Experimental Eosinophilia V. Specificity of Regional Lymph Node Responses to Antigen-Antibody Systems.*† (28266)

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Data obtained in guinea pig experiments suggest a responsible role for antigen-antibody union in eosinotactic mechanisms(1-2). On the other hand, failure to demonstrate development of eosinophilia in passively sensitized-challenged mice has been cited as evidence in favor of alternate explanations for eosinophil cell etiology and function(3-4). Therefore the design of this study has had the purpose of utilizing an additional species experimental model to evaluate the nature of cellular responses to antigen-antibody systems of varying character.

Materials and methods. Antigens. (1) Bovine serum albumin (BSA, Armour); (2) bovine gamma globulin (BGG, Armour); and (3) diphtheria toxoid (diph. tox., Lilly).

Antisera. Pooled sera were prepared from blood taken by cardiac puncture from immunized animals. Rabbits and guinea pigs received BSA or BGG emulsified in Freund's adjuvant, or alum precipitated diphtheria toxoid by subcutaneous routes. Chickens received BSA or BGG by intravenous injection. Diphtheria antitoxin as horse globulin was obtained in a concentration of 3000 units per ml (Eli Lilly). Antisera of different species origins to common corresponding antigen demonstrated reactions of identity in Ouchterlony(5) agar immunodiffusion tests indicating qualitative similarity of character. However, further resolution of precipitin bands through the more sensitive technique of Preer(6) gave evidence for multiple components in BSA, BGG, and in diph. tox. antigen-antibody systems (Table I). Precise determinations of antibody nitrogen content for quantitation of dosages were therefore not possible and precipitin curves could only be utilized for relative measures of administered antisera.

Experimental procedure was based upon the demonstration of inflammatory cell infiltrations within regional lymph nodes draining subcutaneous sites of hypersensitivity reactions in the rabbit(7). One-half ml solutions of either BSA, BGG, or diph. fluid tox. were injected into the hind foot pad of one extremity. Thirty minutes later 0.5 ml of corresponding antiserum was given into the same site. Four hours later popliteal lymph nodes from both treated and untreated hind extremities were removed and prepared for histologic study. Fixed tissue sections were stained with hematoxylin and eosin. Undritz's modification II of the peroxidase reaction(8) was additionally employed for selective granule staining and verification of eosinophil identification in cell smears(9) prepared from surfaces of fresh cut nodes.

White male albino rabbits were subjected to procedures of reversed passive sensitization with each group of 6 receiving a different antigen-antibody system. Dosages and characterizations of reactants are given in Table I. Horse antitoxin was given in both full strength and diluted to dosages estimated equivalent to precipitating antibody content of the guinea pig and rabbit antitoxins. Serums taken from blood of rabbits prior to treatment were tested for possible presence of preformed precipitins to the antigen or to components of the foreign species normal serum by the technique of agar immunodiffusion in capillary tubes(6). Control series of three rabbits each received foot pad injections of identical doses of either: one of the antisera; or BSA, BGG, or diph. fluid tox.; alone, and in combination with a sample of normal foreign serum corresponding to the species antiserum employed.

Results. Cell infiltrations when found in serial sections of popliteal lymph nodes occupied both peripheral cortical and medullary

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TABLE I. Eosinophilic Granular Cell Infiltrations into Sinuses of Popliteal Lymph Nodes Regional to Locally Sensitized Foot Pads of Rabbits.

Antigen dosage	Corresponding antiserum: Species origin	Corresponding antiserum: No. of precipitins*	Corresponding antiserum: Dosage†	No. of 6 rabbits demonstrating eosinophilia: Equivocal	No. of 6 rabbits demonstrating eosinophilia: Diffuse
BSA, 10 mg	Rabbit	6	117 μg AbN		6
"	Guinea pig	6	60 " "	1	5
"	Chicken	2	199 " "		6
BGG, 10 mg	Rabbit	4	59 " "		6
"	Guinea pig	5	51 " "		6
"	Chicken	2	97.5 " "		6
Diph. tox. L_f25	Horse	2	1500 units 3.5 mg AbN	2	2
"	"	2	15 units 35 μg AbN		2
"	Guinea pig	2	35 " "		6
"	Rabbit	2	35 " "	1	5

* Precipitin bands demonstrated in agar-gel double diffusion tests by capillary tube technique of Preer(6).

† Estimated in terms of mg antibody nitrogen per ml determined by standard quantitative precipitin test techniques. Those involving chicken antisera were carried out in 8% saline.

sinuses. These consisted of diffuse and uniform accumulations of eosinophilic granular leukocytes (Fig. 1). Eosinophils were clearly differentiated from pink-staining polymorphonuclear leukocytes, the rabbit pseudo-eosinophil, by the presence of typical coarse cytoplasmic granules and eccentrically-placed bilobed nuclei. Selective peroxidase reaction staining(8) of cell smear preparations aided in cell identification (Fig. 1).

