High Incidence of Adjuvant Arthritis as a Secondary Lesion to Peritonitis in Rats (35886)

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(Introduced by R. G. Herrmann)

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Pearson (1) produced the animal model of arthritis by injecting tubercle bacilli into the foot pad of rats. Since that initial study in the development of adjuvant arthritis, other investigators have also induced the disease with injections of Mycobacterium into the foot pad and tail of the rat (2–3). The disease has been characterized with respect to severity, drug effects, sites of injections, animal age, sex, strain, and biochemical parameters (1–12). The secondary lesions of polyarthritis occur approximately 2 weeks after the initial inflammatory reaction produced by the injection of antigen. When antigen is injected into a site which ultimately becomes involved in arthritic lesions, it is difficult to follow the development of the primary inflammatory reaction, the immune response, and the development of the secondary lesions. This report presents a study of polyarthritis in rats produced remotely as result of an intraperitoneal injection of heat-killed *Mycobacterium tuberculosum* in mineral oil. Because of the separation of the antigen injection site and the developing secondary lesion site, we were able to study the primary inflammation (peritonitis) and the secondary lesion (polyarthritis) simultaneously.

Experimental procedures. The results reported in this study were obtained from two experiments. In the first experiment 40 Cox-strain male rats (140–160 g) were injected intraperitoneally with 0.25 mg of heat-killed *M. tuberculosum* in 0.05 ml of mineral oil (Group I animals). Forty control animals were injected the same day with 0.05 ml of mineral oil. In a second experiment, 44 animals were injected with 1.0 mg of *M. tuberculosum* in 0.2 ml of mineral oil (Group II). Forty-four control animals were injected with 0.2 ml of mineral oil.

Paw volumes were measured by mercury displacement using a pressure transducer. Volumes were determined daily in both experiments until day 21, when volumes were taken at 2-day intervals. Four animals were selected randomly from each experimental and control group and sacrificed at intervals (1 week in Group I and 4 days in Group II) for the determination of peritoneal leukocyte counts and measurement of plasma inflammation units and β-glucuronidase. X-Rays of hind limbs were made on remaining animals at 7 weeks of Group I and at 5 weeks of Group II.

Animals selected for daily experimentation were injected subcutaneously with 100 units of heparin (Lilly) and were bled within 15 min. After sacrifice, peritoneal cells were obtained with two 10-ml washes of Krebs–Ringer phosphate buffer (KRP). The cells were sedimented at 200*g* for 5 min and resuspended in KRP containing 1% bovine serum albumin. Total and differential cell counts were made for each peritoneal exudate. Before sacrifice, blood was collected by orbital puncture into tubes containing 2.0 ml of isotonic saline with 100 units of heparin. The blood was diluted twofold with saline and centrifuged for 10 min at 3000*g* to remove cells. The diluted plasma was used for the determination of inflammation units (13) and β-glucuronidase (14). Paw volumes and body weights were measured prior to animal sacrifice.

Parameters measured for the primary lesion were leukocyte count and cell population. Parameters for the secondary lesion were paw volume/body weight and

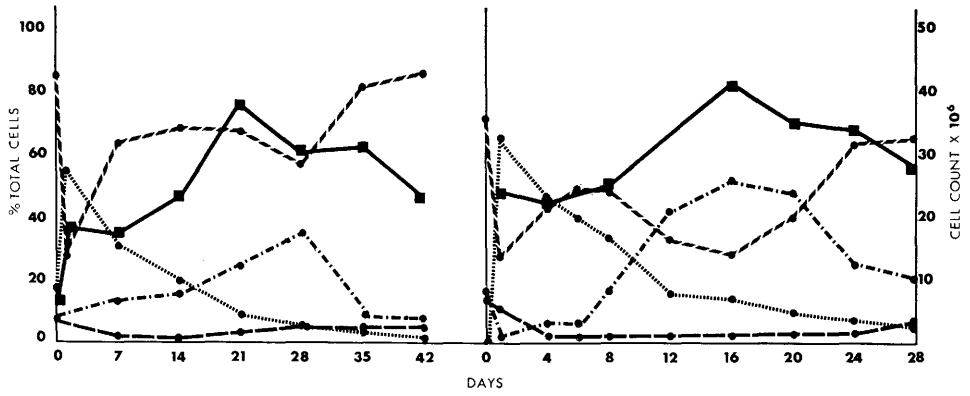

FIG. 1. Temporal relationship of peritoneal cell populations and total cell count during the development of peritionitis-polyarthritis: (left) Group I; (right) Group II; (▬) total peritoneal count; (◣◥) monocytes; (▮▮▮▮▮▮▮▮▮) polymorphonuclear leukocytes (PMN); (▬ ▮ ▬) lymphocytes; (▬ ▬ ▬) eosinophils.

percentage of swollen hind limbs. Percentage swollen limbs = (no. of limbs with index $\geqq$ 1.4/total no. of limbs) $\times$ 100. Swelling index was defined as paw volume on a given day/paw volume at day zero. The paw volume index for control animals ranged from 1.05 $\pm$ 0.04 (day 0) to 1.34 $\pm$ 0.04 (day 40).

