Abnormal Lipid Metabolism in Adjuvant Arthritic Rats

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Accepted April 20, 1989

Abstract—This study examined the altered lipid metabolism and effects of drug treatments during the development of adjuvant arthritis in rats. Before its onset (day 9 post-adjuvant), large decreases were noted in the serum total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride levels and, in particular, a large decrease in the lecithin-cholesterol acyltransferase activity. A large increase in the serum phospholipid level was also noted. As the arthritis progressed, the serum total cholesterol and HDL-cholesterol levels were rapidly reversed, finally reaching a level significantly higher than normal, together with rises in the serum free-cholesterol and lipid peroxide levels. These changes in serum lipids and enzyme activity could be normalized by treatment with cyclophosphamide, an immunosuppressive agent, but other than the serum triglyceride level, were not affected by treatment with indomethacin, a nonsteroidal anti-inflammatory drug, despite the fact that both drug treatments almost completely suppressed the progression of arthritis. These findings suggest that the abnormal lipid metabolism induced by adjuvant injection is not associated with the inflammatory activity, but associated with the immunopathologic response.

Chronic inflammatory diseases are fundamentally disorders of immunological regulation, but they affect the lipid and protein metabolism as well as other biochemical components in the serum (1–4). Lipid metabolic disturbance, characterized by a reduction of the cholesterol and triglyceride levels in different lipoprotein moieties, has been found in patients with rheumatoid arthritis (5, 6). A recent report suggested (7) that the abnormal lipoprotein metabolism in chronic rheumatoid arthritis is part of the inflammatory process. Extensive studies have been conducted in humans, but few have examined the lipid metabolism in arthritic animals, despite their use as disease models. Adjuvant-induced arthritis in rats, an experimental chronic inflammatory disease, has been widely used as a model of rheumatoid arthritis (8, 9). In a previous study, we found abnormal lipid levels in the serum of adjuvant arthritic rats (10).

The present study was undertaken to obtain detailed information on the alteration of the lipid metabolism with time as adjuvant arthritis developed in rats, and also to determine whether the mechanism is involved in the immune response or the intensity of the inflammation activity by using two drugs with different modes of action, cyclophosphamide (CY), an immunosuppressive agent, and indomethacin (IND), a nonsteroidal anti-inflammatory drug, both of which are known to produce clinical improvement of adjuvant arthritis in rats (8, 9).

Materials and Methods

Animals: Male inbred Fischer F344/N rats (Japan SLC, Inc.), 6 weeks of age at the start of the experiment, were used. They were housed in an air-conditioned room (23±2°C and 60±10% humidity) and were weighed twice a week during the experiment.

Induction and assessment of adjuvant arthritis: Adjuvant arthritis was induced by a single s.c. injection of 0.6 mg of heat-killed Mycobacterium butyricum (Difco Laboratories) suspended in 0.1 ml liquid paraffin into the base of the rat tail.
The severity of adjuvant-induced arthritis was assessed by paw volume change. Both hind paw volumes were measured with a plethysmometer (TK-101, UNICOM) by water displacement to the malleolus (ankle) just before adjuvant injection and then, to follow the development of the inflammatory lesions, at intervals of 3–4 days. The percentage changes in paw volume were calculated as \[ \frac{(Y-X)}{X} \times 100\% \], where \( X \) = the mean value of both hind paw volumes before injection and \( Y \) = the mean value of both hind paw volumes after injection.

**Drug treatment:** CY (Nakarai Chemicals) was dissolved in water just before use and administered orally at a dose of 10 mg/kg/day, on days 4 to 10 post-adjuvant. IND (Sigma Chemicals) was suspended in 0.5% tragant gum solution and administered orally at a dose of 1.5 mg/kg/day on days 0 to 18 post-adjuvant. The dose levels of the two drugs used in this study were based on the antiarthritic potency of CY and IND (8, 11). All the rats were fed a commercial diet (Oriental Yeast Co., Ltd.), which was given by pair-feeding to synchronize the body weight changes with those of adjuvant-treated animals. Water was freely available.

