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Reexamination of the Difference in Susceptibility to Adjuvant-Induced Arthritis among LEW/Crj, Slc/Wistar/ST and Slc/SD Rats

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Abstract: The present investigations were performed to assess the differences among rat colonies commonly used for neurophysiological research regarding the development of complete Freund's adjuvant (CFA)-induced arthritis. Inflammatory signs including edema in the paw fluctuated remarkably among individual Wistar (Slc/Wistar/ST) and Sprague-Dawley (Slc/SD) rats, while the inflammatory signs of Lewis (LEW/Crj) rats appeared earlier and was severer and more consistent than Slc/Wistar/ST and Slc/SD rats. Edema in the hind paw developed in 100% of LEW/Crj rats with the lowest dose of CFA (0.6 mg/rat) used as compared with 64% of Slc/Wistar/ST (CFA 1 mg/rat) and 38% of Slc/SD rats (CFA 1.2 mg/rat). Retardation of weight gain was observed in Slc/Wistar/ST and Slc/SD rats in contrast to a severe weight decrease in inflamed LEW/Crj rats after the development of arthritis.

Key words: complete Freund's adjuvant, hind paw volume, rat

Chronic pain is an intriguing challenge for modern medicine. To understand the pathophysiology of chronic inflammation and for the evaluation of anti-inflammatory drugs, animal models of chronic pain are required which can serve as a counterpart for human diseases. Thus, in 1956, Pearson observed that polyarthritis could be produced in rats by parenteral injection of live attenuated antigen (mostly bacterial) in oil immersion [12]. Thereafter, it has been shown that subcutaneous injection of *Mycobacteria* in mineral oil into the rat tail induces chronic inflammation in the ankle joint, which has many similarities to the human

disease, rheumatoid arthritis [13]. This model has been validated as a model of chronic pain [3]. Studies using different strains or colonies of rats have shown that the present model induces profound biochemical changes in central and peripheral nervous systems [1] together with modification of the activity of neurons receiving noxious input [5, 8, 10]. However these data can not be well correlated with each other since different strains or colonies of rats were used in these studies which are not similar in their susceptibility to the present model [4]. Differences in susceptibility between inbred Lewis and randomly bred Sprague-Dawley rats [15], and be-

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Table 1. Comparison of adjuvant-induced arthritis in LEW/Crj, Slc/Wistar/ST and Slc/SD rats

Strain	CFA ^a (mg/rat)	Present Incidence (%) ^b	Overall Incidence (%) ^c	Latency (Days) ^b
LEW/Crj	0.6	100	100 ± 0	11.5 ± 1.2
Slc/Wistar/ST	1	58 [*]	64.6 ± 16.8 [¶]	15.0 ± 5.0 [¶]
Slc/SD	1.2	45 [*]	38.2 ± 24.8 [¶]	15.1 ± 3.6 [¶]

All the results are means ± S.D. a) Complete Freund's adjuvant. b) Twelve LEW/Crj, 12 Slc/Wistar/ST, 20 Slc SD rats were used in the present series. c) Retrospective analysis of data from other experiments including the present series in which total 40 LEW/Crj in 9 series 30 Slc/Wistar/ST in 3 series and 70 Slc/SD in 14 series were used. ^{*} $P < 0.05$, [¶] $P < 0.0001$, χ^2 test and [¶] $P < 0.05$, Dunnett's test, compared with LEW/Crj rats.

tween Lewis (LEW/N) and Fischer 344/N rats [18] in developing arthritis have been reported, but the data for Wistar rats is missing. Many neurophysiological experiments have been done on the present model using Wistar rats and it is necessary to link these data with other strains. Therefore, the present study aimed to detect the differences in the development of arthritis among Lewis (LEW/Crj), Wistar (Slc/Wistar/ST) and Sprague Dawley (Slc/SD) rats- commonly used rat colonies.

All the experimental procedures were approved by the Animal Care Committee, Research Institute of Environmental Medicine, Nagoya University. Male rats, 4–8 weeks old, were used throughout. Inbred Lewis rats (LEW/Crj) were obtained from Charles River, Japan, and Sprague-Dawley (Slc/SD) and Wistar (Slc/Wistar/ST) rats which were bred in closed colonies, were obtained from SLC Inc., Japan. The rats were kept in conventional animal facilities and the temperature of the animal rooms was maintained at $24 \pm 2^\circ\text{C}$ with 12-hr light/dark cycles. Two or three rats were housed in each rodent cage. Twelve LEW/Crj rats, 20 Slc/SD rats, and 12 Slc/Wistar/ST rats were used. Three intact rats of each colony were used as control animals. To compare the incidence of the development of inflammation more precisely, in addition to the present series, a retrospective analysis was done with data from other experiments (neurophysiological experiments and the collection of specimen e.g. dorsal root ganglia) by the same investigators. In these experiments 28 LEW/Crj rats were used in 8 series, 50 Slc/SD rats in 13 series and 18 Slc/Wistar/ST rats in 2 series. In every series 2–10 rats were used.