Results. a. Primary lesion (peritonitis). An early acute inflammation developed in the peritoneum of 65% of Group I, 85% of Group II, and to a very mild extent in all the control rats. This inflammatory response was shown by the sharp rise in the number of polymorphonuclear leukocytes in the peritoneal exudates during the first day (Fig. 1). In control animals, the leukocyte population returned to normal after 2 or 3 days. In the adjuvant-treated animals, the cell number continued to increase and the percentage of PMN leukocytes decreased slowly until the appearance of the secondary lesions. A rapid increase in total leukocyte count occurred with lymphocytes reaching 45–50% of the total population (18–24 days for Group I and 12–15 days for Group II).

b. Blood parameters. Plasma inflammation units and β-glucuronidase patterns were similar for control and experimental animals for 21 days in Group I and 8 or 9 days in Group II (Fig. 2). β-Glucuronidase reached a maximum level in 35 days in Group I and 16 days in Group II animals. Peak activities of plasma enzyme levels in both experimental groups ranged between 3.0 and 3.5 times that of control levels.

Inflammation units showed similar patterns with peaks at 35 days and 16 days for the two groups. Values for inflammation units remained near zero throughout the experiment for control animals. Group I started to show an increase in inflammation units between days 16–18, while Group II demonstrated an increase as early as 8 to 10 days. Several animals from both groups had inflammation units as high as 350 during the period of maximal swelling in the limbs.

c. Secondary lesion. Paw volume changes were seen at approximately day 18 in Group I and days 8 to 10 in Group II (Fig. 3). The ratio of paw volume/body weight gives an exaggeration of the extent of swelling since there was a weight loss in the experimental animals which parallels paw swelling. First increases in paw volumes were observed around day 18 in Group I and days 8–10 in Group II. The average maximum paw volume/body weight index was the same in both groups (1.3). Control values ranged between 0.70–0.93. Peak volumes were observed in 35 days in Group I and between 15 and 25 days in Group II.

The incidence of significant swelling was determined by the percentage of hind limbs having a critical index, *i.e.*, 1.4 or greater. Figure 3 shows that the extent of the secondary lesions reached 60% in Group I and 95%

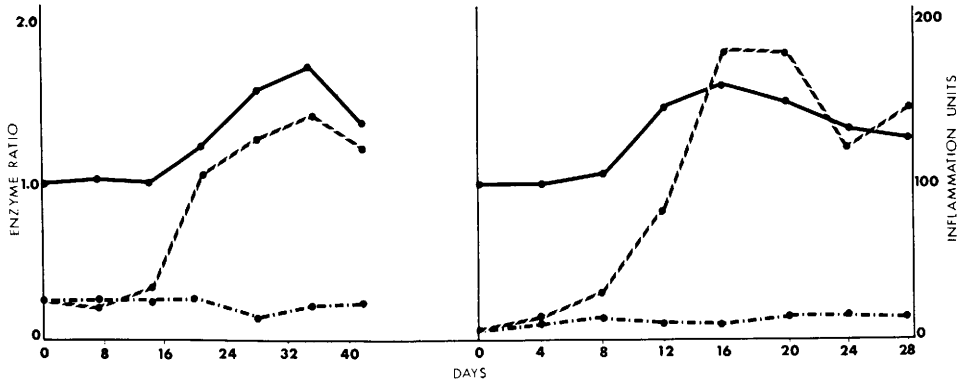

FIG. 2. Temporal relationship of plasma β-glucuronidase and inflammation units during the development of peritonitis-polyarthritis in rats. Fifty percent plasma was incubated with 1 m*M* phenolphthalein β-glucuronide, pH 5.0 60 min, 45° to determine β-glucuronidase level. Enzyme ratio (■) obtained by dividing experimental values by control values. Fifty percent plasma was diluted 10-fold with isotonic saline and heated at 55° for 30 min. (◥◤) experimental inflammation units; (■■■) control inflammation units.

in Group II. We have no explanation for the first peak of incidence followed by a second peak in Group II.

Bone damage was determined by X-rays at 7 weeks in Group I and at 5 weeks in Group II animals. Damage was rated on a 0–5 scale. Control animals showing no bone damage rated 0 (Table I). The remaining animals in Group I rated an average of 1.3; those in Group II, 2.2. Paw injected animals rated 3.0 at 5 weeks. No severe damage was observed in Group I. In Group II, approximately 40% of the limbs evaluated had ratings of 3 or 4.

