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Pain Threshold Changes in Adjuvant-Induced Inflammation: A Possible Model of Chronic Pain in the Mouse

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LARSON, A. A., D. R. BROWN, S. EL-ATRASH AND M. M. WALSER. *Pain threshold changes in adjuvant-induced inflammation: A possible model of chronic pain in the mouse.* PHARMACOL BIOCHEM BEHAV **24**(1) 49–53, 1986.—A chronic hyperalgesic condition was induced in mice by the injection of Freund's complete adjuvant (FCA) into the lower lumbar region or directly into the hind footpads. Although little or no visible inflammation was observed after a single intradermal injection of FCA into the lower lumbar area of rats or mice, significant alterations in nociceptive thresholds occurred in each species as determined by decreases in response latency in tail-flick and hot-plate assays. Unilateral intraplantar administration of FCA in mice resulted in visible inflammation in the area of the tibiotarsal (ankle) joint. Changes in the response latency to a noxious stimulus in the areas surrounding the inflamed joint were similar to those observed in non-inflamed limbs, suggesting that changes in sensitivity to noxious stimuli were not merely the result of local hypersensitivity of the inflamed tissue, but may also be due to alterations in nociception at the level of the central nervous system (CNS). When the chronic inflammatory condition induced in the mouse tibiotarsal joint was evaluated by histological and morphological techniques, it was found to have the same characteristics as described in the early stages of FCA-induced arthritis in rats. The similarities between the response to FCA in rat and mouse suggest that injection of FCA in mice may prove to be a useful model for the study of chronic pain in mice as well as in rats.

Inflammation Freund's adjuvant Nociception Mouse Chronic pain Arthritis

FREUND'S complete adjuvant (FCA), which consists of heat-killed mycobacteria suspended in a mineral oil vehicle, has been employed to produce an arthritic condition in rats which has served as a useful laboratory model in studies of chronic pain [4,6] and inflammation [8, 10, 13]. Adjuvant-induced arthritis was first produced in rats by an intradermal (ID) injection of a combination of spleen cells and adjuvant [16]. Later, complete Freund's adjuvant, which contains heat-killed mycobacterium only, was found to be sufficient to induce arthritis [13]. Since then, chronic arthritis has been shown to be experimentally produced by injections of cell walls or their components from a variety of bacteria [15].

Although Freund's adjuvant has been widely used to induce an arthritic condition in rats, an arthritic condition in mice has been typically induced by the intravenous (IV) injection of live mycoplasmal bacteria [2,3]. The resulting arthritic model in mice differs from that produced by FCA in rats as it is migratory and typically lasts throughout the lifetime of the mouse. Thus the use of mice has been disadvantaged by the complications of handling live cultures and by the potential variability compared to the use of Freund's adjuvant, which is available commercially and therefore introduces less variability.

In the present investigation, we examined the effects of Freund's adjuvant in mice to compare the response in this more economical species to that previously reported in rat, which has been extensively used as a model of chronic pain. The degree of inflammation was quantified and the animals examined histologically. As previous studies have shown that induction of arthritis in rats using FCA results in hypersensitivity, as assessed by the intensity of spontaneous vocalization [14], we also studied changes in nociceptive thresholds during the development of the arthritic-like condition.

METHOD

Subjects

The experimental animals were Swiss-Webster mice of both sexes, weighing between 15 and 20 g, and male Sprague-Dawley albino rats weighing 375 to 425 g (Biolabs, White Bear Lake, MN). Rats were housed individually and

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TABLE 1

TAIL FLICK LATENCIES IN FCA- AND FIA-TREATED RATS AND MICE

Species	(n)	Treatment[a]	Tail Flick Latencies (sec)[b] at 14 days	at 21 days
Rat	15	FIA	5.54 ± 0.18	5.64 ± 0.12
Rat	15	FCA	5.08 ± 0.26	4.75 ± 0.11
Mouse	7	FIA	4.64 ± 0.09	4.92 ± 0.22
Mouse	7	FCA	3.57 ± 0.01†	4.09 ± 0.17*

[a]All animals received a single (50 μl) injection of either Freund's complete adjuvant (FCA) or Freund's incomplete adjuvant (FIA) ID in the lumbar area of the back.

