Adjuvant-Induced Arthritis in Rats
I. Temporal Relationship of Physiological, Biochemical, and Hematological Parameters (35392)

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Experimental models of arthritis used during the past decade or more have several inherent limiting factors. The variability and lack of specificity observed in various *in vivo* and *in vitro* procedures have caused investigators to search for more acceptable animal models of rheumatoid arthritis. It would be particularly desirable to have a model that could elucidate the mechanisms of the disease processes rather than one that is only capable of screening compounds for effectiveness after these processes have been evoked.

The similarities of adjuvant arthritis to human arthritis (1, 2) and the wide acceptance of the adjuvant arthritic rat model for detecting and evaluating anti-arthritic agents (3–11) dictate the importance of further investigation and elucidation of this model. This report presents the temporal relationship of various physiological, biochemical, and hematological alterations in adjuvant arthritis.

A. Methods and Materials. Adjuvant arthritis was produced by a single intradermal injection of 0.75 mg of *M. butyricum* (Difco) suspended in 0.1 ml of white paraffin oil (NF) into a foot pad of the left hindpaw of male Lewis inbred rats (Microbiological Associates) weighing between 150 to 200 g. The rats were anesthetized with ether inhalation prior to and during adjuvant injection.

1. Edema (hindleg) volume. Hindleg volume was determined by a modified method of Van Arman *et al.* (12). The hindlegs were immersed to the anatomical hairline in a mercury reservoir. The mercury column was connected to a Statham pressure transducer (Model P23BB, 0–5 cm Hg). The output from the transducer was led through an amplifier to a Hewlett-Packard digital voltmeter (Model HP-3440A) and a high gain/auto range unit (Model 3443A) and finally to a digital recorder (Model J74562A). The digital recordings were calibrated, and a linear relationship between millivolts and milliliters was obtained by placing cylinders of known volumes into the mercury reservoir.

2. Local hyperpyrexia. Paw temperature was measured by an infrared thermometer (Model MT-3 Barnes Engineering Co.). The specifications of this instrument are as follows: temperature range, 20–40°; sensitivity 0.1°; field of view ¼ in. at 1-in. range; absolute accuracy, 0.5°.

3. Grip function. Grip function was measured by a modified method of Wiesinger (13). The rats were placed on a vertical screen, this was enclosed with a curved metal screen to form a vertical cylinder which limited locomotion. The grip function was measured by determining the length of time (0–60 sec) the animals were able to remain on the screen.

4. Serum lysozyme. Lysozyme was measured in the serum by the method of Piliero *et al.* (10). The rats were anesthesized with ether, and blood samples were obtained by cardiac puncture. The sera were assayed for lysozyme by measuring the lytic action produced on a cell wall suspension of *Micrococcus lysodiekticus* (Worthington Biochemical Corp., Freehold, N.J.). The lysis produces a change in the optical density of the bacterial suspension which is proportional to the lysozyme concentration. The change in optical density (0–3 min) was measured spectrophotometrically at 645 mμ.

5. Peripheral white blood cells. Blood was obtained from the tail of unanesthetized adjuvant arthritic rats. Total peripheral white

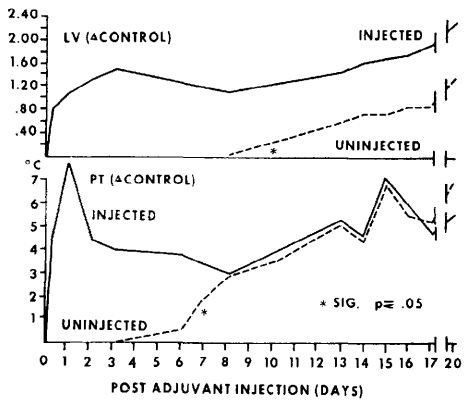

FIG. 1. Adjuvant arthritic (AA) rat hindleg volume (LV) and paw temperature (PT): Temporal relationship between increases in hindleg volume and paw temperature in adjuvant arthritis. Measurements made daily exclusive of days 4, 5, 9, 11, and 12. Plotted values represent the mean difference from untreated control rats (10 rats/group). *Earliest significant difference $p \leq .05$

blood cells were counted with the aid of a Coulter counter. Polymorphonuclear cells and lymphocytes were counted microscopically after being stained with Wright's stain.

