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The Anti-Inflammatory Effect of Heterologous Anti-Polymorphonuclear Leucccyte Serum on Adjuvant-Induced Arthritis (37949)

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Adjuvant-induced arthritis is an excellent model of chronic inflammation. Its pathogenesis is unknown, but immunological mechanisms are believed to play a major role in its initiation and perpetuation (1). Classical immunosuppressant drugs inhibit adjuvant disease when given prophylactically (2), but are ineffective when administered therapeutically (3). Antilymphocyte serum (ALS) prevents the development of adjuvant disease and reduces the intensity of established arthritis (4). Antimacrophage serum (AMS) produces a moderate reduction in the intensity of the clinical disease (5). There is a positive correlation between the lymphopenia and anti-inflammatory effects of the antiserum. By contrast, serum from arthritic donors is not arthritogenic and titers of antiserum against old tuberculin are unrelated to the severity of the adjuvant-induced arthritis (6).

Polymorphonuclear (PMN) leucocytes and mononuclear cells emigrate to the site of inflammation (7). The PMN cells predominate during the early stages of acute or chronic inflammation and the mononuclear cells become the dominant cell type as the PMN leucocytes move from the inflammatory lesion. Peripheral PMN leucocytes are increased in adjuvant disease (8) and in other models of chronic inflammation (9). Humphrey (10) demonstrated a relationship between peripheral blood PMN leucocytes and the intensity of inflammation, but more recently Willoughby and Spector (11) showed that animals depleted of peripheral PMN cells are capable of mounting a vigorous inflammatory reaction.

Urate crystal-induced acute synovitis has been shown to be suppressed by depleting peripheral PMN leucocytes with vinblastine or anti-PMN leucocyte serum (12). By contrast, the effect of anti-PMN leucocyte serum on adjuvant-induced arthritis has not been reported previously.

The present study demonstrates that heterologous anti-rat PMN leucocyte serum suppresses the acute phase of adjuvant disease (primary lesions), but is without effect when given during the chronic phase. It reduces the intensity of the arthritis when given prophylactically, but not when administered therapeutically despite comparable decreases in peripheral blood PMN leucocyte levels. Anti-rat PMN serum is without effect on cutaneous sensitivity to purified protein derivative (PPD).

Materials and Methods. Young adult male Lewis rats (Microbiological Associates), weighing between 200–250 g, were used. Arthritis was induced by a single subcutaneous injection of 0.1 ml of a 0.5% suspension of killed *Mycobacterium butyricum* in light mineral oil in the left hind paw. Hind leg volume on the injected (primary lesion) and contralateral, uninjected (secondary lesion) side was measured by mercury displacement (13). Secondary lesions appeared between 10 and 12 days after adjuvant injection (Day 0).

Anti-rat PMN leucocyte serum was prepared in rabbits as described by Humphrey (10). Peritoneal exudates rich in PMN leucocytes were induced by a single intraperitoneal injection of 10 ml of 0.1% glycogen in sterile saline according to the method of Cohn and Morse (14). Four hours later, the peritoneal cavity was washed twice with buf-

fered Hanks' salt solution, pH 7.4, containing 100 U of heparin. The cell suspension was mixed with an equal volume of light mineral oil containing 0.5% *M. butyricum*. Ten milliliters of the resulting suspension, containing 2×10^8 PMN, were injected into the foot pads and nuchal region of adult male New Zealand rabbits. Three weeks later the animals were bled terminally. The blood was allowed to clot, and the serum was decanted and inactivated by heating for 30 min at 56°. The serum was absorbed with rat erythrocytes, rat lymphocytes, rat platelets, and rat serum proteins. After absorption, the serum was divided into aliquots and frozen. Antilymphocyte serum was obtained using washed rat thymocytes and an immunization schedule identical to that described for the anti-PMN serum. Control serum was obtained from rabbits injected only with complete Freund's adjuvant (5).

The prophylactic effect of anti-PMN leucocyte serum on adjuvant disease was studied in groups of 6 rats given 2 ml of antiserum intraperitoneally, on days −1, 0, and +1, postadjuvant injection. The therapeutic effect was studied in groups of 6 rats, each given 2 ml of anti-PMN leucocyte serum intraperitoneally on Days 14–17 inclusive. Another group of 6 rats received 1 ml of antilymphocyte serum intraperitoneally on Days 14–17 inclusive. Control animals received 2 ml of control rabbit serum intraperitoneally.

Total leucocyte counts were made by means of a Coulter counter. Differential counts were performed on thin smears of tail vein blood stained with Wright's stain.

Cutaneous sensitivity to purified protein derivative (PPD, Parke–Davis) was measured according to the procedure described by Flax and Waksman (15). Fifty micrograms of PPD was injected intradermally on the shaved abdomen on Day 15 postadjuvant and the induration measured 24 hr later.