Intradermal injection of a suspension of heat-killed *Mycobacterium butyricum* (Difco, Detroit, USA) and mineral oil commonly known as complete Freund's adjuvant (CFA) was injected into the distal one third of the rat tail. Initially, 0.1 ml of a 6 mg/ml concentration of CFA was injected into rats from every colony. The concentration of CFA was increased in Slc/SD to 12 mg/ml and in Slc/Wistar/ST to 10 mg/ml because the inoculation of the initial concentration of 6 mg/ml had little or no effect in preliminary experiments. Volumes of hind paws and body weights were measured for evaluation of inflammation. In addition, inspection for development of plantar erythema and decreased mobility of rats was done to detect the onset of inflammation. Great care was taken, particularly with regard to housing conditions, to minimize the discomfort of the animals, i.e., animals that developed acute signs of inflammation were isolated in separate cages. The volume of the right hind paw was measured by a mercury plethysmograph. Body weights and paw volumes were measured every other day for up to 4 weeks after the inoculation of CFA. All body weights and paw volume records were normalized, respectively, by subtracting the control value (obtained before inoculation) and dividing by it. Statistical analyses involved one way ANOVA, χ^2 test, Dunnett's test and ratio of variances (F-test) were done using the Prism 2.0 software. The results were regarded significant when P values were less than 0.05.

Incidence: Highly significant differences (Table 1) were evident in the incidence of development of arthritis between different colonies. In the present series 12 out of 12 (100%) LEW: Crj, 7 out of 12 (58%) Slc/

Fig. 1. The time course of paw volume (PV) in the rats which developed arthritis and body weight (BW) in both untreated and complete Freund's adjuvant (CFA) treated rats. All data are expressed as means $\pm$ S. E. Asterisks in A and B indicate the time points when significant differences ($P < 0.05$, one way ANOVA) were evident in PV and BW of LEW/Crj rats ($\circ$) when compared with Slc/Wistar/ST ($\triangle$) and Slc/SD ($\square$) rats after development of arthritis. Significant ratio of variances ($P < 0.05$, F-test) were also present between PV of arthritic LEW/Crj rats and the other two arthritic groups in days indicated by (+). PV and BW values are normalized as $PV + PV_b$, $BW - BW_b$; b , value obtained before inoculation.

Wistar/ST and 9 out of 20 (45%) Slc/SD rats developed arthritis due to inoculation of the effective doses of CFA. The retrospective analysis of data from other experiments together with the present data showed that in 9 series ($N=40$), 100% LEW/Crj rats were affected with the lowest dose of CFA (0.6 mg/rat). Using the same dose of CFA, no Slc/SD rats and few Slc/Wistar/ST rats were affected. With increased doses of CFA, different series of Slc/Wistar/ST and Slc/SD rats showed different sensitivities to the development of arthritis. The variations in incidence among the series were 50–84% (64.6% on average) in Slc/Wistar/ST rats with a dose of 1 mg CFA/rat, and 0–70% (38.2% on average) in Slc/SD rats with the highest dose of CFA (1.2 mg/rat) (Table 1). Nineteen out of 30 Slc/Wistar/ST rats in 3 series and only 28 out of 70 Slc/SD rats in 14 series developed arthritis.

Latency: After the inoculation of CFA, all affected rats had a latent period when animals looked apparently normal. In no instance, did the initial signs of arthritis (redness or swelling of paw) appear before the 11th day after inoculation. Ten of 12 LEW/Crj rats developed arthritis on the 11th day after inoculation and this latent period was the shortest among the rats from

different breeding colonies (Table 1, $P < 0.05$, Dunnett's test). The latent periods for the development of arthritis in Slc/Wistar/ST and Slc/SD rats were in the range of 11–28 days.