B. Results and Discussion. 1. Edema formation and local hyperpyrexia. The temperal relationship of edema formation and local hyperpyrexia in the hindlegs of adjuvant arthritic rats is shown in Fig. 1. As shown in the upper portion of Fig. 1, the injected hindleg becomes inflamed within 3 hr and reaches maximal size within 3 days (primary lesion). The secondary lesions of adjuvant arthritis occur after a delay of approximately 8 days and are characterized by inflammation of the noninjected sites (right hindleg) and further increases in the volume of the injected hindleg which parallel the increase in volume of the noninjected leg. Figure 1 (lower portion) shows the increase in hindpaw temperatures. As shown, the paw temperature of the injected leg follows the biphasic pattern of the leg volume increases. In contrast to volume increases, however, the paw temperature of the injected leg is maximum on day 1. Similarly, significant increases in the paw temperature of the noninjected leg occur on day 7 which is 3 days prior to significant volume increases. Another difference between these parameters which is evident in Fig. 1 is that, although the volumes of the injected and noninjected legs are markedly different in the secondary phase of the disease, their paw temperatures are essentially identical.

2. Grip function. The temporal relationship of grip function impairment and increases in hindleg volume are shown in Fig. 2. As shown, significant impairment of grip function occurs on day 10 and parallels the onset and severity of the secondary lesions. These results are in agreement with the reported observations of Perrine and Takesue (11) who used a rotarod to assess the grip strength of adjuvant-treated rats. The impairment of grip function in the secondary phase of adjuvant arthritis probably reflects the development of inflammation in both the forepaws and hindpaws which occurs during this period.

3. Body weight changes. Body weight changes (from day 0) of untreated and adjuvant arthritic rats are compared in Fig. 3. The impairment of body weight gain in adjuvant arthritic rats follows a biphasic pattern corresponding to the primary and secondary phases of the disease. The adjuvant arthritic rats exhibited an initial body weight loss on day 1; then, their body weight gain paralleled the body weight gain of untreated rats until day 6, after which the adjuvant arthritic rats failed to gain weight. The un-

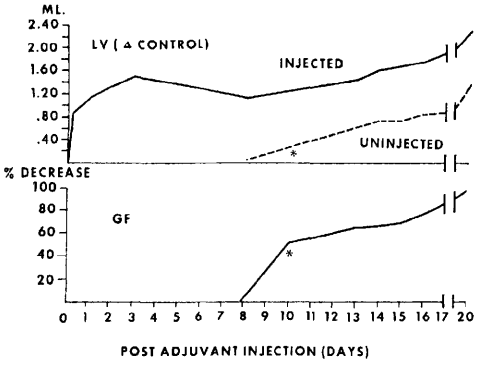

FIG. 2. AA rat hindleg volume (LV) and grip function (GF): Temporal relationship between increases in hindleg volume and impairment of grip function in adjuvant arthritis. Measurements made daily exclusive of days 4, 5, 9, 11, and 12. Leg volume values represent the mean difference from untreated control rats (10 rats/group). *Earliest significant difference $p \leq .05$

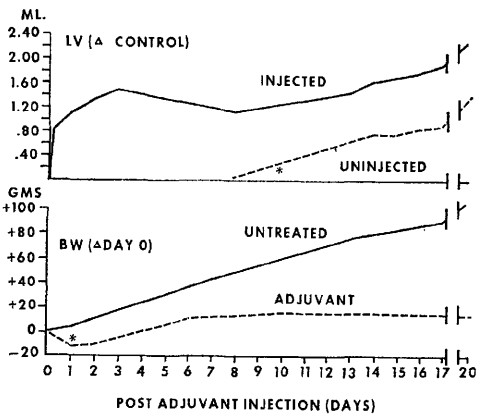

FIG. 3. AA rat hindleg volume (LV) and body weight change (BW): Temporal relationship between increases in hindleg volume and body weight gain in adjuvant arthritis. Measurements made daily exclusive of Days 4, 5, 9, 11, and 12. Leg volume values represent the mean difference from untreated control rats (10 rats/group). *Earliest significant difference $p \leq .05$

treated rats exhibited a continuous gain in body weight throughout the test period. It is interesting to note that the body weight impairment in the secondary phase on day 6 corresponds temporally with the onset of hyperpyrexia in the uninjected hindleg (Fig. 1).