[b]Values indicate the mean ± S.E.M. of responses in (n) animals at 14 and 21 days after adjuvant administration. Significant differences between the mean response latencies of FCA- and FIA-treated animals of each species, as determined by Student's *t*-test, are indicated as $*p<0.05$ and $\dagger p<0.005$.

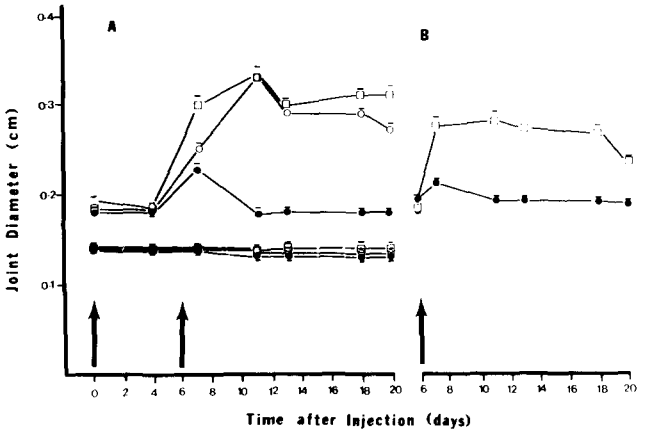

FIG. 1. A. Hindlimb tibiotarsal (solid lines) and forelimb radiocarpal (stippled line) joint diameters in mice at various times after injection of 0.05 ml of FCA (open squares), FIA (open circles) or saline (closed circles), administered first ID on day zero and then injected again directly into the foot (intraplantar) again on day six. Each symbol represents the average ±S.E. diameter of 3 to 13 mice.
B. Tibiotarsal joint diameter in mice at various times after a single intraplantar injection of 0.05 ml of FCA (open squares) or saline (solid circles) on day 6. In contrast to the above, these mice were *not* injected with adjuvant on day zero. Each symbol represents the average ±S.E. diameter of 10 mice.

mice were housed in groups of 8 per cage. Standard laboratory rodent chow and water were freely available to all animals.

Materials

Freund's complete adjuvant (FCA), containing heat-killed *Mycobacterium butyricum* in Bayol F paraffin oil, and Freund's incomplete adjuvant (FIA), comprised of only the paraffin oil vehicle (Difco Laboratories, Detroit, MI) were used in these studies.

Adjuvant Administration

In the first experiment, rats and mice were injected ID in the lumbar area at the base of the tail with 0.05 ml of FCA or FIA, as described previously in rats [1, 4, 6]. Pain thresholds were determined in both species using the tail-flick assay (described below) at 14 and 21 days after injections.

A second set of mice were then injected ID with 0.05 ml of FCA, FIA or saline in the lumbar area, followed 6 days later by a second injection of 0.05 ml of the same agent directly into the right hind footpad, as described previously by Newbould [11]. Nociceptive thresholds were then assessed in these mice by the hot plate and tail flick assays. The diameters of the right tibiotarsal (ankle) and radiocarpal (wrist) joints were also measured for 20 days after the first ID injection.

The last set of mice were injected directly into the footpads of both hind legs with either FCA, FIA or saline in a volume of 0.05 ml/foot. Joint diameters were measured at 0, 1, 5, 13 and 15 days after the injections.

Evaluation of Inflammation

The diameters of the tibiotarsal and radiocarpal joints were measured with a caliper at various times after the initial adjuvant or saline treatment. Mice from the second set of experiments were sacrificed for histopathological examination at various time after their injection. Both radiocarpal and tibiotarsal joints, as well as the liver and lungs were excised and preserved in 10% neutral buffered formalin. The foot and tibiotarsal region were decalcified in 10% formic acid. Tissues were embedded in paraffin, sectioned at 6 microns, and stained with hematoxylin and eosin. Tissues from 3 mice were injected with FCA, two mice treated with FIA and four mice injected with saline were examined.

