PRODUCTION OF A RABBIT ANTIMOUSE EOSINOPHIL SERUM WITH NO CROSS-REACTIVITY TO NEUTROPHILS*

BY ADEL A. F. MAHMOUD, KENNETH S. WARREN, AND DOV L. BOROS
(From the Departments of Medicine and Community Health, Case Western Reserve University School of Medicine, and University Hospitals, Cleveland, Ohio 44106)

(Received for publication 26 March 1973)

For approximately 100 yr the eosinophil has been recognized as a distinct type of polymorphonuclear leukocyte (1), but despite an extensive literature, its function remains largely unknown. An accumulation of eosinophils is evoked by a wide variety of immunological responses including anaphylaxis (2), immune complex reactions (3), and cell-mediated hypersensitivity (4). Eosinophilia is the hallmark of helminthic infections, and occurs in dermatoses, bronchial asthma, drug reactions, and several other conditions grouped as the hypereosinophilic syndrome (5).

The availability of antisera against cells of the polymorphonuclear series, e.g., neutrophils (6, 7), has greatly facilitated investigation of their function and kinetics. Similar antisera have not been available previously for the study of eosinophils. We have been attempting to produce an antieosinophil serum for some time, but have failed because of the difficulty in obtaining pure preparations of eosinophils in sufficient quantity. Recently Colley (8) reported a method of producing an eosinophil-rich peritoneal exudate from mice infected with Schistosoma mansoni. The peritoneal exudate was purified by a modification of the techniques described by Day (19) and Gleich and Leogering (10). To our knowledge this is the first communication reporting the preparation and characterization of a specific antieosinophil serum (AES).

Materials and Methods

Collection of Eosinophils.—Eosinophil-rich peritoneal exudates were obtained from mice 8 wk after exposure to 100 S. mansoni cercariae (8). The peritoneal cavity was injected with 1.5 ml of 10% proteose peptone (Difco Laboratories, Inc., Detroit, Mich.) and 48 h later it was washed with 5 ml of Hanks’ balanced salt solution (Grand Island Biological Co., Grand Island, N. Y.) containing 2.5 IU heparin/ml (Upjohn Co., Kalamazoo, Mich.). A total of $6 \times 10^7$ to $8 \times 10^7$ cells of which 55-78% were eosinophils was obtained from each mouse. Exudates from several mice were pooled, layered over a solution of Hypaque 50 (sodium diatrizoate USP, Winthrop Laboratories, New York), diluted 1:2 with distilled water, and centrifuged at 400 g for 40 min at 4°C. Eosinophils collected from the pellet at the bottom of the centrifuge tube were resuspended in pyrogen-free saline, washed twice, and counted in a hemacytometer using Discombe’s diluting fluid (11).

*This work has been supported by U.S. Public Health Service Grants nos. AI 08163 and RR 05410-12.
Preparation of AES.—Each of two adult male New Zealand albino rabbits weighing 4.5–5.0 kg received three weekly subcutaneous injections, each consisting of $4 \times 10^8$ cells averaging 90.3% eosinophils, 5.8% macrophages, and 3.4% lymphocytes suspended in 1 ml physiological saline and mixed with an equal amount of Freund’s complete adjuvant (Difco). 1 wk after the last injection the animals were bled and the sera pooled, inactivated at 56°C for 40 min, and stored frozen at −20°C until further use.

Titration of AES.—Agglutinating titers of crude and purified AES against eosinophils, neutrophils, macrophages, lymphocytes, and red cells were determined in siliconized test tubes by serial twofold dilutions from 1:10 to 1:10,240 as described by Simpson and Ross (12).

Absorption of AES.—Macrophages and lymphocytes were obtained from mouse peritoneal exudates (13). A 4 ml sample of pooled serum was absorbed twice for 1 h at 23°C and overnight at 4°C with $6 \times 10^6$ cells consisting of 75–80% macrophages and the remainder lymphocytes. The absorbed serum was retested for macrophage and lymphocyte agglutination. Eosinophils were obtained as described above; neutrophils were obtained by stimulating peritoneal exudates with 1.5 ml 10% proteose peptone for 18 h. Samples of 0.5 ml of AES were absorbed with $6 \times 10^7$ neutrophils or eosinophils for 1 h at 23°C. The absorbed sera were then titrated against eosinophils.

Cytotoxicity of Absorbed AES.—Cytotoxicity testing was carried out as described previously (13) using fresh guinea pig serum as a source of complement.

In Vivo Activity of AES.—The effect of unabsorbed and absorbed AES on the peripheral eosinophil counts was compared to that of normal rabbit serum (NRS) on normal and schistosome-infected mice. Each animal received a single intraperitoneal dose of 0.1 ml of either serum. Absolute eosinophil, total leukocyte, and differential counts were done before injection and at given intervals thereafter.

