Relations between functional, inflammatory, and degenerative parameters during adjuvant arthritis in rats

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Philippe, Lionel, Pascale Gégout-Pottie, Corinne Guingamp, Karim Bordji, Bernard Terlain, Patrick Netter, and Pierre Gillet. Relations between functional, inflammatory, and degenerative parameters during adjuvant arthritis in rats. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1550–R1556, 1997.—We assessed the time-course of adjuvant arthritis (AA) in Lewis rats, using biotelemetry to monitor the rat's spontaneous locomotor activity and body temperature, and studied the evolution of the arthritic index, circulating concentrations of inflammation-promoting cytokines, cartilage proteoglycan synthesis, and the effect of indomethacin as a cyclooxygenase inhibitor to evaluate prostaglandin (PG) contribution in AA. The injection of complete Freund's adjuvant on day 0 (D0) induced a marked, transient loss of locomotor activity (D1–D4; initial phase) and then a second phase of hypomobility peaking on D15 and thereafter irreversible (D16–D20; arthritic phase). Fever peaked first on D1 and again between D13 and D17. The primary hyperthermia was associated with increases in plasma interleukin-6 and tumor necrosis factor-α concentrations and seemed to be partly PG dependent. Proteoglycan synthesis inhibition in the patellar cartilage increased gradually, spreading from the injected paw to the contralateral paw. It was corrected on D20 by preventive and curative indomethacin treatments. Indomethacin also greatly relieved hypomobility during the systemic phase of AA (D10–D15). The combination of information about cartilage metabolism, body temperature, locomotor activity, and cytokine in this study permits analysis of analgesic, antipyretic, anti-inflammatory, and chondroprotective properties of drugs in the various phases of AA. Thus, using a new methodology, we have discriminated the different phases of the disease and confirmed the symptomatic and systemic inhibitory effect of indomethacin on fever, activity, and cartilage metabolism.

proteoglycan synthesis; locomotor activity; indomethacin; experimental model

ARTICULAR HANDICAP is currently assessed in patients with arthritis on the basis of, for example, joint tenderness, articular and functional indexes (Lee, Ritchie, Health Assessment Questionnaire, etc.), and/or clinical examination. Such assessment by the patients themselves and the clinicians provides a sensible estimate of joint impairment. In animals, the severity of the arthritic disease is classically evaluated from the arthritic score or paw volume, but locomotor handicap is more difficult to assess. Biotelemetry provides a useful and original index of function by allowing, without stress, continuous evaluation of the rat's spontaneous mobility (34), especially in experimental models of arthritis, and also permits the simultaneous measurement of body temperature as an index of inflammation (24).

Adjuvant arthritis (AA) of rats is a widely used experimental model because in many respects it mimics arthritis in humans. This T cell dysimmune-mediated disease is one of the most important pharmacological models of rheumatoid arthritis and is commonly used to select classic nonsteroidal anti-inflammatory drugs (NSAIDs) (6). The model is also used to evaluate pain (11) and to elucidate immunological phenomena relating to the arthritic process, antigen presentation, and various cellular aspects (18). However, few studies have assessed the degeneration of articular cartilage in AA and little is known about the effect of NSAIDs on such degeneration (6) as a pertinent index of their putative chondroprotective potencies.

Therefore, we have used biotelemetry to characterize continuously changes in locomotor activity, used as a “clinical-like” index of articular function, and in body temperature during AA. We also wished to shed light on these biotelemetrically measured changes by use of classic measures such as the clinical index, plasma concentrations of inflammation-promoting cytokines [interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α)]. We also assessed the preventive and curative effect of indomethacin (30) as a prostaglandin inhibitor on the original parameters developed hereafter (spontaneous mobility, body temperature, and proteoglycan anabolism). We have promoted this original clinical-like approach in classic AA to provide evidence about the chronological course of underlying pathological mechanisms that induce articular dysfunction, either reactional (primary response vs. the phlogistic agent) or dysimmune, through the arthritic process from the injected paw to the other joints (secondary response).

