Differences in the Severity of Adjuvant Arthritis in Four Strains of Rats (34270)

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(Introduced by J. W. Miller)
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The polyarthritis which develops in rats inoculated with mycobacterial adjuvant has been regarded as an experimental approximation to human rheumatoid arthritis (1). The familial tendency to rheumatoid arthritis in humans (2), suggesting genetically determined factors in susceptibility to the disease, prompted the following study comparing the evolution of adjuvant arthritis in four strains of rats.

Materials and Methods. Ten male and 10 female rats, weighing approximately 160 g, of each of four rat strains (Simonsen Sprague-Dawley, Holtzman, Charles River, and Buffalo) were injected in the plantar surface of the right hind foot with 0.5 mg of *Mycobacterium butyricum* (Difco) suspended in 0.05 ml of Drakeol 6-VR (Penn. Refining Co.). Right and left hind foot volumes were determined on the third, sixth, tenth, seventeenth, and twentieth days after adjuvant injection with a plethysmograph (Plethysmographe ΔV-3, Ugo Basile, Milano). Secondary lesions which occurred on the ears, nose, front paws, contralateral (noninjected) hind foot, and tail were separately scored according to their severity on a 0 to +3 scale. The maximum total score per rat was 15.

In a separate experiment, the effect of certain anti-inflammatory drugs on hind foot volumes and secondary lesions was determined in male Holtzman rats. Drugs were administered daily by gastric intubation as 4% acacia suspensions. Concentrations of the drugs were such that all animals received a volume of 0.5 ml/100 g of body weight. Control animals received a like volume of 4% acacia. For this experiment, a negative control group of animals was included. This group was injected with 0.05 ml of Drakeol 6-VR in the right hind foot and received daily oral doses of 4% acacia.

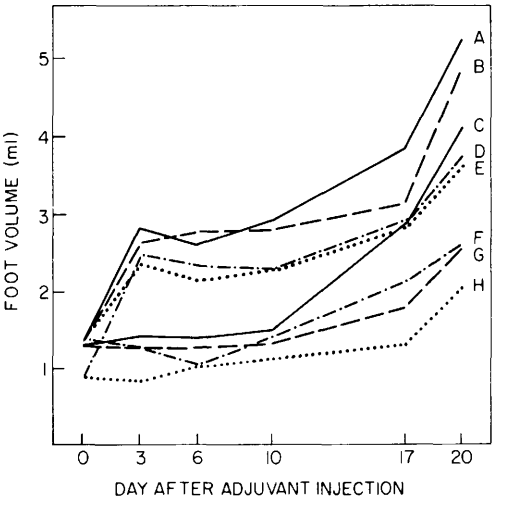

FIG. 1. Hind foot volumes for male rats of four strains on various days after the injection of adjuvant; Holtzman injected foot (A), Charles River injected foot (B), Holtzman contralateral foot (C), Simonsen injected foot (D), Buffalo injected foot (E), Simonsen contralateral foot (F), Charles River contralateral foot (G), and Buffalo contralateral foot (H). Each value is the mean of 10 rats; standard errors were generally 3–8% of the mean, the higher values occurring on days 17 and 20.

Results. Injected and noninjected hind foot volumes for the four strains of male rats are shown in Fig. 1. Similar results were obtained with female rats of the four strains. There was no marked difference in the magnitude of foot swelling between male and female rats of any particular strain confirming the lack of sex differences in the response of the rat to adjuvant injection (3). Swelling of both the injected and noninjected feet was greatest in Holtzman rats. Mean foot volumes of males or females for each of

TABLE I. Secondary Lesion Scores for Arthritic Rats on Various Days after the Injection of Adjuvant.

Rat strain	Sex	Mean secondary lesion score ± SE (day): 10	14	20
Simonsen	Male	1.8 ± 0.4	3.0 ± 0.5	5.1 ± 1.0
Buffalo	Male	1.2 ± 0.2	1.5 ± 0.4	2.4 ± 0.7
Holtzman	Male	1.9 ± 0.3	8.0 ± 0.8	9.3 ± 0.4
Charles River	Male	0.9 ± 0.2	4.5 ± 1.4	5.0 ± 0.9
Simonsen	Female	1.2 ± 0.4	4.8 ± 1.0	5.3 ± 1.1
Buffalo	Female	1.9 ± 0.2	1.1 ± 0.3	1.6 ± 0.3
Holtzman	Female	2.1 ± 0.6	6.5 ± 1.0	9.2 ± 1.0
Charles River	Female	3.0 ± 0.5	5.2 ± 1.6	6.3 ± 1.7