Results. Anti-PMN leucocyte serum, given prophylactically at a dose that produced a significant neutropenia but no lymphopenia, reduced adjuvant-induced hind leg swelling on the injected and unin-

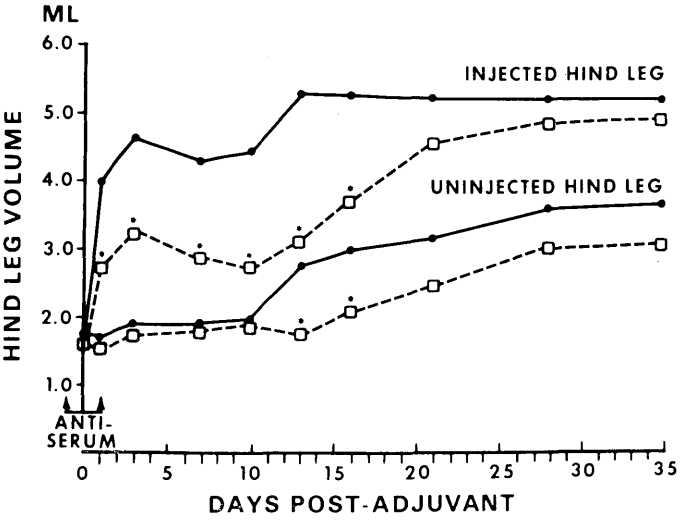

FIG. 1. The anti-inflammatory effect of anti-PMN leucocyte serum, given prophylactically, on hind leg volumes of adjuvant arthritic rats: complete Freund's adjuvant was injected into the left hind paw on Day 0. Two milliliters of rabbit anti-rat PMN leucocyte serum (□ - - - □) or control rabbit serum (●——●) were injected intraperitoneally on Days −1, 0, and +1 postadjuvant. Hind leg volumes were measured on Days 0, 1, 3, 7, 10, 13, 16, 21, 28, and 35. *Significant ($P < 0.05$) difference between controls and anti-PMN leucocyte serum-treated rats.

TABLE I. The Effects of Rabbit Anti-PMN Leucocyte Serum, Given Prophylactically or Therapeutically, on Peripheral Blood Leucocyte Levels and Cutaneous Sensitivity to Purified Protein Derivative (PPD) in Adjuvant Arthritic Rats.

Treatment	Days post-adjuvant	Peripheral blood leucocytes, percent change from controls			Cutaneous sensitivity to PPD induration diameter, MM[c]	
		Total	PMN[a]	LYM[b]	Anti-PMN serum	Control serum
Prophylactic	1	−43**	−65**	+ 2	n.d.[d]	n.d.
(Days −1, 0, +1)	10	− 6	+ 9	−21*	n.d.	n.d.
	16	−19	−23	−16	13.3 ± 2.9	11.0 ± 2.4
Therapeutic						
(Days 14–17)	16	−51**	−72**	−26*	13.0 ± 2.5	11.8 ± 1.0

[a] = Polymorphonuclear leucocyte.
[b] = Lymphocyte.
[c] = Mean ± SD.
[d] n.d. = not determined experimentally.
* = $P < 0.05$ versus controls.
** = $P < 0.005$ versus controls.

jected sides (Fig. 1). Hind leg volume was significantly below control values between days 1 and 15, but no significant difference was observed thereafter.

Table I shows that prophylactic treatment with anti-PMN leucocyte serum significantly and selectively decreased peripheral PMN leucocytes on Day 1 postadjuvant. No significant difference in peripheral blood PMN leucocyte levels was seen between treated and control animals on Days 10 or 16 postadjuvant. Given prophylactically, anti-PMN leucocyte serum was without effect on cutaneous sensitivity to PPD measured on Day 16.

Anti-PMN leucocyte serum, given therapeutically, did not alter hind leg volumes on either the injected or uninjected side (Fig. 2), although the antiserum significantly decreased peripheral PMN levels (Table I). By contrast, antilymphocyte serum, given therapeutically, produced a significant, transient reduction in hind leg volume, in accord with the findings of Ziff and his associates. Anti-PMN serum was also without effect on cutaneous sensitivity to PPD when administered therapeutically, i.e., coincident with the injection of the challenging dose of PPD. Antilymphocyte serum did not affect cutaneous sensitivity to PPD when administered prophylactically, but did suppress the induration when administered therapeutically, i.e., during the time cutaneous sensitivity to PPD was measured (Zuccarello and Wolff, unpublished observations).

Discussion. The generalized inflammatory reaction induced by a suspension of *M. butyricum* in oil is inhibited by heterologous anti-PMN leucocyte serum given prophylactically, but not therapeutically. Cutaneous sensitivity to PPD was unaffected by either treatment.

Inhibition of the primary lesion could be related to a significant reduction in peripheral blood PMN leucocyte levels, while a comparable neutropenia in rats with established arthritis did not influence the intensity of the hind leg edema. Prophylactic treatment with anti-PMN leucocyte serum delayed the appearance of the secondary lesions, suggesting a possible role for the PMN leucocyte in the early events of adjuvant disease. Its effect differs, however, from antilymphocyte serum which, prophylactically, prevents the development of the arthritic symptoms.