Assessment of Nociceptive Threshold

In the first and second set of experiments, nociceptive thresholds were determined in all subjects at various time intervals prior to and after adjuvant injection. In both rats and mice, the latency of a tail flick response to a radiant heat source was measured using the method of D'Amour and Smith [5]. In the present study, a heat lamp was adjusted to an intensity sufficient to produce an average response latency of 4.4 and 5.3 sec in naive, untreated mice and rats respectively. In mice only, nociception was additionally tested using a modification of the hot plate assay [7]. Animals were placed on a hot plate (Model 475, Technilab Instruments, Pequannock, NJ) maintained at 52.5°C. The response latency was measured as the time preceding licks of a hindpaw and a forepaw.

Data Analysis

Data from measurements of joint diameter and nociceptive threshold were analyzed by analysis of variance for repeated measures. Preplanned individual comparisons across time and treatments were made with $p<0.05$ as the criterion for statistical analysis. The difference between means in Table 1 were evaluated by the Student's *t*-test.

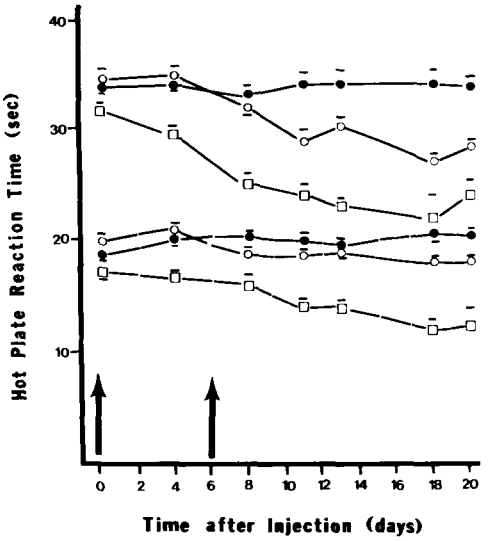

FIG. 2. Effect of two administrations of 0.05 ml of FCA (open squares), FIA (open circles) or saline (closed circles) on the tail flick latency of mice at various times after their first injection. Mice were injected initially ID in the lumbar area of the back on day zero and then injected again directly into the foot on day six. Each symbol represents the average ±S.E. latency of 3 to 13 mice.

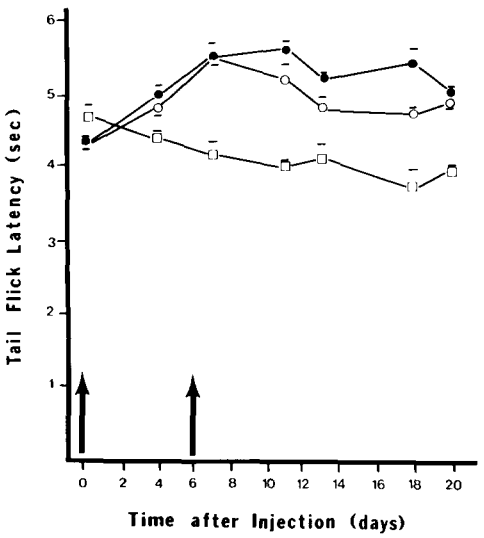

FIG. 3. Effect of two administrations of 0.05 ml of FCA (open squares), FIA (open circles) or saline (closed circles) on the hot plate reaction time of mice at various times after the first injection. Mice were injected initially ID in the lumbar area of the back on day zero and then injected again directly into the foot on day six. Latency of forelimb response (stippled lines) and hindlimb response (solid lines) were determined simultaneously in the same group of animals. Each symbol represents the average ±S.E. reaction time of 3 to 13 mice.

RESULTS

No gross pathological changes were visible in mice at any time after the single ID injection of either FCA or FIA. Nevertheless, mice treated with FCA were found to have significantly shorter tail flick latencies relative to FIA-treated controls at 14 and 21 days after the injection of adjuvant (Table 1). Rats injected in an identical fashion were also found to have shorter tail flick latencies after injections of FCA than after FIA (Table 1). However this apparent increase in nociceptive responsivity in this species was observed only at 21 days following adjuvant treatment.