Results of microscopic examinations of serial histologic sections of affected popliteal nodes within members of each experimental group are summarized in Table I. The popliteal nodes of all animals receiving foot pad injections of either an antiserum; or BSA, BGG, or diph. tox.; alone or with the foreign species normal sera, were free of eosinophil cell infiltrations.

It was found that some samples of normal rabbit sera precipitated with horse serum. Also eosinophil cell infiltrations into regional lymph nodes occasionally were observed following foot pad injections of whole horse serum. Therefore experimental results in those rabbits receiving diph. toxoid and horse antitoxin were accepted only if their axillary lymph nodes were found free of eosinophil cell infiltrations after simultaneously receiving horse antitoxin alone into a homolateral anterior foot pad.

Discussion. Intraperitoneal eosinophilic cellular responses to passive sensitization have been reported for the guinea pig(2), but were found lacking in mouse experiments(3-4). It is not known whether differences related to species of the recipient animals or of the donor antisera, or to the character of antigens employed might explain these conflicting experimental findings. Several variations in each of these factors did not inhibit the emergence of eosinophilic cellular infiltrations within rabbit lymph nodes. However, lesser responses to diph. tox.-horse antitoxin systems in contrast to those of rabbit and guinea pig origins were noted even when in equivalent antigen-antibody proportions. The general findings in this experimental model are in agreement with that described by Litt but differ in the respect that conditioning through preliminary exposure to an unrelated system was not required(2).

Eosinophilic granular leukocytes were generally observed in subcutaneous injected foot pad sites of responding animals. The pattern of peripheral-cortical-medullary sinus cell distribution suggested lymphatic routes of migration from the primary site of inflammatory stimulus. Secondary hematogenous involvement was indicated by the occasional finding of these cells within lumina of small blood vessels within affected nodes and associated