**Blood samples:** Blood samples were taken from the tail vein on days 4, 9, 14, 21, 28, 35 and 42 post-adjuvant after the animals had fasted overnight. The serum was separated by centrifugation (1500×g for 10 min), and the high-density lipoprotein (HDL) fraction was separated immediately from the serum by the heparin-manganese precipitation procedure (12).

**Analytical methods:** Total and free cholesterol levels in the serum were fluoroenzymatically determined as described previously (13). Lectin:cholesterol acyltransferase (LCAT) activity was obtained by measuring the rate of esterification of free cholesterol in the serum, as described by Dieplinger and Kostner (14). The activity was expressed as nmoles of esterified cholesterol per ml of serum per hour. Triglycerides were determined by the acetylated method of Fletcher (15). Phospholipids were determined by the method of Yoshida et al. (16). Lipid peroxides were fluorometrically determined by the thiobarbituric acid method of Yagi (17).

**Statistics:** The results obtained were expressed as the mean±S.E. of data from 6 rats per group. Student's t-test for paired observations was used to test for significance.

**Results**

**Changes in body weight**

Changes in the body weight are shown in Fig. 1. Despite pair-feeding, the growth rate of adjuvant arthritic rats was significantly inhibited compared to the normal control animals after day 13 post-adjuvant. However, CY- and IND-treated animals gained weight relatively steadily, although CY-treated animals showed a tendency of weight decrease from day 10 to 20 post-adjuvant.

**Effects of CY and IND on the development of adjuvant arthritis**

Figure 2 shows the time curves for the development of inflammation as reflected by edema formation on the hind paw. Adjuvant arthritic rats developed severe polyarthritis which became apparent at day 10, peaked at day 21 and persisted until day 42 post-adjuvant. Both CY and IND treatments almost completely suppressed the development of adjuvant arthritis.

**Effects of CY and IND on serum LCAT activity**

As shown in Fig. 3, on day 4 post-adjuvant, adjuvant arthritic rats already showed a marked decrease (34% of the normal control) in the LCAT activity, which persisted until day 28. CY treatment rapidly restored the decreased LCAT activity induced by adjuvant injection after day 14 post-adjuvant and returned to the normal value, which was reached on day 42. With IND treatment, no effect was noted.

**Effects of CY and IND on the serum lipid levels**

**Total cholesterol level:** As shown in Fig. 4, the total cholesterol level displayed a diphasic change in adjuvant arthritic rats. First, it significantly decreased as found on days 9 and 14 post-adjuvant, and then it began to rapidly increase with the development of arthritis, finally reaching a significantly higher level than that of the normal animals after day 28 post-adjuvant. CY treatment completely prevented the latter change, but not significantly the former one. On the other hand, IND
Fig. 1. Change in body weight in rats after adjuvant injection. Normal group (○): Untreated rats. ADJ group (●): Rats injected with adjuvant (Mycobacterium butyricum, 0.6 mg). ADJ+CY group (▲): Rats injected with adjuvant and given CY (10 mg/kg/day, p.o.) on days 4 to 10 post-adjuvant. ADJ+IND group (▲): Rats injected with adjuvant and given IND (1.5 mg/kg/day, p.o.) on days 0 to 18 post-adjuvant. Each point represents the mean±S.E. of 6 rats per group. *P<0.05, **P<0.01, ***P<0.001 vs. normal group.

Fig. 2. Changes in hind paw swelling in rats after adjuvant injection. Each point represents the mean±S.E. of 6 rats per group. *P<0.05, **P<0.01, ***P<0.001 vs. normal group. See Fig. 1 for the explanation of groups.
Free cholesterol level: In adjuvant arthritic rats, the free cholesterol level markedly increased after day 14 post-adjuvant (Fig. 5). CY treatment produced marked inhibition of such change, whereas IND treatment had no effect on it. The data on both total and free cholesterol levels obtained above showed that the ester ratio (the ratio of esterified cholesterol to total cholesterol) significantly decreased.

