PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 183-185 (1976)

Adjuvant Arthritis in T Lymphocyte Depleted Rats[1] (39356)

J. W. HOLLINGSWORTH, DIANE S. GREENBERG[2], AND MARTHA DAWSON[3]

Department of Medicine, University of Kentucky College of Medicine, Lexington, Kentucky 40506

Adjuvant arthritis in rats, induced by injection of oil and tubercle bacilli, is mediated through lymphoid cells. Passive transfer into syngenic recipients of cells from lymph nodes of rats with adjuvant disease transferred the disease (1, 2). Similarly, transfer of the disease followed infusion of thoracic duct lymphocytes from sensitized donors (3). From these observations, it seemed likely that the T lymphocyte was the important cell in induction of adjuvant arthritis, and the disease has been considered as some type of delayed sensitivity reaction in which T cell function is crucial. Against this general hypothesis, however, was the finding of Arnason *et al.* that neonatal thymectomy markedly suppressed tuberculin skin sensitivity but had little if any effect on adjuvant arthritis (4). Also, recent studies have indicated definitely that rat thoracic duct lymph contains both T lymphocytes and long-lived recirculating B cells (5, 6). Finally, Lennon and Byrd (7) recently reported adjuvant arthritis occurring after attempts to develop allergic encephalomyelitis (EAE) in rats, but with arthritis developing *only* in neonatally thymectomized animals. In those experiments, adjuvant arthritis developed in rats with lymphopenia, evidence of depressed T cell function, and poor humoral antibody responses to myelin protein used to produce EAE.

Our experiments were designed to study adjuvant arthritis in a highly susceptible rat strain, in animals made specifically T cell deficient but with normal B cells.

Materials and methods. Male and female Long-Evans rats, inbred at the University of Kentucky by Dr. Katherine Sydnor, were used in these experiments, because studies in our laboratory revealed that this strain regularly developed severe adjuvant disease. T lymphocytes were defined by their unusual affinity for uptake of tritiated uridine by methods described in previous studies from this laboratory (6).

T depleted rats were prepared by the method of Howard and colleagues (8), somewhat modified as described below. At age 7 or 8 days, a time when thymocytes in rats have migrated to nonthymic sites, thymectomy was performed using hypothermic (crushed ice) anesthesia, as described for newborn rats (9). The animals were returned to their mothers and grew normally. At about 3 months of age, blood was drawn for T cell determinations by [^{3}H]uridine incubation, and 1 week later the rats received 850-rad total body irradiation from a cobalt-60 source. Within a few hours of otherwise lethal irradiation for rats of this age, hematopoietic cells and B lymphocytes were repopulated by intravenous injection of 10^7 viable bone marrow cells from a syngenic donor that had been depleted of T lymphocytes by 5 days of thoracic duct drainage.

Blood counts and [^{3}H]uridine studies were followed for 6 weeks until the total lymphocyte count and T and B populations were stable. At that point, the left hind paw of 14 rats was injected with 0.1 ml of Freunds complete adjuvant (3 mg tubercle bacilli/ml). Normal Long-Evans rats of the same age and sex (about 4½ months) were injected at the same time. Every other day thereafter, the animals were observed for the onset of arthritis and the severity quantitated by a cumulative severity index using a slight modification of the scoring technique described by Pearson (10).

At the end of the experiment, when the arthritis was inactive, rats were again studied for T and B lymphocytes in blood, and a few animals underwent thoracic duct drainage for evaluation of quantitative and qualitative changes in cells of their thoracic duct lymph.

Results. A. Studies of the extent of T cell

[1] Supported by a grant from the Kentucky Chapter of the Arthritis Foundation.

[2] Present address: University of North Carolina, Chapel Hill, North Carolina 27514.

[3] Present address: 1201 Heyburn Building, Louisville, Kentucky 40202.

depletion. Unlike neonatal thymectomy which leads to near absence of T cells with runting disease and severe defects in cellular immune reaction, these animals were less severely depleted and appeared grossly normal. In Table I blood small lymphocytes and total T and B cells are shown for normal Long-Evans rats, and for the experimental group 3 months after thymectomy and before irradiation, at 6 weeks after irradiation and bone marrow repopulation, and again 8 weeks later at the end of the arthritis experiment. In looking closely at the data in Table I, T cell depletion by thymectomy at 8 days of age was only slightly enhanced by thymectomy + irradiation + reconstitution with T depleted bone marrow. B cells remained relatively intact in all groups. At least 22 weeks after thymectomy, irradiation, reconstitution, and arthritis, T cells remained low.

Thoracic duct lymph drainage, performed 16 weeks after thymectomy, irradiation, and marrow replacement in a few rats, confirmed the significant and persistent T lymphocyte depletion. Total cell count declined from about 12,000/mm^3 to about 3000/mm^3, and percentage T ([^{3}H]uridine-labeled small lymphocytes) from 70 to about 8%.

B. Severity and incidence of adjuvant arthritis. All 12 T-depleted rats and their 10 controls developed some degree of arthritis, and the severity index is plotted in Fig. 1. At all points, the T-deficient rats had less severe arthritis. Statistical validity was checked for the Day 20 scores, and the differences were significant ($P = < 0.05$).

Tuberculin sensitivity was checked in all rats on Day 14 by intradermal injection of 0.1 ml of P.P.D. (720 μg/ml) in saline, and 8/10 control animals and 6/12 T-depleted rats reacted. In those rats who did develop tuberculin sensitivity, the size of the reaction 48 h after test was similar in both groups (12 and 11 mm erythema, respectively).