Discussion. Under the conditions reported in this study, a high incidence of polyarthritis was induced by intraperitoneal injections of adjuvant. This is in contrast to reports by

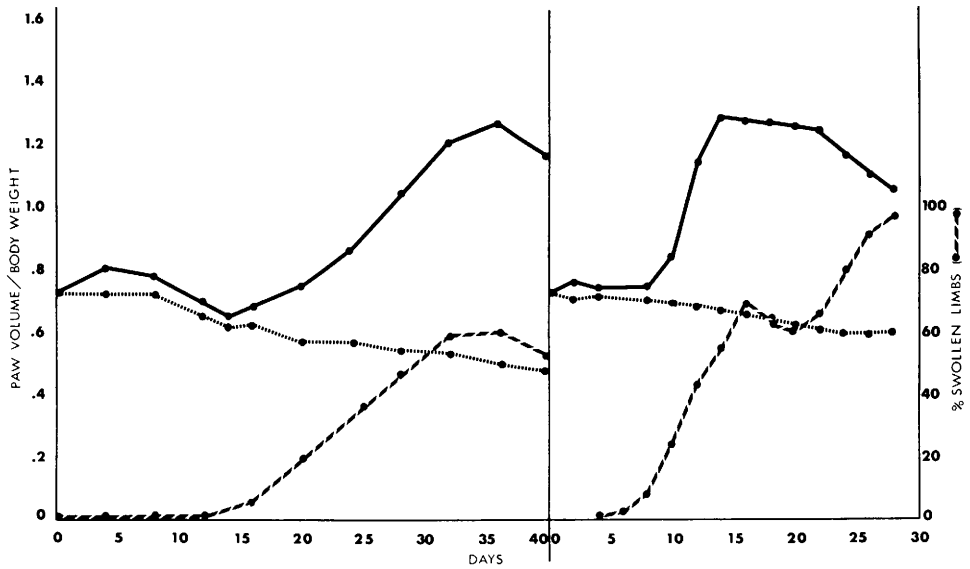

FIG. 3. Temporal relationship of paw volumes and degree of swelling during the development of peritonitis in rats. Percent swollen limbs has been defined in Methods. (■) paw volume/body weight (experimental); (IIIII) paw volume/body weight (control); (◥◤) percent swollen limbs.

TABLE I. Evaluation of Bone Damage in the Hind Limbs of Peritonitis-Polyarthritis Rats.

Animal group	Bone damage rating[a]
Control (noninjected)	0 ±
Adjuvant (paw injected)	3.0[b] ±
Group I	1.3 ± 0.18[c]
Group II	2.2 ± 0.25[d]

[a] Rating determined by visual inspection of X-rays. Values range from 0 (no damage) to 5 (maximum damage).

[b] Value represents the average taken from a number of animals on a routine basis for several years at the Lilly Research Laboratories.

[c] Average of 18 limbs.

[d] Average of 24 limbs.

previous investigators (1, 9). The results show a definite correlation between certain changes in the primary lesion (peritonitis) and the development of the secondary lesion (polyarthritis) in rats injected intraperitoneally with *M. tuberculosum* in mineral oil. The sharp increase in the number of peritoneal lymphocytes and the mesenteric lymph node hypertrophy suggest the immune involvement in the development of the secondary lesions. The increase of plasma β-glucuronidase and inflammation units at the time of the appearance of the secondary lesion indicates that these parameters are related to the inflammation and swelling in the limbs and not to the peritonitis. The major differences in the two doses of adjuvant given appear to be in the time required to develop secondary lesions and the extent of the lesions. In general, all parameters were less marked in animals given the lower dose of adjuvant even though isolated animals in that group showed swelling and bone damage equal to those found in Group II. The lower incidence of arthritis in Group I may have been due to technical problem of injecting small volumes (0.05 ml) of the oil into the peritoneum. This is supported by the fact that peritoneal cell counts and differential populations in those experimental animals not developing secondary lesions were similar to those of control animals.

The significance of this study is the fact that a high incidence of polyarthritis can be produced by peritoneal injection of adjuvant. The primary and secondary lesions are separated and can be studied independently. Thus, peritonitis-adjuvant arthritis can serve as a convenient model for studying drug action and the immunological perspectives of the disease.

Summary. Heat-killed *M. tuberculosum* injected intraperitoneally in rats produced peritonitis as a primary lesion and polyarthritis as a remote secondary lesion. Rats given 0.25 mg of the adjuvant developed paw swelling 21 to 28 days after the injection. Those given 1 mg developed severe swelling in 12 to 15 days. Parameters investigated were peritoneal leukocyte population and cell count, plasma β-glucuronidase, inflammation units, paw volumes, and body weights.

A sharp increase in peritoneal leukocyte counts and percentage lymphocytes was observed prior to swelling in the hind limbs. Plasma inflammation units and β-glucuronidase increased sharply at about the time paw volume increased. Bone damage was less severe than in animals injected in foot or tail.

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