the four strains were compared by the least significant difference criterion (4) on each of the various days after adjuvant injection. The volume of the injected foot of Holtzman males was significantly greater ($p < .05$) than the corresponding volume for Simonsen males on all days, for Buffalo males on 4 of the 5 days, and for Charles River males on day 17 after adjuvant injection. The volume of the injected foot of Holtzman females was significantly greater than the corresponding volume for Buffalo, Simonsen, and Charles River females on 3, 2, and 3 of the 5 days, respectively. The volume of the contralateral (noninjected) foot of Holtzman males on days 10, 17, and 20 after adjuvant injection was greater than the corresponding volumes for Charles River and Buffalo males on all 3 days and for Simonsen males on 2 of the 3 days. For Holtzman females, the volume of the contralateral foot on day 20 was greater than the corresponding volumes for Simonsen and Buffalo females and not different from Charles River females. For days 10 and 17 differences among the strains of females were not significant.

The secondary lesion scores on the tenth, fourteenth, and twentieth days after adjuvant injection for the four strains are given in Table I. Holtzman rats were the most severely affected and Buffalo rats the least using this score as a parameter of the disease. Mean secondary lesions scores for the four strains were compared by the least significant difference criterion. There was no difference in secondary lesion scores between male and female rats of any particular strain. On day 20 after adjuvant injection, Holtzman males and females had significantly greater scores ($p < .05$) than any of the other strains of either sex except for Charles River females for which the difference was not significant ($p > .05$). Only Holtzman rats showed a 100% incidence of secondary lesions in the front and contralateral hind paws. Both sexes of all strains showed a high frequency of involvement of the contralateral hind paw. The ears and tail were affected in at least half of the rats for all strains except Buffalo rats in which the frequency of involvement was somewhat less. Buffalo rats showed much less frequent involvement of the front paws

TABLE II. "Type" of Arthritis Developing in Four Strains of Rats after the Injection of Adjuvant.

Strain	"Type" of arthritis, intensity
Simonsen	Nodular (*i.e.*, secondary lesions characterized for the most part by small nodules), moderate
Buffalo	Allergic?, mild
Holtzman	Edematous (*i.e.*, secondary lesions characterized for the most part by edema; nodules that occur are confined to ears and tail), severe
Charles River	Mixed nodular and edematous, moderate to severe

TABLE III. Effect of Certain Anti-Inflammatory Drugs on Adjuvant Arthritis in Holtzman Male Rats.

Treatment	Daily oral dose (mg/kg)	N	Mean body wt (g)		Vol increase[a] (% ± SE)		Secondary lesions		Thymus wet wt on day 21 (mg/100 g ± SE)
			Day 0	Day 21	Injected foot, day 7	Contralateral foot, day 21	Incidence[b]	Score ± SE, day 21	
Negative control[c]		5	170	275	14.1 ± 7.8[d]	3.0 ± 5.4[d]	0/5	0[d]	200.6 ± 11.6[d]
Positive control[c]		10	185	197	137.9 ± 10.7	121.8 ± 15.7	10/10	7.4 ± 0.9	149.0 ± 18.7
Indomethacin	0.50	10	184	227	73.7 ± 5.0[d]	18.1 ± 6.6[d]	9/10	4.9 ± 1.0	187.1 ± 17.2
	0.25	10	186	231	77.7 ± 3.9[d]	46.9 ± 7.4[d]	9/10	5.3 ± 0.9	194.1 ± 10.2[d]
Phenylbutazone	50	10	183	245	97.9 ± 6.3[d]	18.7 ± 5.0[d]	10/10	3.7 ± 0.8[d]	200.3 ± 9.6[d]
	25	10	182	232	95.1 ± 7.7[d]	30.9 ± 4.6[d]	7/10	5.0 ± 0.6[d]	217.1 ± 13.8[d]
Prednisone	2.0	10	184	208	118.7 ± 6.4	60.0 ± 15.3[d]	9/10	4.4 ± 0.8[d]	112.8 ± 19.3
	1.0	9	174	198	103.2 ± 13.5	47.7 ± 13.9[d]	8/9	5.7 ± 1.1	124.8 ± 15.5

[a] (Volume of foot on day_x—volume of foot on day_0/volume of foot on day_0) × 100.

[b] Number of rats with any secondary lesion/number of rats tested.