MATERIALS AND METHODS

Laboratory animals. Male Lewis rats (Charles River) weighing 180–200 g were housed in individual cages with free

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access to standard laboratory diet and drinking water. They were kept in a 12:12-h light-dark cycle (lights on 6:00 AM to 6:00 PM) in a temperature-controlled room (25 ± °C). One hundred fifty-four rats were used for these studies: four groups for the assessment of locomotor activity (n = 6/group), fever (n = 6/group), and cartilage anabolism (n = 5/group for each time point) and two groups for the measure of arthritic score (n = 10) and cytokine levels (n = 5/group for each time point).

Induction of polyarthritis with complete Freund’s adjuvant. Arthritis was induced [on day 0 (D0)] by a single subcutaneous injection (100 µl) of heat-killed Mycobacterium tuberculosis H37Rv (Difco Laboratory, Detroit, MI) suspended in sterile mineral oil (10 mg/ml; paraffin oil; NaCl 0.9% Tween 80) into the right hindpaw. The contralateral hindpaw of the adjuvant-sensitized rats and both hindpaws of control rats were injected with a sterile saline solution (100 µl).

Arthritic score. Clinical lesions were assessed by scoring each paw from 0 (no sign) to 4 (severe signs), yielding a maximum score of 16 per animal (D0 to D18). Scoring was based on the severity and extent of erythema, the swelling of periarticular tissues, and the enlargement and distortion of the joints. A sensitized animal was considered to have arthritis when at least one noninjected paw was inflamed.

Biotlemetry. Body temperature and locomotor activity were monitored between 6:00 PM and 6:00 AM (dark cycle) and recorded from D0 (nocturnal control data) to D20 using battery-operated biotlemetry devices (Mini-Mitter, model VMFH) implanted intraperitoneally (25). Complete Freund’s adjuvant (CFA) was injected between 9:00 AM and 10:00 AM, just after the recording of nocturnal control data. D1 includes the first dark cycle after the adjuvant injection. The activity index is expressed as a percentage of mean nocturnal activity relative to the control mean nocturnal activity (D0), with a negative percentage representing the percentage of activity lost (16).

Cartilage proteoglycan synthesis. As we previously described (15), cartilage metabolism was studied through proteoglycan synthesis of patellae (37). The rats were killed, and the patellae were dissected and incubated for 3 h at 37°C in a 5% CO2 atmosphere in 2 ml of RPMI-N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid-bicarbonate medium (Sigma) supplemented with L-glutamine, penicillin, streptomycin, and 2.5 µCi Na35SO4 (Amersham) per patella. At the end of the incubation period, the patellae were washed with saline and fixed overnight in 0.5% cetylpyridinium chloride (Sigma) in phosphate-buffered Formalin. After decalcification in 2% formic acid (Sigma), the patellae could be easily punched out from the surrounding tissue with a 2-mm biopsy punch (Stiefele). Each patella was solubilized in Soluene 350 (Packard), and its 35S content, a reliable measure of the sulfated glycosaminoglycan content, was measured by liquid scintillation counting (Hionic Fluor, Packard).

IL-6 and TNF-α assays. Blood samples were collected in sterile tubes by cardiac puncture and centrifuged, and the plasma was stored at −20°C until testing. The samples were assayed for their ability to support the proliferation of the IL-6-dependent B9 cell line, as previously described (1). Results were expressed as units per milliliter; 1 U/ml produced half-maximal cell proliferation. Plasma TNF-α was measured by enzyme-linked immunosorbent assay for rat TNF-α (kit from Genzyme).

Treatment with indomethacin. Indomethacin (Indocid, Merck Sharp & Dohme-Chibret, France) was given daily (subcutaneously at a dose of 3 mg·kg−1·day−1) to the adjuvant-injected rats in an attempt to either prevent (D0 to D20) or cure (D15 to D20) the arthritis. Indomethacin effect was studied comparing indomethacin-treated AA rats to AA rats that received saline (untreated AA rats).