In the second set of experiments, mice received an ID injection of either FCA, FIA or saline in the lumbar area of the back followed by an intraplantar injection 6 days later. Irrespective of the composition of the administered test solution, the tibiotarsal joints of injected limbs of all animals were markedly enlarged on day 1 after intraplantar injection. At day 4, tibiotarsal joint diameters in the adjuvant-treated mice remained significantly enlarged relative to saline-treated animals throughout the 21 day course of the experiment (Fig. 1A). Diameters of the radiocarpal joints were not significantly altered by any treatment.

Histopathological examination of subjects in experiment 2 revealed that mice injected with FCA manifested an extensive chronic active purulent cellulitis in the treated footpad. This was characterized by a dense infiltration of macrophages and neutrophils in the intermetatarsal soft tissue with a tendency to form microabcesses. One FCA-treated mouse exhibited a multifocal vasculitis in the footpad. Moreover, a chronic active periarticular synovitis and tenosynovitis in the tibiotarsal region was apparent in the 3 FCA-treated mice; the lesion consisted of increased numbers of macrophages, neutrophils, lymphocytes, plasma cells and eosinophils. The infiltrate did not extend into the articular space. In the tibiotarsal region of one animal, multifocal arteritis was present which was characterized by infiltration of the arterial walls by similar cell types. The liver of one FCA-treated mouse exhibited a mild diffuse fatty change; those of the other 2 animals were not remarkable. Mild multifocal peribronchiolar infiltration of mononuclear cells was present in the lungs of these subjects. These areas of infiltration by lymphocytes and plasma cells were small and quiescent and judged of no diagnostic importance.

The character of the lesions in the tibiotarsal joint and footpad of two mice treated with FIA was similar to that of the FCA-treated mice. These subjects manifested severe lesions in the footpad, and a mild to severe periarticular synovitis and tenosynovitis; the liver and lungs were not remarkable. One of the 4 mice which were injected with saline had mild plasmacytic cellulitis in the intermetatarsal space of the foot pad. No other lesions were present in the tissues of the saline-treated mice.

In both the tail flick (Fig. 2) and hot plate assays (Fig. 3), mice treated with FCA displayed a significant decrease in the threshold for nociception relative to mice administered either FIA or saline. Response latencies in the tail flick paradigm progressively decreased in FCA-treated mice over the 21 days of the experiment to values that were approximately 70 to 80% of saline-treated controls, while those of FIA- or saline-treated mice increased over time. This divergent response profile became manifest by day 4 after intralumbar injection and reached a maximum at day 18, at which time FCA- and saline-treated mice exhibited mean tail flick response times of 3.7±0.2 and 5.4±0.2 sec, respectively (Fig. 2). Mice injected with FIA had tail flick response latencies that were not significantly different from saline-treated controls (Fig. 2).

All subjects, regardless of pretreatment, exhibited a greater response latency in licking the hindpaws than in lick-

ing the forepaws in the hot plate paradigm (Fig. 3). As in the tail flick assay, FCA-treated mice displayed a general pattern of progressive decline in the latency to lick both the hindpaws and forepaws compared to the relatively stable latencies to paw lick in the saline-treated mice over the 21 day experimental period. Time-response curves between saline- and FCA-treated mice in latency to lick the hindpaws and forepaws were significantly different when compared by repeated-measures ANOVA. The maximum decrease in response latency determined by either the forepaw or hindpaw lick occurred at day 18 after intralumbar injection of FCA (Fig. 3). In contrast to FCA-treated mice, forepaw lick latencies of FIA-treated animals were not significantly different from controls injected with 0.9% saline. Moreover, hindpaw lick latencies in these mice did not differ from their saline-treated counterparts at day 4 after intralumbar injection of FIA. However, after intraplantar FIA injection, these mice displayed latencies to lick the hindpaws that progressively declined to levels intermediate between those of saline- and FCA-treated animals (Fig. 3). The difference in the time-response curves of the saline- and FIA-treated mice in latency to lick the hindpaws was statistically significant.