Fig. 3. Changes in serum LCAT activity in rats after adjuvant injection. Each point represents the mean±S.E. of 6 rats per group. **P<0.01, ***P<0.001 vs. normal group. See Fig. 1 for the explanation of groups.

Fig. 4. Changes in serum total cholesterol level in rats after adjuvant injection. Each point represents the mean±S.E. of 6 rats per group. *P<0.05, **P<0.01, ***P<0.001 vs. normal group. See Fig. 1 for the explanation of groups.
in adjuvant arthritic rats after day 14. CY treatment led to recovery of the ester ratio to the normal value, whereas IND treatment had no recovery effect.

**Triglyceride level:** As shown in Fig. 6, adjuvant arthritic rats showed a gradual decrease in the triglyceride level until day 28 post-adjuvant and thereafter a gradual return.

**Fig. 5.** Changes in serum free cholesterol level in rats after adjuvant injection. Each point represents the mean±S.E. of 6 rats per group. **P<0.01, ***P<0.001 vs. normal group. See Fig. 1 for the explanation of groups.

**Fig. 6.** Changes in serum triglyceride level in rats after adjuvant injection. Each point represents the mean±S.E. of 6 rats per group. *P<0.05, **P<0.01 vs. normal group. See Fig. 1 for the explanation of groups.
to the normal levels. These changes were inhibited by both IND and CY treatments.

**Phospholipid level:** Figure 7 shows that the adjuvant injection caused a marked increase in the phospholipid level from day 4 post-adjuvant to the end of the experiment compared to the normal control. These changes were completely prevented by CY treatment.

![Figure 7](image_url)

**Fig. 7.** Changes in serum phospholipid level in rats after adjuvant injection. Each point represents the mean ± S.E. of 6 rats per group. *P < 0.05, **P < 0.01, ***P < 0.001 vs. normal group. See Fig. 1 for the explanation of groups.

![Figure 8](image_url)

**Fig. 8.** Changes in serum HDL cholesterol level in rats after adjuvant injection. Each point represents the mean ± S.E. of 6 rats per group. *P < 0.05, **P < 0.01, ***P < 0.001 vs. normal group. See Fig. 1 for the explanation of groups.
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Fig. 9. Changes in serum lipid peroxide level in rats after adjuvant injection. Each point represents the mean±S.E. of 6 rats per group. *P<0.05, **P<0.01, ***P<0.001 vs. normal group. See Fig. 1 for the explanation of groups. TBA-RS: thiobarbituric acid-reacting substance.

but scarcely by IND treatment.

HDL-total cholesterol level: As shown in Fig. 8, adjuvant arthritic rats showed a marked decrease in the HDL-total cholesterol levels from day 4 to 28 post-adjuvant. CY treatment tended to inhibit such changes, whereas IND treatment had no effect.

Lipid peroxide level: The serum lipid peroxide level in adjuvant arthritic rats markedly increased after day 9 and reached a maximum value at day 21 post-adjuvant when arthritis also was at its peak (Fig. 9). CY-treatment normalized the enhanced lipid peroxide production in the arthritic rats, whereas IND treatment was ineffective.

Discussion

Adjuvant arthritis in rats is generally known as an immunologically induced disease mediated by T-cells (18–20). Several studies (9, 21–25) have shown that it involves not only the obvious signs of inflammation, but also produces many other changes in a variety of biological systems such as a decrease in serum albumin; increases in serum globulin, α1-acid glycoprotein and fibrinogen; and decreases in liver lipid peroxidation, drug metabolizing enzyme activities and the cytochrome P-450 level. These changes in protein metabolism implicate hepatic involvement in adjuvant disease.

