The Adjuvant Arthritic Rat: Inflammatory Parameters during Development and Regression of Gross Lesions (33048)

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In 1956, Pearson (1) described a migratory polyarthritic condition in rats produced by the intradermal injection of a killed mycobacterium organism. Subsequently, reports on the clinical course (2) the histopathology (3–5) and the response to anti-inflammatory drugs (6–10) have established rat adjuvant arthritis as an excellent model of chronic inflammation. The assay procedure is demanding both with respect to time and material, and for these reasons, a study was undertaken of parameters which possibly might reflect more sensitively the inflammatory state of the adjuvant arthritic rat. This report describes the sequential changes in inflammatory parameters during the development and steroid-induced regression of gross arthritic lesions in adjuvant injected rats.

Methods. Seventy-two male Charles River CD rats (175–200 gm; 8 per group) were injected subcutaneously in the tail with 0.1 ml of a 1% suspension of *M. butyricum* (Difco) in Primol (Humble Oil and Refining Co., New York, New York). Controls (40) were injected with 0.1 ml of Primol alone. Rats were periodically removed from either test or control groups, fasted overnight, anesthetized with pentobarbital sodium, and bled from the abdominal aorta into heparinized syringes; the plasma was separated by centrifugation. Fourteen days post injection, 40 rats in 5 groups were dosed orally with a suspension of dexamethasone, 1 mg/kg per day. The remaining test and control rats received vehicle alone. One group received four daily doses of dexamethasone and was examined 7 days after drug withdrawal. Spleen and iliac lymph nodes were excised and weighed. Plasma inflammation units (11), sialic acid (12), and glycoprotein (13) concentrations were measured by the published procedures. Copper concentrations were determined by atomic absorption. Erythrocyte sedimentation rates (ESR) were determined in Westergren tubes filled with citrated whole blood. The amount of settling was measured after 1 and 2 hours with the tubes inclined at 45°. After the sedimentation rate was established, tubes were centrifuged at 3000 rpm for 15 min, the cell volume was measured and hematocrit was expressed as a percentage of the total. Arthritic lesions were evaluated as either present or absent by gross examination.

Results. Lymphoid organ weights, ESR, plasma inflammation units, sialic acid, glycoprotein, and copper concentrations were moderately elevated above controls within 3 days after adjuvant injection, and gradually increased to day 13 (onset of gross lesions). There was an immediate fall in all parameters 24 hours after dexamethasone treatment began (Table I). The ESR, plasma glycoprotein concentrations and inflammation units were more closely correlated than all other parameters examined. One group of animals was dosed with dexamethasone for 4 days and taken off drug for 7 additional days. At this time their inflammatory parameters were similar to the corresponding values of adjuvant-injected but untreated rats (Table I).

Discussion. Reduction of prearthritic lymphoid hyperplasia by dexamethasone administration was a confirmation of previously reported observations (5) on adjuvant-induced polyarthritis in rats. This concurs with the observations that lymphocytes, produced in lymphoid tissues, appear in the synovia in increasing numbers before arthritic development (4) and that lymphoid hyperplasia parallels the severity of arthritis (14).

Erythrocyte sedimentation rates (ESR), plasma inflammation units, sialic acid, glycoprotein, and copper concentrations have all been reported to fluctuate proportionally with inflammation in human rheumatoid arthritis. As indicated in Table I, the parameters examined in this study were elevated within

TABLE I. Inflammatory Parameters in the Adjuvant Arthritic Rat.

Day	Treatment	Body wt. (gm)	Spleen (gm)	Iliac L.N. (mg)	ESR (mm/hour)	Hemato-crit (%)	Sialic acid (μg/0.4 ml)	Glyco-protein (OD units)	Plasma copper (ppm.)	Gross lesions	Inflamm. units
2	Control	192.0	612.3	48.3	29.0	40.9	0.015	0.322	0.7	0/8	33.9
3	ADJ-injected	200.0	690.9	70.7	54.1	35.5	0.019	0.392	1.1	0/8	83.3
6	ADJ-injected	208.4	—	—	66.1	38.1	0.015	0.329	1.6	0/8	73.6
7	Control	227.3	673.6	38.9	—	47.4	0.011	0.241	0.68	0/8	33.1
9	ADJ-injected	228.0	798.5	97.6	44.0	—	0.0165	0.329	1.56	0/8	72.5
10	Control	253.6	658.6	41.0	19.5	40.1	0.012	0.277	0.7	0/8	33.1
13	ADJ-injected	218.4	1103.1	192.9	55.2	35.4	0.019	0.427	1.25	6/8	93.1
14	ADJ-injected; treated, Rx1	220.9	533.4	91.7	76.6	34.9	0.0215	0.431	2.45	5/8	106.4
15	Control	268.4	799.4	60.9	18.4	39.1	0.010	0.272	0.5	0/8	23.3
15	ADJ-injected; treated, Rx2	209.1	420.7	63.5	29.5	40.1	0.017	0.410	1.1	3/8	63.4
16	ADJ-injected	222.0	1617.7	248.2	69.7	33.2	0.023	0.531	1.53	4/4	103.5
16	ADJ-injected; treated, Rx3	207.7	379.4	45.5	55.0	36.3	0.017	0.402	1.58	2/8	73.7
17	ADJ-injected; treated, Rx4	209.1	330.6	42.9	35.7	39.1	0.0145	0.355	1.0	2/8	59.2
17	Control	267.1	650.5	57.1	21.4	40.9	0.011	0.263	0.95	0/8	25.4
23	ADJ-injected; treated 4 days; drug withdrawn 7 days	242.9	829.7	95.1	54.6	35.4	0.021	0.468	1.3	5/7	93.2

3 days after adjuvant injection, probably due to the inflammation produced by the Primol injection, and continued to increase up to the time of gross arthritic development. However, there was no marked change in any parameter either immediately before the appearance of gross lesions or after dexamethasone treatment. Furthermore, no convincing correlation between levels of any one parameter and gross arthritic development could be discerned from the data; in other words, no plateau appeared above which arthritis was invariably present.

A close correlation was noted among plasma inflammation units, sedimentation rates, and plasma glycoprotein concentrations, which apparently follow parallel courses during lesion development and regression, and any one parameter could conveniently serve as a reliable indicator of the disease. Since determination of inflammation units requires an amount of plasma which can easily be obtained from the living anesthetized animal, it is obviously the anti-inflammatory parameter of choice in following the progression of the arthritic state.

Summary. Inflammatory parameters were followed during the prearthritic stage of adjuvant-injected rats. Plasma inflammation units, erythrocyte sedimentation rates, and plasma sialic acid, glycoprotein and copper concentrations were raised 3 days post-dose and continued to increase up to 13 days, when gross lesions appeared. A prearthritic lymphoid hyperplasia occurred in the same manner. All these lesions regressed with dexamethasone treatment. It was concluded that none of the parameters investigated showed a unique sensitivity to the inflammatory state of the animal.

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Inhibition of Triglyceride Synthesis in Everted Intestinal Sacs* (33049)

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It has been reported that in the lymph-fistula rat, the concurrent administration of 2-ethyl-*n*-caproic acid inhibits the incorporation of oleic acid-1-^{14}C into lymph triglyceride (1). It was also shown that this branched-chain acid increased the portal absorption of oleic acid from 15 to 40% of the administered dose; the newly absorbed oleic acid in portal blood was predominantly in the form of the unesterified fatty acid. It was therefore suggested that 2-ethyl-*n*-caproate

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