The relative unimportance of the peripheral blood PMN leucocytes in the secondary

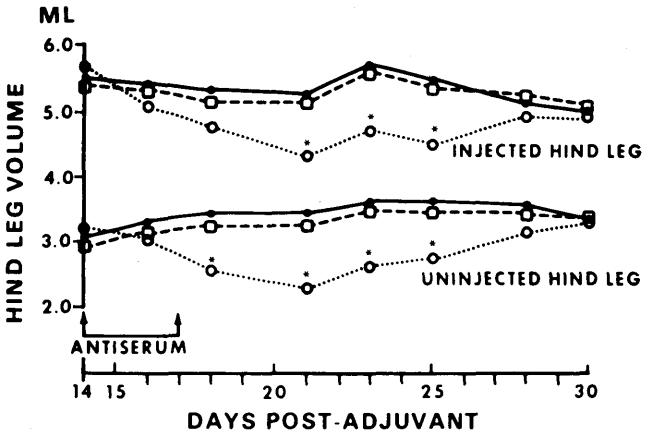

FIG. 2. The effects of rabbit anti-PMN leucocyte serum or rabbit antilymphocyte serum, given therapeutically, on hind leg volumes of adjuvant arthritic rats: complete Freund's adjuvant was injected into the left hind paw on Day 0. Two milliliters of anti-PMN leucocyte serum (□ - - - □), antilymphocyte serum (○ - - - ○), or control rabbit serum (●——●) were injected intraperitoneally on Days 14 through 17 inclusive. All animals exhibited arthritic lesions in the injected and uninjected, contralateral hind legs when treatment was initiated. Hind leg volumes were measured on Days 14, 16, 21, 23, 25, 28, and 30. *Significant ($P < 0.05$) difference between controls and rats treated with specific antiserum.

lesion is apparent from the dissociation of antiserum effects on the PMN and hind leg volumes. As we have noted, antilymphocyte serum, given therapeutically, does reduce hind leg volume transiently.

Our observations of a biphasic ability of anti-PMN leucocyte serum to inhibit adjuvant disease are in general accord with the findings of Willoughby and Spector (15) who demonstrated temporal changes in the tissue levels of PMN leucocytes during chronic inflammation. Our data also suggest an important function of the PMN leucocyte in the initiation of the disease, but that these cells are of minor importance in the maintenance of the arthritic lesions.

Summary. Prophylactic treatment with heterologous anti-PMN leucocyte serum at a leucopenic dose inhibited the intensity of the primary lesion, and delayed but did not prevent the development of the secondary joint lesions. When given therapeutically, anti-PMN leucocyte serum did not reduce the hind leg edema, despite a significant reduction in peripheral blood PMN leucocytes. Cutaneous sensitivity to PPD was unaffected by anti-PMN serum. The data suggest that the PMN leucocytes may play a role in the initiation, but not the maintenance, of adjuvant disease.

1. Pearson, C. M., J. Chron. Dis. **16**, 863 (1963).

2. Ward, J. R., Cloud, R. S., Krawitt, E. L., and Jones, R. S., Arthritis Rheum. **7**, 654 (1964).

3. Newbould, B. B., Brit. J. Pharmacol. **24**, 632 (1965).

4. Currey, H. L. F., and Ziff, M., *ibid.*, **127**, 185 (1968).

5. Jasin, H. E., Lennard, D., and Ziff, M., Clin. Exp. Immunol. **8**, 801 (1971).

6. Waksman, B. H., Pearson, C. M., and Sharp, J. T., J. Immunol. **85**, 403 (1960).

7. Paz, R. A., and Spector, W. G., J. Path. Bact. **84**, 85 (1962).

8. Glenn, E. M., Gray, J., and Kooyers, W., Amer. J. Vet. Res. **26**, 1195 (1965).

9. Spector, W. G., Lykke, A. W. J., and Willoughby, D. A., J. Path. Bact. **93**, 101 (1967).

10. Humphrey, J. H., Brit. J. Exp. Pathol. **36**, 283 (1955).

11. Willoughby, D. A., and Spector, W. G., Nature **219**, 1258 (1968).

12. Chang, Y., and Gralla, E. J., Arthritis Rheum. **11**, 145 (1968).

13. Walz, D. T., DiMartino, M. J., Kuch,

J. H., and Zuccarello, W., Proc. Soc. Exp. Biol. Med. **136**, 907 (1971).

14. Cohn, A. Z., and Morse, S. I., J. Exp. Med. **110**, 419 (1959).

15. Flax, M., and Waksman, B. H., J. Immunol. **89**, 496 (1962).

16. Flax, M., and Waksman, B. H., Biochem. Pharmacol. Suppl. 123 (1968).

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