In the present study, we characterized the time course of the spectrum of serum lipid levels and enzyme activity in adjuvant arthritic rats: marked decreases in total cholesterol, HDL-cholesterol, triglycerides and LCAT activity, and a marked increase in phospholipids before the onset of arthritis (day 9 post-adjuvant), followed thereafter by reverse changes of total cholesterol and HDL-cholesterol together with increases in free cholesterol and lipid peroxides. These biphasic changes also seem to reflect an altered liver function because lipids and LCAT are mostly of hepatic origin. It is of interest to note that the lipid changes observed before the onset of arthritis (in the acute phase) closely resemble those in patients with rheumatoid arthritis (1, 3, 5, 6). We also observed that the extremely reduced LCAT activity appeared in the early stage of adjuvant disease (at least on day 4 post-adjuvant), although the changes in the serum lipid levels were still moderate at that time. This suggests that the changes in LCAT activity offer the first evidence of a prearthritic reaction. Since LCAT is responsible for converting free cholesterol into esterified cholesterol in the serum, its low activity may
lead to an increase in free cholesterol, a decrease in esterified cholesterol and consequently, a decrease in the ester ratio. Thus, LCAT activity may be useful as a new parameter for the adjuvant disease activity.

We also found that the abnormalities in the lipid metabolism of the arthritic rats were normalized by CY treatment, but not affected by IND treatment, except for the serum triglyceride level, despite the fact that adjuvant arthritis induction was almost completely suppressed with either drug treatment. CY treatment alone had little influence on the lipid metabolism except for mild reductions in the LCAT activity and the HDL-cholesterol level at the dose used in this study (data not shown). These findings suggest that the abnormal lipid metabolism induced by adjuvant injection does not result from the inflammatory activity, but rather from the immunopathologic response. Svenson et al. (7) have recently reported similar results in patients with rheumatoid arthritis. Abnormal serum lipoprotein lipid levels were found to be normalized in parallel with a reduction of the inflammatory activity when CY or penicillamine was administered, but not when nonsteroidal anti-inflammatory drug therapy was given (7). They concluded that reduction of the inflammatory activity is a prerequisite for normalization of the disturbed lipoprotein metabolism. However, in our study, since we could give an excess dose of IND to adjuvant arthritic rats, paw edema might be sufficiently inhibited without an inhibitory effect on the abnormal lipid metabolism.

Adjuvant arthritis is currently thought to occur through cell-mediated autoimmunity by structural mimicry between mycobacteria and cartilage proteoglycans in rats (20, 26, 27). Thus activated macrophages and/or lymphocytes with adjuvant injection or their products, monokines, may be involved in the abnormal lipid and protein metabolism as well as degradation of the joint cartilage. Lee et al. (28) have reported that production of interleukin-1 (IL-1) by splenic macrophages is markedly increased during the development of adjuvant arthritis. IL-1 has a number of nonimmunologic effects on liver function (29, 30). Other monokines, IL-6 (31) and tumor necrosis factor (32), also have a hepatocyte-stimulating effect similar to IL-1. Thus, a few such monokines may affect hepatic synthesis of LCAT and lipoproteins as well as other acute-phase proteins.

The functional significance of the abnormal lipid metabolism that developed in adjuvant arthritic rats is not clear. Serum lipids or lipoproteins have been known to exert regulatory effects on the immune response (33). Most are attributed to an immunosuppressive role with a few exceptions (34, 35), for example, inhibitory responses to lymphocytes by the low-density-lipoprotein fraction (36) and an inhibitory effect of dietary-induced hypercholesterolemia on nonspecific immune responses of macrophages and monocytes (37). Therefore, significant changes in serum lipid levels of adjuvant arthritic rats may play a homeostatic role in the progression and regression of the adjuvant disease. Some support for this comes from our finding that cholesterol feeding inhibits the induction of adjuvant arthritis in rats (10).

In summary, the results of the present study demonstrate that adjuvant injection in rats induced abnormal changes in lipid metabolism in the acute as well as chronic phases of arthritis and that these changes are associated with an immune response. However, it still remains to be established whether the changes observed can also be generally observed in chronic inflammatory disease and whether they can be induced by the administration of monokines.

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