In the third set of experiments, mice were subjected to a single treatment consisting of bilateral injections of FCA or physiological saline into both hindpaws in order to determine whether intraplantar administration of FCA alone (i.e., in the absence of adjuvant injections into the lumbar area) is sufficient to produce an inflammatory response in the tibiotarsal region. Within one day after the intraplantar injection of FCA, the diameter of the right tibiotarsal joint abruptly increased from 0.18 ± 0.01 to 0.28 ± 0.01 cm and remained enlarged over the 14 day course of the experiment (Fig. 1B). In contrast, tibiotarsal joint diameter in control mice was not significantly altered by the intraplantar injection of 0.9% saline (Fig. 1B).

DISCUSSION

The production of a chronic inflammatory condition in the rat by administration of FCA ID at the base of the tail is a well established model for studies of chronic pain [4,6]. The present study is in agreement with previous reports indicating that such ID injections into the base of the tail of mice fail to produce any visible inflammation in this species [8]. The findings account for the abandonment of the mouse as a model for adjuvant-induced arthritis. The absence of a gross inflammatory response to an ID injection of FCA prompted us to test the effect of a direct injection of adjuvant into the foot, which has been previously shown to result in a more rapid onset of inflammation in rats [17]. Intraplantar injection of either FCA or FIA caused an inflammatory reaction in the limb which appears to be an effect of the vehicle locally (Fig. 1B). Similar local inflammatory responses to adjuvant have been previously observed in rat [8,13].

In previous studies, rats injected ID with FCA were found to exhibit pronounced increases in nociception, as indicated by an increase in the intensity of spontaneous vocalization [14]. While this treatment of mice did not result in any overt signs of inflammation, the effect of FCA on pain thresholds, as measured by a decrease in the tail flick latency, was similar in mice as that previously reported in rats [14,18]. In mice, the decrease in tail flick latency was readily apparent at 14 and 21 days, however the decrease in that of rats was not significant until 21 days after their injection, indicating an even greater sensitivity of mice to this effect than that seen in rats.

Only FCA, injected either ID at the base of the tail or directly into the footpad, appeared to result in an increase in the rate of response to nociception, when compared to the effect of FIA (Table 1) or saline (Fig. 2). In contrast, only intraplantar injection of either complete or incomplete adjuvant produces inflammation (Fig. 1). Even within groups while intraplantar injection of FCA resulted in inflammation of the hindpaws but not the forepaws (Fig. 1A), both forepaws and hindpaws were found to be hyperalgesic when tested on the hot plate assay (Fig. 3). Together these results indicate a lack of correlation between inflammation and changes in pain threshold, which is in agreement with the results previously reported in the rat [9,18]. The effect of FCA on pain perception is thus independent of the ability of the vehicle to induce a local inflammatory reaction.

The rationale for using rats rather than mice in previous studies of pain was that ID injections of adjuvant do not produce an arthritic response in other species than rats, and would therefore not result in chronic pain. However our results indicate that *direct* injection of *either* FCA or FIA in the feet of mice by two weeks after injection results in a constellation of changes consisting of dense infiltration of macrophages and neutrophils, microabcesses and periarticular synovitis, in addition to marked inflammation in the joints, indicating that this syndrome closely resembles the acute stages of the adjuvant-induced arthritic condition in rats [2, 3, 15]. As our examination of the tissue was limited to 14 days only, the usefulness of this technique for studies of arthritis remains to be determined by examination of joints at longer time intervals after injection.

The present study, as well as other recent reports [12], indicate that use of FCA in mice as well as in rats, produces a chronic hypersensitivity to nociceptive stimuli which may reflect a chronically enhanced pain perception in the mouse, as it does in the rat. As FCA-induced decreases in nociception did not correlate with the presence of inflammation and were detected in noninflamed tissue, far from the site of injection, as well as in areas of inflammation, hyperalgesia may be due to alterations by FCA in nociception at the level of the CNS. Thus the injection of FCA in mice appears to be deserving of further evaluation and consideration as a useful model of chronic pain, regardless of the presence of absence of gross or histologic inflammatory responses to the injection.

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