Statistical analysis. Results are presented as means ± SE. AA indomethacin (preventive and curative)-treated rats, AA untreated rats, and control rats were compared using a two-way analysis of variance test for biotelemetric data, with P < 0.01 taken as the level of significance. The significance of differences in proteoglycan synthesis data between groups was calculated using Student’s unpaired two-tailed t-test, with P < 0.05 taken as the level of significance.

RESULTS

Clinical examination of the arthritic process. As shown in Fig. 1, an increase in the clinical score of the right hindpaw 24 h after adjuvant injection reflected severe local inflammation and swelling. In this initial inflammatory phase, the arthritic index of the right hindpaw peaked on D2 (score 3.3 ± 0.2). During the systemic phase, beginning on D10, the arthritic index of the (noninjected) left hindpaw rose slightly from zero (score 0.2 ± 0.1 on D10). From D10, rats were affected in all four paws, with the arthritic index increased until the end of the experiment (total score 9.8 ± 0.8 on D18).

Variation of spontaneous activity and body temperature in arthritic rats. A biotlemetry unit constantly recorded the spontaneous locomotor activity and the body temperature of the unrestrained rats. There was a large, transient febrile response (Fig. 2A) during local, acute inflammation, with a peak on D1 (38.9 ± 0.1°C) followed by a return to the control level on D3. During the systemic phase of the arthritic response, from D10, a second bout of fever peaked on D15 (38.5 ± 0.1°C). Then the body temperature fell toward normal levels until the end of the experiment (38.0 ± 0.1°C on D20).

"Preventive" treatment with indomethacin (subcutaneous administration of 3 mg·kg−1·day−1 from D0 to D20) of adjuvant-injected rats diminished the first bout of fever (38.4 ± 0.1°C on D1) and totally abolished the second peak (38.1 ± 0.1°C on D15) observed in untreated arthritic rats. Immediately on its administration, indomethacin "curative" treatment (the same dose administered by the same route, but from D15 to D20) abolished the second bout of fever (37.8 ± 0.1°C on D16) observed in untreated arthritic rats.
Nocturnal spontaneous locomotor activity (Fig. 2B) decreased greatly from D1 to D2 during the local acute inflammatory phase, reaching its lowest level for this phase (−65.5 ± 2.9%) on D2, and then, between D5 and D9, remained steady to an intermediate level. Then a marked loss of mobility appeared gradually from D12 until D15 and settled irreversibly until D20 (maximum hypomobility −93.2 ± 2.0% on D19).

Preventive treatment with indomethacin did not affect the first hypomobility low point (most severe hypomobility −66.3 ± 5.4% on D1), but it totally abolished the marked loss of mobility from D12 until the end of the experiment compared with untreated arthritic rats. Curative treatment with indomethacin markedly lessened hypomobility in arthritic rats during its administration period.

Variations in proteoglycan cartilage anabolism during AA. We studied proteoglycan synthesis in the central part of the cartilage of both patellae in arthritic and control rats throughout the experiment (Fig. 3A). Proteoglycan synthesis in right patellae decreased gradually and significantly in the adjuvant-injected rats from D7 (−19.2%, P < 0.01) to D20 (−42.9%, P < 0.001) compared with patellae of control rats. The proteoglycan synthesis of the left patellar of arthritic rats relative to patellae of control rats gradually and significantly decreased from D14 (−22.5%, P < 0.05) to D20 (−37.0%, P < 0.001). Thus damage to the patellar cartilage was noted from D7 on the right injected side and from D14 on the left contralateral side.