Since the level of circulating eosinophils in normal mice is low, the technique of Archer and Hirsch (14) was used to stimulate exudation of eosinophils in the peritoneal cavity of normal mice. Three groups of five animals each were used: group 1 received 0.1 ml AES intraperitoneally and group 2 a similar dose of NRS before stimulation of the peritoneal cavity; the 3rd group was untreated. Saline stimulation [5 ml of 0.85% NaCl ip (intraperitoneally)] was done for 3 consecutive days and the animals were killed 30 min after the last injection. The total eosinophil count of the peritoneal exudate was then determined.

RESULTS

Titration of AES.—The unabsorbed AES contained agglutinating antibodies to eosinophils, macrophages, and lymphocytes. The titers were 1:5,120 for eosinophils, 1:80 for macrophages, and 1:20 for lymphocytes. The serum did not agglutinate either neutrophils or red cells in dilutions as low as 1:2.

Absorption and Specificity of AES.—Agglutinins against macrophages and lymphocytes were eliminated by two absorptions with 48 h peritoneal exudate cells. Absorbed monospecific AES retained the same agglutination titer against eosinophil (1:5,120). The absorbed AES contained cytotoxic antibodies to eosinophils in dilutions up to 1:5,120 as demonstrated by uptake of trypan blue by 50% or more of the cells.

Matched single absorptions of equal samples of AES with identical numbers of polymorphonuclear cells (either eosinophils or neutrophils) revealed that the eosinophils reduced the eosinophil agglutination titer from 1:5,120 to 1:80 while the neutrophils had no effect whatsoever.
Hematologic Response to AES and NRS in Normal and Schistosome-Infected Mice.—

Normal mice: AES produced no significant change in the total white cell or differential counts in comparison with either animals that received NRS or untreated controls (Table I).

Mice with schistosomiasis mansoni of 7 wk duration: AES caused a drop of peripheral eosinophils to 11.5% of their original level in 1 h (Fig. 1). Eosinopenia was maintained for 5 days, followed by a gradual recovery to near initial levels in 15 days. NRS had no significant effect on the counts of circulating eosinophils. Except for a slight and temporary drop of total leukocyte count (11.9% at 12 h), probably related to the fall in eosinophils, AES effect was not significantly different from NRS (Fig. 2).

Effect of AES and NRS on Peritoneal Exudation of Eosinophils in Normal Mice.—After three consecutive daily stimulations of the peritoneal cavity with

### TABLE I

| Differential leukocyte counts Mean ±SE | Total leukocyte count/mm³ Mean ±SE |
|---------------------------------------|----------------------------------|
| Lymphocyte                            | AES 5,440 ± 717                  |
| Neutrophil                             | 71.6 ± 3.0                       |
| Monocyte                               | 24.0 ± 3.0                       |
| Eosinophil                             | 4.4 ± 0.4                        |
| Controls                               | NRS 6,468 ± 576                  |
|                                        | 74.6 ± 1.3                       |
|                                        | 19.0 ± 1.1                       |
|                                        | 5.2 ± 0.5                        |
|                                        | 1.2 ± 0.6                        |

Fig. 1. Effect on the absolute eosinophil count of one dose (0.1 ml) of AES or NRS administered intraperitoneally to mice with schistosomiasis mansoni of 7 wk duration. Each point represents a mean of five animals.
saline, five animals previously injected with NRS, each produced an average of $3.6 \times 10^5$ eosinophils/peritoneal cavity and five untreated controls $3.4 \times 10^5$ eosinophils/peritoneal cavity. Among the five mice treated with AES before peritoneal stimulation, three had no eosinophils in the exudate and the remaining two had approximately 5,000 cells each.

**DISCUSSION**

The polymorphonuclear eosinophil remains a mystery; its relation to the polymorphonuclear neutrophil and its role in immunological reactions are essentially unknown. Eosinophils are believed to progress through the same maturation sequence as neutrophils (15). The granules, which are the most striking feature of eosinophils, begin to appear at the promyelocyte level. Nevertheless, the present study reveals complete antigenic individuality of the mouse eosinophil and neutrophil. AES markedly lowered circulating eosinophil counts in infected animals and also depressed the exudation of eosinophils in the peritoneal cavity of normal mice after saline stimulation. As the time required for maturation of mouse eosinophils was calculated to be 32 h (16), and as AES-produced eosinopenia was maintained for about 5 days, the serum may have caused a maturation block or depletion of eosinophil precursor cells in the bone marrow.