4. Serum lysozyme and peripheral blood leukocytes. The temporal relationship of edema formation (increased hindleg volumes), elevated serum lysozyme levels, and increases in peripheral blood leukocytes is shown in Fig. 4. The serum lysozyme levels in adjuvant arthritis increase in a biphasic manner and parallel the edema formation in the primary and secondary phases of the disease. The concentration of polymorphonuclear leukocytes was significantly elevated after a delay of 7 days and continued to increase until day 17; whereas the blood lymphocyte concentration was significantly elevated only on day 17. Serum lysozyme levels have been previously reported to be elevated in adjuvant arthritis (10) and rheumatoid arthritis (14). This elevation is believed to be a reflection of lysosomal enzyme release from leukocytic cells following intense endocytosis. In our studies, the increased lysozyme levels in adjuvant arthritic rat sera were not dependent upon the elevation of polymorphonuclear leukocytes, since the levels of these parameters were not significantly correlated

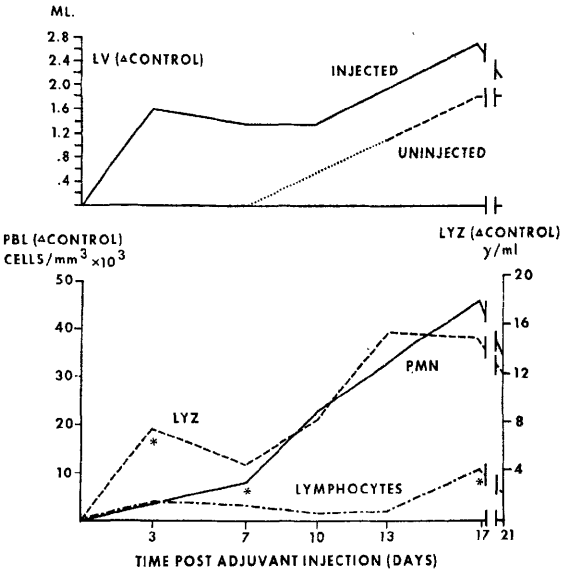

FIG. 4. AA rat hindleg volume (LV), serum lysozyme (LYZ), and peripheral blood leukocytes (PBL): Temporal relationship between increases in hindleg volume, serum lysozyme, and peripheral blood leukocytes. Measurements made on days 3, 7, 10, 13, 17, and 21. Plotted values represent the mean difference from vehicle (oil)-treated control group (6–7 rats/group). *Earliest significant difference $p \leq .05$; PMN = polymorphonuclear leukocytes.

(correlation coefficient = 0.5). There was also no correlation between serum lysozyme levels and edema volume in either the injected or uninjected hindlegs of arthritic rats.

5. Further correlation of parameters. Local hyperpyrexia, edema volume, and grip function were measured in 30 adjuvant arthritic rats on day 14 following adjuvant injection. The temperature and volume of both the paw and ankle region of the hindlegs were measured. There was no significant correlation (correlation coefficient <0.7) between edema volume, local hyperpyrexia, or grip function. Thus, it appears that these parameters occur as independent expressions of inflammation in adjuvant arthritis.

C. Summary and conclusions. The temporal relationship of various physiological and biochemical alterations in adjuvant arthritis was presented. The parameters measured included hindleg edema volume, local hyperpyrexia, grip function, serum lysozyme, peripheral blood leukocytes, and body weight. The injection of *M. butyricum* (in oil) into the hindpaw of Lewis rats produced increases in the injected hindleg volume and temperature, elevation of serum lysozyme, and loss of body weight gain. These alterations occurred in a biphasic pattern which corresponded temporally to the primary and secondary phases of adjuvant arthritis. Increases in the uninjected hindleg volume and temperature, impairment of grip function, and increases in peripheral blood leukocytes occurred mainly in the secondary phase of the disease. Local hyperpyrexia, elevated polymorphonuclear leukocytes, and impairment of body weight gain were the earliest detectable signs of the onset of the secondary phase in adjuvant arthritis. Edema volume, local hyperpyrexia, impairment of grip function, and elevated serum lysozyme levels appear to be diverse expressions of the inflammatory process in adjuvant arthritis as well as the human disease. The assessment of these parameters in the animal model may have utility in defining the profile of potential antiarthritic agents.

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