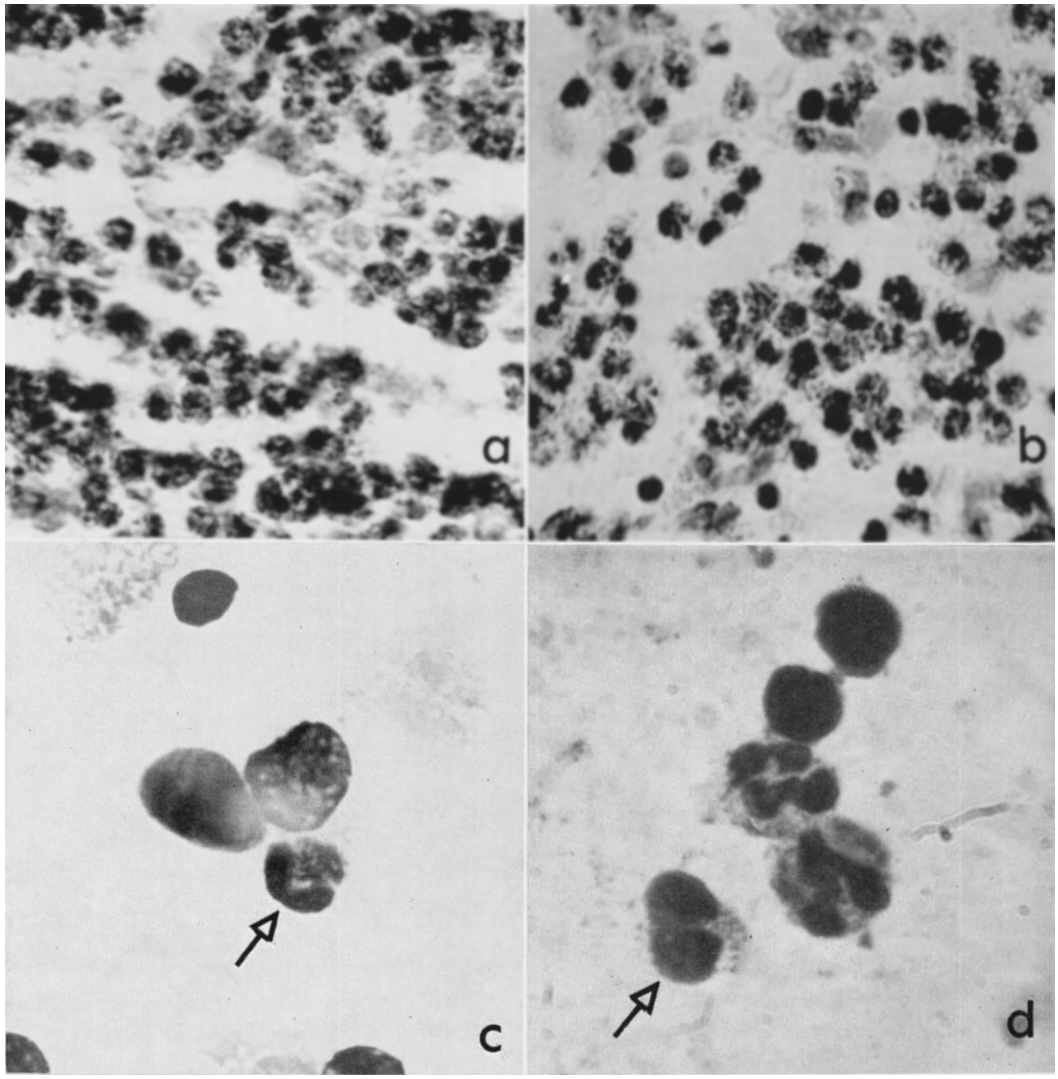

FIG. 1. Rabbit popliteal lymph node regional to injected foot pad site. (a) and (b). Sections taken from animal who received BSA and rabbit antiserum to BSA showing granular leukocytes within a medullary sinus (Hematoxylin and Eosin) (×470). (c) and (d). Brush smears taken from cut surface of lymph node. Cells with bilobed eccentrically-placed nuclei characteristic of typical eosinophil as distinguished from pseudoeosinophil are indicated by arrows (Undritz's modification II of the peroxidase reaction) (×1200).

elevation of peripheral blood eosinophil counts.

Not all antigen-antibody systems could be utilized in this experimental procedure since some single reactants in themselves effected eosinophil cell infiltrations. These included extracts of *Ascaris lumbricoides* var. *suum* and *Ambrosia elatior* (ragweed pollen) and some samples of horse, bovine, and human sera. It was subsequently found that many normal rabbit sera precipitated with these materials. In evaluating possibilities for this reactivity the presence of preformed precipitins arising through prior antigenic exposure of parasitic infection and cross-related dietary grains and the participation of natural isoagglutinins and species-related blood group substances(10-11) are cause for suspicion. Failure to carry out control serologic studies on test animals prior to treatment suggests one valid objection to accepting the concept that a biologically-derived material may be pri-

marily eosinotactic(12-13) apart from function as an immune reactant.

Attempts to modify this inflammatory cellular reaction with antihistamines, chemical inhibitors of histamine-liberation, and anti-serotonin and anti-heparin agents have been without effect. Identification of a possible physico-chemical factor responsible for eosinotactic responses to antigen-antibody union is the subject of our continued investigation.

Summary. Eosinophilic granular cell infiltrations into sinuses of regional popliteal lymph nodes of the rabbit were demonstrated. These followed reversed passive sensitization experiments at foot pad sites in response to antigen-antibody union. Systems of varying character found effective were: bovine serum albumin and bovine serum gamma globulin with respective corresponding antisera of rabbit, guinea pig, and chicken origins; diphtheria toxoid with corresponding rabbit, guinea pig, and horse antitoxin; and tetanus toxoid-horse antitoxin.

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Size of Minimal Reproductive Units of Bacterial L Forms. (28267)

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The size of the minimal reproductive units of the stable Proteus L form L9 was determined by Weibull and Lundin(1). Samples of liquid cultures of this L form were spread on agar plates. Blocks of agar were transferred to slides and covered with cover slips. The slide cultures thus obtained were sealed with paraffin and incubated for 24 hours or longer. The size of the individual L elements in the slide cultures was determined photographically before and after incubation. It was thus found that only L elements having a diameter > 0.6 to $0.7\ \mu$ enlarged measurably during growth. Similar experiments carried out on 3 other Proteus L forms and L forms of a staphylococcus and a diphtheroid will be described in this report.

Materials and methods. A. *Organisms.* The Proteus L forms used (strain L VI, L 18, and L D52) have been described(2). Stock cultures were grown in the serum-free medium described by Abrams(3). L forms derived from a staphylococcus and a diphtheroid were provided by Dr. L. Dienes, Massachusetts General Hospital, Boston, Mass.(4). Stock cultures were grown on plates containing meat broth supplemented with 10% inactivated horse serum, 3% NaCl, 1,000 units of penicillin g/ml, and 1% agar.

B. *Photomicrography.* A Leitz 90 X phase contrast objective and a 8 X compensating ocular was used. L forms moving freely in liquid medium between slide and cover slip were photographed with a microcamera equipped with 35 mm Gevaert Duplo Ortho film, and with the Leitz Multiblitz-Mikro 300 W electronic flash as the light source. Photographs of L forms in slide cultures were taken