Blood eosinophilia is common in helminthic diseases, and is often found in allergic reactions (17), dermatoses, and occasionally in so-called eosinophilic leukemia (18). Eosinophils have been demonstrated in anaphylactic (19), antigen-antibody complex (20), and cell-mediated granulomatous hypersensitiv-
Antieosinophil serum (AES), obtained by immunizing rabbits with a highly purified suspension of mouse eosinophils, contains high titers of specific agglutinating and cytotoxic antibodies to eosinophils. Absorption with macrophages, lymphocytes, and neutrophils does not affect the antieosinophilic activity while it is markedly lowered by absorption with eosinophils. One dose of AES (0.1 ml) injected into mice with schistosomiasis mansoni caused a mean decrease in circulating eosinophils of 90% within 1 h which was maintained for 5 days, followed by gradual recovery. No other changes in the total or differential white cell counts were noted. In normal mice AES markedly depressed exudation of eosinophils in the peritoneal cavity after repeated saline stimulation. Some of the immunological and clinical implications of a monospecific AES are discussed.

The research assistance of Pierre A. Peters is gratefully acknowledged.

REFERENCES

1. Ehrlich, P. 1880. Methodologische Beitrage zur Physiologie und Pathologie der verschiedenen Formen der Leukocyten. Z. Klin. Med. 12:553.
2. Samter, M. 1949. The response of eosinophils in the guinea pig to sensitization, anaphylaxis and various drugs. Blood. 4:217.
3. Archer, G. T., and N. Bosworth. 1961. Phagocytosis by eosinophils following antigen-antibody reactions in vitro. Aust. J. Exp. Biol. Med. Sci. 39:157.
4. Basten, A., and P. B. Beeson. 1970. Mechanism of eosinophilia. II. Role of the lymphocyte. J. Exp. Med. 131:1288.
5. Samter, M., and D. Czarny. 1971. Secondary cells involved in the allergic reactions: Eosinophils, basophils, neutrophils. In Immunological Diseases. M. Samter, editor. Little, Brown, and Co. Inc., Boston, Mass. 2nd edition. 375.
6. Cochrane, C. G. 1968. Immunologic tissue injury mediated by neutrophilic leucocytes. Adv. Immunol. 9:97.
7. Lawrence, J. W., C. G. Craddock, Jr., and T. N. Campbell. 1967. Antineutrophilic serum, its use in studies of white blood cell dynamics. J. Lab. Clin. Med. 69:88.
8. Colley, D. G. 1973. Lymphokine-induced eosinophil stimulation. J. Immunol. 110: In press.
9. Day, R. P. 1970. Eosinophil cell separation from human peripheral blood. Immunology. 18:955.
10. Gleich, G. J., and V. A. Leogering. 1972. Purification of eosinophils from the peritoneal cavity of the guinea pig. Fed. Proc. 31:792. (Abstr.)
11. Discombe, G. 1946. Criteria of eosinophilia. Lancet. 1:195.
12. Simpson, D. M., and R. Ross. 1971. Effects of heterologous antineutrophil serum in guinea pigs, hematologic and ultrastructural observations. Am. J. Pathol. 65:79.
13. Boros, D. L., and K. S. Warren. 1971. Effect of antimacrophage serum on hypersensitivity (Schistosoma mansoni egg) and foreign body (divinyl-benzene copolymer bead) granulomas. J. Immunol. 107:534.
14. Archer, G. T., and J. G. Hirsch. 1963. Isolation of granules from eosinophil leukocytes and study of their enzyme content. J. Exp. Med. 118:277.
15. Archer, R. K. 1970. Regulatory mechanisms in eosinophil leukocyte production, release and distribution. In Regulation of Hematopoiesis. A. S. Gordon, editor. Appleton-Century-Crofts Inc., New York. 2:917.
16. Fiedner, T. M., I. Fache, and C. Adolphi. 1966. Über die Umsatzkinetik der Leukocyten bei Keimfreien Mäusen. Schweiz. Med. Wochenschr. 69:1236.
17. Speirs, R. S. 1970. Function of leukocytes in inflammation and immunity. In Regulation of Hematopoiesis. A. S. Gordon, editor. Appleton-Century-Crofts Inc., New York. 2:995.
18. Pierce, L. E., A. H. Hosseinan, and A. B. Constantine. 1967. Disseminated eosinophilic collagen disease. Blood. 29:540.
19. Parish, W. E., and R. R. A. Coombs. 1968. Peripheral blood eosinophilia in guinea pigs following implantation of anaphylactic guinea pig and human lung. Br. J. Hematol. 14:425.
20. Litt, M. 1968. Studies on experimental eosinophilia. VIII. Induction of eosinophilia by homologous 7S VI antibody and by extremely minute doses of antigen. Excerpta Med. Int. Congr. Ser. 162:5.
21. Warren, K. S., E. O. Domingo, and R. B. T. Cowan. 1967. Granuloma formation around schistosome eggs as a manifestation of delayed hypersensitivity. Am. J. Pathol. 51:735.
22. Boros, D. L., and K. S. Warren. 1973. The bentonite granuloma, characterization of a model system for infectious and foreign body granulomatous inflammation using soluble Mycobacterial, Histoplasma and Schistosoma antigens. Immunology. 24:511.