[c] Negative control (nonarthritic, daily oral doses of vehicle); positive control (arthritic, daily oral doses of vehicle).

[d] Significantly different from positive control ($p < .05$) when compared by Student's t; body weight and incidence of secondary lesions not compared; for all other values, $p > .05$ when compared to positive control.

(only 4 of 20 rats) and no strain showed marked involvement of the nose.

A subjective evaluation of the "type" of arthritis for each strain is given in Table II. The response of Buffalo rats to adjuvant injection was tentatively designated "allergic" since this strain gave a high incidence of nasal discharge around the twelfth day after adjuvant injection. Holtzman rats were adjudged to develop the most severe arthritis of the strains studied and were consequently selected for use in assessing the effects of anti-inflammatory drugs on the disease.

Table III lists certain data obtained at 7, 14, or 20 days after adjuvant injection for rats treated with indomethacin, phenylbutazone, and prednisone. A two-dose level assay is shown for each drug. Phenylbutazone and indomethacin at the doses used significantly reduced the swelling of the injected foot on days 7, 14, and 21. Prednisone was not effective in reducing swelling of the injected foot at the doses used on any of these days. All drugs significantly reduced swelling of the contralateral foot on day 21 following adjuvant injection. The finding that prednisone reduced swelling in the noninjected foot while having nonsignificant effects on the swelling of the injected foot suggests a lower threshold to inhibition by the steroid of the delayed swelling. All drugs reduced the secondary lesions score on days 14 and 21 following adjuvant injection although the reduction in score was not significantly different from the positive control for either dose of indomethacin or the low dose of prednisone. The incidence as distinct from the severity of secondary lesions did not seem to be affected by the three drugs (Table III). Although immunosuppressants reduce the incidence of secondary lesions (5), the dose of prednisone used here was apparently too low. In a separate experiment, 6-mercaptopurine at a dose of 30 mg/kg/day orally prevented completely the appearance of any secondary lesion (mean score $= 0$) in adjuvant injected rats. Rats treated with nonsteroidal drugs tended to maintain thymus weight (Table III) which may result from an alleviation of the stress brought about by the disease process.

Discussion. Similarities between rat adjuvant arthritis and human rheumatoid arthritis are apparent in certain blood changes. These include a depressed albumin/globulin ratio, and increased "inflammation units," glycoprotein, mucoprotein, and α-2-globulin (6), an elevated erythrocyte sedimentation rate, and increased plasma copper and sialic acid concentrations (7). Certain histopathologic changes in the joints are similar in rat and human arthritis including pannus formation (3). Traumatizing a joint makes the arthritis more severe in rats (3) as well as humans. Both the human and rat diseases are relieved by steroidal and nonsteroidal entiinflammatory drugs (8). Pearson (9) reported cyclic regression followed by exacerbation in the rat disease, a characteristic of the human disease.

Differences between the human and rat diseases include the absence of rheumatoid factor in arthritic rats (1), the absence of a sex difference in rats (3), and the apparent lack of effect of pregnancy or jaundice in the rat disease (3).

The familial tendency to rheumatoid arthritis in humans (2) may have its counterpart in rats. For example, Glenn and Gray (3) reported a difference in incidence of the disease in different rat litters of the same strain as well as differences in severity between rat strains. The findings reported here confirm the variability in response of different rat strains to adjuvant injection.

The similarities between rat adjuvant arthritis and human rheumatoid disease would appear to be superficial at best. The rat model is a useful adjunct to other screening methods of testing for anti-inflammatory activity (steroidal or nonsteroidal) since certain components of the disease appear to represent a chronic inflammatory condition capable of being ameliorated by nontoxic doses of anti-inflammatory drugs. The model would appear to be of definitive value in detecting immunosuppressants although less time-consuming methods do exist for identifying drugs with this activity. The fact that nonsteroidal anti-inflammatory agents do not reduce the incidence of secondary lesions but only their severity makes assessment of these changes of rather limited value in anti-

inflammatory drug evaluation. Primary and secondary foot swelling (*i.e.*, swelling of the injected and noninjected hind foot, respectively) would appear to be useful parameters in assessing activity of drugs against an "acute" and "chronic" inflammatory condition, respectively.

Summary. The development of adjuvant-induced arthritis was studied in four strains of rats. Using injected and noninjected hind foot volumes and a secondary lesion scoring system as parameters of the disease, Holtzman rats were adjudged to develop the most and Buffalo rats the least severe arthritis of the four strains.

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