On D20, preventive and curative treatments with indomethacin (3 mg·kg⁻¹·day⁻¹) have beneficial effects on the inhibition of patellar cartilage proteoglycan synthesis of arthritic rats compared with patellae of untreated arthritic rats (Fig. 3B).
 Variation of plasma TNF-\(\alpha\) and IL-6 concentrations in AA rats. In the plasma of control rats (D0), a basal TNF-\(\alpha\) concentration (27.5 ± 2.7 pg/ml) was observed (Fig. 4A). The systemic TNF-\(\alpha\) concentration had significantly increased by 6 h after adjuvant injection, peaked at 12 h (183.5 ± 6.3 pg/ml), returned to near control concentrations on D2 (38.0 ± 1.3 pg/ml), and increased slightly until D20 (50.2 ± 3.3 pg/ml).

A basal systemic IL-6 concentration (40.0 ± 5.0 IU/ml) was observed in control rats (Fig. 4B). The systemic IL-6 concentration in adjuvant-injected rats increased by 6 h after injection, peaked at 12 h (517.5 ± 37.1 IU/ml), returned to control concentrations by D6 (35.0 ± 5.0 IU/ml), and then rose to 323.3 ± 66.4 IU/ml by D20.

**DISCUSSION**

In the present study, biotelemetry was used to study the effect of developing AA on nocturnal body temperature and spontaneous locomotor activity of the experimental rats. In fact, we have observed that this technique permits the quantification of the effect of developing AA on these original parameters accurately and continuously under less stressful conditions versus more classical indexes such as clinical score or cytokine profile. In addition, the course of the disease from the injected paw (primary response) to the contralateral paw (secondary dysimmune process) was assessed via patellar cartilage metabolism, as previously studied in other experimental arthritides (15, 37). In the view of the results reported here, these original parameters appear mostly prostaglandin dependent with regard to the beneficial influence of indomethacin on the course of the disease.

Biotelemetry has only been used previously in rats with AA mainly to assess diurnal sleep disturbance (20). The few previous reports of changes in mobility during AA have been of observed behavior (8, 13), of exploratory phenomena, or of actimetry using infrared and photocells during daytime (9, 21, 34, 35) paradoxically in this nocturnal animal. In the results reported here, the use of biotelemetry was extended to provide a continuous record of the mobility and body temperature of Lewis rats with AA, to evaluate disability of spontaneous articular function by means that do not stress these nocturnal animals (33), and to establish correlations between original functional indexes (mobility and fever) and a more classic parameter (clinical score) during the different phases of the disease.

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Injection of CFA into the right hindpaw induced a marked hyperthermia and greatly decreased locomotor activity characterizing the 4-day initial phase of AA. Interestingly, this phase is also observed with biotelemetry when the induction procedure is performed in the base of the tail either with complete or incomplete Freund's adjuvant (data not shown), thus minimizing the crippling role of induction procedure in the paw. During the quiet second phase, D5 to D9, temperature and mobility did not change. During the third phase, from D10 to D15, systemic AA was accompanied by deepening hypomobility and a peak in body temperature at D15. The fourth phase, D16 to D20, coincided with an irreversible and marked loss of mobility, with a gradual return to normal body temperature on D20.

These four phases defined with the use of biotelemetry are similar to those previously reported by others, usually with slight differences depending on the parameters studied. For example, Baumgartner et al. (5) distinguished three phases according to biochemical criteria: one of acute, local inflammation (D1–D4), one of remission and prearthritis (D7–D12), and one of chronic inflammation with periarthritis and osteogenic activity (D15–D28). As in our study, the primary, local reaction (until D3) was followed by secondary swelling (after D10), reflecting the arthritic response (31).

