

Production of anaphylaxis with ragweed in nonatopic dogs

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Ten nonatopic dogs were sensitized by weekly injections of ragweed extract in Freund's incomplete adjuvant. Skin tests for immediate wheal and flare type of hypersensitivity were done weekly, and hemagglutinating antibody titers were done every 2 weeks. Within 4 weeks all dogs developed positive skin tests to ragweed. Following intravenous challenge with ragweed, all the dogs developed anaphylaxis. More severe reactions were observed in dogs with low hemagglutinating antibody titers. No systemic reactions were observed in control dogs, and passive anaphylaxis with induced antibody could not be elicited. Thus, anaphylaxis was produced in nonatopic dogs with ragweed, further establishing the use of a canine model for the study of atopic diseases.

Induced homocytotropic antibody in nonatopic dogs has characteristics similar to the homocytotropic antibody (HCA) mediating the immediate type of spontaneous hypersensitivity in atopic dogs.¹ The characteristics common to both include: skin-sensitizing ability for immediate wheal and flare type of reaction both by direct challenge and passive transfer, heat lability, persistence at skin sites, and specific neutralization of reactivity by the appropriate antigen. A difference between the induced and spontaneous HCA was the inability to produce systemic anaphylaxis in animals with induced HCA in response to antigenic challenge.¹

The present study was undertaken to attempt to produce systemic anaphylaxis in dogs with induced HCA following ragweed sensitization.

MATERIALS AND METHODS

Antigen

Ragweed extract (RW) was prepared in buffered saline from whole defatted pollen. The amount of protein nitrogen in the extract was determined by the Markham modification of the micro-Kjeldhal method.² The protein nitrogen contained in RW was expressed as protein nitrogen units (PNU) with one PNU equalling 0.00001 mg. of protein nitrogen (PN).

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Skin test antigen

Ragweed antigen in concentrations of 0.01 mg. of PN per milliliter and 0.001 mg. of PN per milliliter were used as previously described.¹ Five milliliters of 0.25 per cent Evans blue dye was given at the same time intravenously to make the reaction more apparent. Buffered saline was used as a control for skin tests.

Sensitization of dogs

Ten adult mongrel dogs were used. To exclude spontaneous ragweed sensitivity, each dog was tested intracutaneously with RW in a concentration of 0.01 mg. of PN per milliliter. Hemagglutinating antibody (HA) titers were determined before challenge. The dogs with negative skin tests and HA titer of 1:80 or less were used for the study.

The dogs were injected with 1 ml. of RW containing 20,000 PNU (0.2 mg. of PN) emulsified in 1 ml. of Freund's incomplete adjuvant as previously described.¹ The dogs were skin tested weekly with RW extract in concentrations of 0.01 and 0.001 mg. PN per milliliter. If the skin tests were negative, the dogs received another injection of RW. The dogs were given injections until the skin tests became positive or a total of four injections were given. Blood (20 ml.) was drawn every other week for HA titer. When the dogs became skin test positive, 30 ml. of blood was drawn, and the dogs were challenged with 20,000 PNU of RW (0.2 mg. PN) per kilogram given intravenously and observed for anaphylaxis. A week after antigenic challenge, 40 ml. of blood was drawn from the dogs that survived. Serum collected before and after challenge was stored in aliquots at -70°C .

Tanned sheep red blood cell hemagglutination

The method of Stavitsky,³ as modified by Arbesman,⁴ was used to determine HA titers of serum. Duplicate serial twofold dilutions of serum were incubated with tanned sheep red blood cells previously sensitized with 10 per cent RW. The titer of the antibody was expressed as the reciprocal of the highest serum dilution demonstrating hemagglutination. Controls consisted of sensitized tanned cells incubated with similar dilutions of normal rabbit serum.

Controls

Three dogs with negative skin tests to RW and a HA titer of 1:80 or less were used as controls for ragweed challenge. Each dog was challenged intravenously with 20,000 PNU of RW (0.2 mg. PN) per kilogram and observed for anaphylaxis.

Passive anaphylaxis

Pre- and postchallenge sera from the first 9 dogs were pooled, and the HCA titer of the pooled serum, as determined by passive transfer, was 1:16. This serum was used for passive anaphylaxis studies in three dogs with negative skin tests to RW and a HA titer of 1:80 or less. The first dog received 2 ml. per kilogram, the second dog 4 ml. per kilogram, and the third dog 6 ml. per kilogram of serum intravenously. Twenty-four hours later each dog was challenged intravenously with 20,000 PNU of RW (0.2 mg. PN) per kilogram and observed for anaphylaxis.

RESULTS

The results of the immunologic studies and clinical observations are shown in Table I.

Only 3 dogs needed four injections before becoming skin test positive. Every dog challenged developed symptoms of anaphylaxis with the more severe reactions occurring in the dogs with lower titers of HA. Two dogs in this group died. An autopsy performed on Dog No. 1 revealed petechial hemorrhages in the gastrointestinal tract wall, liver congestion, and petechiae on the pericardium.

TABLE I. Summary of individual dog responses

Dog No.	HA titer before induction*	No. of RW injections required for induction	Minimum RW concentration for positive skin test (mg. PN/ml.)	HA titer at the time of challenge	Degree of anaphylaxis†
1	1:40	3	0.0001	1:640	Died
2	1:40	4	0.01	1:2,560	Mild
3	1:20	3	0.001	1:1,280	Moderate
4	1:40	2	0.01	1:320	Died
5	1:20	4	0.01	1:5,120	Moderate
6	1:80	4	0.01	1:2,560	Moderate
7	1:80	2	0.001	1:5,120	Moderate
8	1:80	3	0.01	1:80	Severe
9	1:40	2	0.001	1:320	Moderate
10	1:40	2	0.001	1:160	Mild

*The titer of the HA was expressed as the reciprocal of the highest serum dilution demonstrating hemagglutination.