Discussion. Adjuvant arthritis is as unique to rats as rheumatoid arthritis is to man, and continued study of the immunological mechanisms involved in the rat model may prove helpful in understanding the human disease. In this study, highly susceptible Long-Evans rats were made T cell deficient by thymectomy at about the end of the first week of life, and by later irradiation and reconstitution with small numbers of bone marrow cells from syngenic donors depleted of marrow T cells. As evidenced by avid [^{3}H]uridine uptake and radioautography, a label quite specific for T cells in the

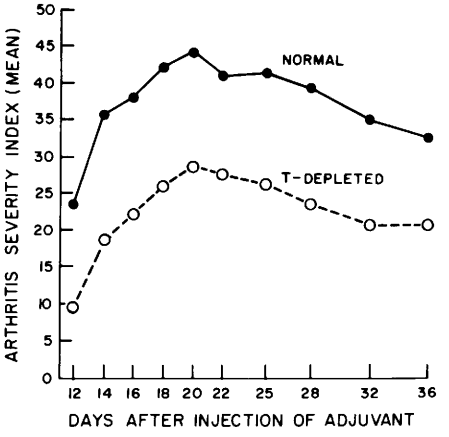

FIG. 1. Arthritis severity index of normal and T lymphocyte depleted Long-Evans rats. All rats in both groups (10 normal and 12 T-depleted) developed arthritis.

TABLE I. LYMPHOCYTE STATUS (T + B CELLS) OF RATS AT DIFFERENT STEPS IN THE STUDY.

Rat status	Number	Total small lymphs per mm^3 ($\times 10^3$)	Total T per mm^3 ($\times 10^3$)	Total B per mm^3 ($\times 10^3$)
Normal	6	3.6 ± 0.8	1.3 ± 0.4	2.4 ± 0.6
Normal, 6 weeks post adjuvant	8	3.6 ± 0.9	1.8 ± 0.5	1.6 ± 0.5
Normal, 16 weeks post adjuvant	6	3.8 ± 1.1	1.9 ± 0.7	1.9 ± 0.5
Post Tx[a], 12 weeks	10	1.8 ± 0.5	0.4 ± 0.2	1.4 ± 0.5
Post Tx, 6 weeks post irrad.[b] + recon.[c]	6	1.6 ± 0.9	0.2 ± 0.05	1.4 ± 0.6
Post Tx, irrad. + recon., 6 weeks post adjuvant	8	1.3 ± 0.5	0.2 ± 0.1	1.2 ± 0.6
Post Tx, irrad, + recon., 16 weeks post adjuvant	6	1.7 ± 1.0	0.3 ± 0.2	1.4 ± 0.9

[a] Tx = thymectomy.
[b] irrad. = 850 rad irradiation.
[c] recon. = Reconstitution with 10^7 T-depleted marrow cells.

rat (6), such rats maintained about 20–30% of normal T cells during the 7–8 months of study. All experimental animals developed adjuvant arthritis, but with modest decrease in severity of the disease when compared to normal rats.

Lennon and Byrd (7) tumbled onto a system in which T cell depletion actually enhanced adjuvant arthritis. Their methods were different; a relatively resistant Lewis strain of rats, slightly different quantity of tubercle bacilli in the adjuvant, presence of myelin basic protein in the Freund's adjuvant, and booster adjuvant in the form of pertussis vaccine. Taken together with our results, however, it is evident that the immunological mechanisms for adjuvant arthritis are complicated. Paterson (11) has reviewed the role of adjuvants in allergic encephalomyelitis (EAE), and also concludes that mechanisms are much more complex than simply a generalized enhancement of T cell function. He suggests that some adjuvants may work by enhancing penetration of the target organ, and others may markedly influence different functional subclasses of T lymphocytes. Lennon and Byrd (7) conclude that their results might be explained by immune complexes formed in the area of antigen excess. Continued work on humoral antibody production and specific T lymphocyte subpopulations may help unravel the growing complexity of the mechanism by which adjuvants produce a rheumatoidlike arthritis specifically in rats.

Summary. Markedly T lymphocyte depleted rats were prepared by thymectomy, irradiation, and repopulation by bone marrow hematopoietic and lymphoid cells. Such rats had persistent T lymphopenia of about 20% of normal. When T depleted and normal rats were injected with adjuvant, all animals developed arthritis but with slightly less severity in the T depleted animals. Such experiments, and other observations, suggest a complex immunological mechanism in the pathogenesis of adjuvant arthritis in the rat.

1. Waksman, B. H., and Wennersten, C., Int. Arch. Allergy **23,** 129 (1963).
2. Pearson, C. M., and Wood, F. D., J. Exp. Med. **120,** 547 (1964).
3. Whitehouse, D. J., Whitehouse, M. W., and Pearson, C. M., Nature (London) **224,** 1322 (1969).
4. Arnason, B. G., Jankovic, B. D., Waksman, B. H., and Wennersten, C., J. Exp. Med. **116,** 177 (1962).
5. Howard, J. C., J. Exp. Med. **135,** 185 (1972).
6. Hollingsworth, J. W., and Carr, J., Cell. Immunol. **8,** 270 (1973).
7. Lennon, V. A., and Byrd, W. J., Nature (London) **244,** 38 (1973).
8. Howard, J. C., Hunt, S. V., and Gowans, J. L., J. Exp. Med. **135,** 200 (1972).
9. Jankovic, B. D., Waksman, B. H., and Arnason, B. G., J. Exp. Med. **116,** 159 (1962).
10. Pearson, C. M., *in* "Mechanisms of Hypersensitivity" (J. H. Shaffer, G. A. LoGrippo, and M. W. Chase, Eds.), p. 647. Little, Brown and Company, New York (1959).
11. Paterson, P. Y., J. Reticuloendothel. Soc. **14,** 426 (1973).

Received January 30, 1976. P.S.E.B.M. 1976, Vol. 152.