Paw swelling: The paw volume in LEW/Crj rats progressively increased up to 3 weeks and remained at this level for up to 6 weeks (partly shown in Fig. 1A). Due to the short latency of the appearance of inflammatory signs, paw swelling of LEW/Crj rats was significantly greater than those of Slc/SD and Slc/Wistar/ST rats on day 16 and day 18 (Fig. 1A; day 16, $P < 0.03$ and day 18, $P < 0.007$, One way ANOVA), indicating rapid and intense inflammatory responses in LEW/Crj rats. Paw volume of Slc/Wistar/ST rats showed a greater rate of increase after 3 weeks of inoculation, although with greater variation (larger S. E. in Fig. 1A); the paw volume of Slc/SD rats also showed greater variation (larger S.E.). After day 25, paw volume of Slc/SD rats had a noticeable remission phase, which remained at the same level up to day 35 (data not shown). A comparison of the ratio of variances (F-test) indicated significant differences in paw volume between LEW/Crj rats and Slc/SD (on days 14, 16, 18, 25 and 28) and Slc/Wistar/ST (on days 14, 18, 25 and

28) rats (Fig. 1A).

Body Weight: The body weights of the normal Slc/SD and Slc/Wistar/ST rats increased faster than the normal LEW/Crj rats. In contrast, retardation of weight gain was observed in injected rats of all colonies. From day 10, the degree of retardation of weight gain was slightly weakened in Slc/SD and Slc/Wistar/ST rats, which gained weight with a surprisingly similar pattern, while a gradual decrease in body weight beginning on day 10 was observed in the LEW/Crj rats after the development of arthritis. On day 28, body weights of the arthritic Slc/Wistar/ST and Slc/SD rats were about 40% of the respective untreated rats, but it was at the same level of day 0 in arthritic LEW/Crj rats (Fig. 1B). The body weight of arthritic LEW/Crj rats was significantly lower (Fig. 1B; $P < 0.01$, One way ANOVA) than those of Slc/SD and Slc/Wistar/ST rats from day 11 to day 28, demonstrating the severity of inflammation in LEW/Crj rats.

The present data confirm previous observations and shed light on the clear differences among commonly used rat colonies in the development of the present model. However, data on the incidence of development of inflammation is not completely in agreement with previous reports. One reason for this might be the use of rat strains from different breeders. In our experience, Lewis rats obtained from SLC Inc. have shown only 93% incidence of inflammation and have had greater individual variations in producing inflammatory signs than those (LEW/Crj) from Charles River, Japan (unpublished observations).

Our observations suggest that inbred LEW/Crj rats are more susceptible to adjuvant-induced arthritis than rats which are bred in closed colonies (randomly bred). Previous studies showed that inbred rat strains were different in many physiological aspects from their progenitors [20]. The high susceptibility of LEW/Crj rats to the present model of arthritis is expected since similar strains of rats have been reported to be susceptible to various experimental auto-immune diseases such as experimental allergic encephalomyelitis [16], experimental autoimmune neuritis [6], monoarthritis [15], streptococcal cell wall-induced arthritis [18], giant cell myocarditis [17], etc. Such peculiar susceptibility of Lewis rats to autoimmune and inflammatory diseases is considered to be due to overproduction of pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor

necrosis factor (TNF- α) [14] and in part, to defective inflammatory and stress mediator-induced activation of the hypothalamic-pituitary-adrenal axis [19]. The relative resistance to the development of arthritis in Slc/SD and Slc/Wistar/ST rats may correlate with evidence that suggests quantitative differences in the distribution of pro-inflammatory and anti-inflammatory cytokines in these strains. Zhu *et al.* reported that in Sprague-Dawley rats resistance to experimental autoimmune neuritis as compared with Lewis rats was associated with decreased interferon- γ (IFN- γ) and TNF- α production [21]. IFN- γ has been shown to play a pathogenic role in experimental models of autoimmune diseases [2, 7]. On the other hand, it has been shown that Wistar rats have lower circulating IL-1 and TNF- α activities and higher circulating corticosterone following injection of lypopolysaccharide as compared with Lewis rats [14]. These observations may help to explain the difference in sensitivities to CFA in the rat colonies presently used, however, the genetic mechanism leading to different cytokine expression in these strains remains unclear.

In our study, although LEW/Crj rats emerged as the most susceptible strain, severe weight decrease in these rats seems to be associated with widespread systemic disease in response to CFA. For this reason, a behavioral or neurobiological study using arthritic LEW/Crj rats could produce results which are related to non-specific effects. Specifically, pain measurements were difficult in these animals because of severe disturbances in their movement (unpublished observation at our laboratory). On the contrary, retardation of weight gain in arthritic Slc/Wistar/ST or Slc/SD rats might parallel the state of mental depression which often accompanies chronic pain in humans [9]. To get a model of rheumatoid arthritis suitable for pain research, the use of Wistar rats (bred in closed colonies) would be more preferable with their moderate susceptibilities to adjuvant arthritis and better general physical condition after the development of arthritis.

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