†Mild, moderate, and severe refer to degrees of anaphylaxis: mild—lethargic, unsteady, rapid respiration and retching with recovery in 15 to 20 minutes; moderate—more lethargic, unable to stand, rapid respiration, vomiting and diarrhea, recovery in 30 to 45 minutes; severe—marked lethargy, collapse with Cheyne-Stokes breathing, cyanosis, repeated vomiting and diarrhea (often bloody), leading either to recovery in one to several hours or death.

These changes are characteristic of canine anaphylaxis.⁵ None of the control dogs had reactions following challenge. Passive anaphylaxis was not elicited in the 3 dogs challenged.

DISCUSSION

Active anaphylaxis has been produced in dogs with animal protein. Magendie is stated to have reported in 1839 the sudden death of dogs repeatedly injected with egg albumin.⁶ In 1902 Richet and Portier⁷ coined the term anaphylaxis after producing shock in dogs with sea anemone as an antigen. To our knowledge, anaphylaxis has not previously been induced in nonatopic dogs with pollens.

Previous studies^{1, 8} have shown that dogs injected with RW produce skin-sensitizing antibodies with characteristics of HCA. It was found that the induced HCA has properties similar to naturally occurring canine HCA. Tennenbaum and associates,⁹ by injecting dogs with large amounts of RW, demonstrated the production of blocking antibody which prevented systemic response in passively sensitized animals. Previous attempts to induce anaphylaxis in dogs with induced HCA antibodies have been unsuccessful.¹ This was believed to be caused by the presence of high titers of "blocking" antibody, as well as to the fact that challenge with antigen was carried out at a time when the titer of HCA was low.

In the present study, we have been able to produce anaphylaxis in every induced dog. The dogs with lower HA titers had more severe anaphylaxis, which suggests that hemagglutinating antibody may play a role as a "blocking" type of antibody. We feel that in this study the dogs developed anaphylaxis because the challenge was given at a time of maximum HCA and low HA titers. In previous studies, the dogs were challenged when the HCA titers had fallen to low levels or were absent and HA titers were high.

Failure to transfer induced hypersensitivity in dogs is most likely caused

by the low HCA titer (1:16). In spontaneous canine atopy, the HCA titers are higher and the HA titers are lower.¹¹

None of the dogs in the present report was given bronchial RW challenge. But in another study,¹¹ the induced dogs were exposed to bronchial challenge, and no bronchospasm was observed.

Fractionation with Sephadex G-200 has demonstrated the localization of HCA in the region between peak I and peak II in both induced and spontaneous dogs. By immunoabsorbent studies, this HCA proved to be IgE in spontaneous dogs.¹⁰ Studies have shown that, in induced dogs, HA activity is located in the IgM area and HCA activity may be IgE.¹¹

REFERENCES

- 1 Arkins, J. A., Bukosky, R. J., and Fink, J. N.: The characterization of skin-sensitizing antibody induced in nonsensitive dogs, *J. ALLERG.* **40**: 50, 1967.
- 2 Kabat, E. A., and Mayer, M. M.: *Experimental immunochemistry*, ed. 2, Springfield, Ill., 1961, Charles C Thomas, Publisher.
- 3 Stavitsky, A. B.: *Micromethods for study of proteins. I. Procedure and general applications of hemagglutination and hemagglutination inhibition reactions with tannic acid and protein treated red blood cells*, *J. Immun.* **72**: 360, 1954.
- 4 Arbesman, C. E., Rose, N. R., Kantor, S. Z., and Breede, R. B.: Immunologic studies of ragweed-sensitive patients. I. Specificity and sensitivity of hemagglutination reactions, *J. ALLERG.* **31**: 317, 1960.
- 5 Dean, H. R., and Webb, R. A.: The morbid anatomy and histology of anaphylaxis in dog. The blood changes in anaphylactic shock in the dog, *J. Path. Bact.* **27**: 51, 1962.
- 6 Davis, B. E., et al.: *Microbiology*, New York, 1967, Hoeber Medical Division, Harper & Row, Publishers, p. 526.
- 7 Portier, P., and Richet, C.: De l'action anaphylactique de certains Venins, *C. R. Soc. Biol. (Paris)* **54**: 170, 1902.
- 8 Bukosky, R. J., Hogan, R. M., Arkins, J. A., and Fink, J. N.: Characterization of ragweed antibodies induced in nonatopic dogs, *J. Lab. Clin. Med.* **72**: 383, 1968.
- 9 Tennenbaum, J. I., Patterson, R., and Pruzansky, J. J.: Canine antiserum analogous to human allergic and blocking antiserums, *Science* **142**: 589, 1963.
- 10 Patterson, R., Roberts, M., and Pruzansky, J. J.: Types of canine serum immunoglobulins, *J. Immun.* **101**: 687, 1968.
- 11 Sunthopal, P., Arkins, J. A., and Fink, J. N.: Characterization of induced homocytotropic antibody to ragweed and timothy pollens in nonatopic dogs, *Clin. Exp. Immunol.* In press.