Various parameters, clinical index, plasma cytokine concentrations (IL-6, IL-1\(\beta\), and TNF-\(\alpha\)), proteoglycan anabolism in the patellar cartilage, and the effect of indomethacin on the time course of the disease, were studied to elucidate the mechanisms underlying the four phases (Table 1). The initial, transient hypomobility could be attributed to an acute phlogistic response due to paw inflammation, as shown by the arthritic index, and the related pain, which is known to be an
important cause of limitation of mobility (21), as well as febrile and ancillary somnogenic responses. This loss of locomotor activity corresponded with the peak of fever, which was accompanied by drowsiness and could have resulted from the increased concentrations of inflammation-promoting cytokines such as TNF-α and IL-6 (19, 23). As shown by the low but significant decrease of hyperthermia with preventive indomethacin treatment in arthritic rats, fever seems weakly prostaglandin dependent in this initial phase at the dosage regimen used here.

In the quiet phase (D5–D9), spontaneous locomotor activity was raised, although by D7 the articular cartilage of the right patella, which was not the anatomic site of the main inflammatory lesions, already showed the first slight alterations. Apparently the defect in cartilage anabolism observed in the knee joint in the quiet phase was not yet sufficient to affect articular joint function. For this purpose, biotelemetric studies provide an interesting tool because they permit the evaluation of all articular joint disabilities (reflecting joint lesion) and also ankle damages, because distal joints are mainly affected in this experimental disease. In contrast, anabolism of patellar cartilage is an interesting, accurate, quantitative, and validated tool (15, 37) and an alternative to autoradiography to study cartilage ex vivo. In addition, no anatomic chondral entity of the ankle is so easily removable. Moreover, this technique is more sensitive than classical (14) and new imaging techniques (6) in assessing chondral degeneration. The absence of fever in this phase coincided with the low plasma concentrations of proinflammatory cytokines (IL-6, TNF-α), although the arthritic score of the injected paw remained elevated.

In the systemic phase (D10–D15), all the parameters studied indicated deterioration. Loss of mobility as well as fever became maximal and seemed strongly prostaglandin dependent, as reflected by the beneficial effect of preventive and curative indomethacin (3 mg/kg). In our experimental conditions, hyperthermia was closely associated with systemic release of IL-6, which is known as a circulating endogenous pyrogen (23), and may have peaked between D12 and D20 in agreement with previous reports (3, 22, 36). Interestingly, the plasma concentration of IL-6 and the body temperature paralleled each other throughout the course of the disease.

The marked hypomobility, which was reversible by treatment with indomethacin, might have been induced by articular degenerative phenomena as reflected by the clinical index and the marked inhibition of proteoglycan synthesis. Indeed, we observed a dramatic decrease in proteoglycan anabolism in both knees from D14, reflecting systemic cartilage alterations and probably corresponding to the maximal hypomobility. In AA, the decrease in cartilage proteoglycan synthesis was time and site dependent, spreading from the injected paw to the contralateral paw. Furthermore, proinflammatory cytokines such as IL-1β, TNF-α, and IL-6 have been implicated in the chondral degenerative process, causing a decrease of cartilage anabolism matrix and an increase in the production of metalloproteases such as collagenase and stromelysin (4, 7).

During the arthritic phase (D16–D20), the body temperature returned to basal levels, although plasma concentrations of IL-6 were quite high. Fever seemed to have been downregulated by the end of the experiment. Maximal and irreversible hypomobility reflects severe degenerative damages at various sites (knee, ankle, hip, and wrist) and impairment of these joints. All of these matrix changes, inflammatory processes, and painful reactions disturb joint articular function and contribute to a loss of spontaneous mobility. The multiplicity of changes makes it difficult to assess the pathological events leading to functional disability. Interestingly, indomethacin treatment significantly improved cartilage proteoglycan synthesis in both knees, with total recovery in the noninjected knee. This marked improvement of cartilage metabolism after indomethacin treatment is certainly correlated with the disappearance of hypomobility characterizing joint impairment in the two last phases of AA.

