody and had the greatest binding capacity, as detected by coprecipitation; it may have thus been a "responsive" animal, or may have had higher titers of homocytotropic antibody than the other experimental animals. Five of the 6 dogs had demonstrable homocytotropic antibody (probably IgE), and all dogs had detectable IgM levels. These findings suggest that the immune response of the dog is variable in its heterogeneity. Some animals seem to respond to immunization by producing one class of antibody preferentially over another, while others have a more general antibody response.

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