Surprisingly, unlike IL-6 and TNF-α, in our experimental conditions IL-1β did not vary in its plasma concentration (CytoScreen Immunoassay kit IL-1β rat, BioSource International) during AA (data not shown). Nevertheless, our results are in complete agreement with those reported during lipopolysaccharide (LPS)-
induced inflammation in the rat air pouch, demonstrating that if IL-1 and IL-6 significantly increased in the pouch, only IL-6 was increased in the circulation (28, 29). In addition, no changes in circulating IL-1 are reported in febrile rats injected with turpentine (25). These data suggest that IL-1 mediates fever through release of IL-6 in the circulation (2, 10, 26). Initial circulating high levels or TNF-α are also consistent with those reported with intraperitoneal injection of LPS by the same authors (peak at 1.5 h). Therefore we could offer the hypothesis that 1) changes in IL-1β concentration were too weak to be detected in rat sera by our method, 2) that the concentration of IL-1β could not increase in this model (systemically) (32), or 3) that IL-1β production was only local, with only ancillary repercussions in sera (12, 17, 27). Systemic concentrations of proinflammatory cytokines weakly reflected intra-articular concentrations, but intra-articular IL-1β, TNF-α, and IL-6 are well known to be involved in cartilage destruction, especially in the inhibition of proteoglycan anabolism (37) and in the increased synthesis of matrix metalloprotease and the decreased synthesis of metalloprotease enzyme-inhibitors (38).

The plasmatic cytokine profile of IL-6 reported here is similar to those published by others (3, 22, 36). Our original contribution was to demonstrate in AA the biphasic response: IL-6 peak occurs very early within the primary phase and is thus not usually detected. IL-6 appears as an early plasmatic indicator of systemic response to adjuvant, and plasmatic increasing of IL-6 appears necessary for pyrogenicity in both primary and secondary responses during AA.

In addition, inhibition of systemic production of IL-6 by treatment with NSAIDs is well documented (3, 22, 36) and accounts for the fact that cyclooxygenase-derived PG mediates IL-6 production. Data concerning TNF are more complex to analyze, because we have only observed an increase in plasmatic levels during the first phase. One hypothesis might be that, during the primary response, TNF and IL-1 are produced at the site of induction and cause release of IL-6 into the circulation and ancillary fever. During the secondary dysimmune process, with regard to the data recently published in this model [mRNA in arthritic paw (3)], IL-6 appears a more prominent and PG-dependent parameter than TNF. These data may reflect the better response to indomethacin during the secondary phase involving mostly IL-6 than during the primary response involving both IL-6 and TNF (non-PG dependent) at the circulating level.

Perspectives

Taken together, the comparative study of the parameters provided a great deal of information. Variations in parameters reflecting function (spontaneous locomotor activity), inflammation (body temperature, arthritic score, systemic TNF-α, and IL-6 concentrations), and degeneration (cartilage anabolism) were not always obviously interrelated in the different AA phases. This new approach to the assessment of AA can advance our understanding of the relations among functional impairments, inflammatory signs, and chondral degeneration in arthritic rats. Although much work is still needed to verify the respective contributions of IL-1-, TNF-, and PG-related local processes and the relations among all the various parameters, either reactional (primary response) or immunological (secondary response), the relative cytokines (IL-1, IL-6, and/or TNF) and prostaglandin dependencies of each parameter remain to be established as a potential target for antirheumatic drugs.

Nevertheless this approach, looking at spontaneous locomotor activity, body temperature, proteoglycan cartilage anabolism, systemic concentrations of inflammation-promoting cytokines, and other experimental parameters, can be used in the classic experimental models to study the respective anti-inflammatory, analgesic, antipyretic, and chondroprotective effects of classic antiarthritis drugs at the various stages of the disease (reflecting articular, systemic and central components). This approach also allows experimental “clinical-like” studies of the properties of new drugs with an impact on the inflammatory cytokine-related versus prostaglandin-dependent phenomena, giving a clearer idea of a drug’s antiarthritic potential due to improvement of mobility impairment and modulation of chondrodegenerative processes in arthritis.

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