

Acute monoarthritis associated with lipid liquid crystals

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SUMMARY We describe three women with unexplained acute monoarthritis and birefringent lipid spherules or lipid liquid crystals in their synovial fluid. Synovial fluid aspirated within 24 hours of onset showed leucocyte counts ranging from 10×10^9 to $46 \times 10^9/l$ with 91-95% polymorphonuclear cells. Numerous positively birefringent 2-6 μm microspherules were seen inside and outside polymorphonuclear cells. These were dissolved in alcohol-ether but were not digested by uricase. Other non-birefringent globules of similar size that stained with Sudan black B were also seen. Transmission electron microscopy identified osmiophilic homogeneous or multilamellated material in phagocytic vacuoles. Serum lipids, lipase, and repeated joint radiographs were normal. Arthritis subsided completely after treatment with colchicine in one patient and non-steroidal anti-inflammatory agents in two. Thus birefringent microspherules can be found in association with acute, otherwise unexplained, arthritis. It seems possible that certain lipid liquid crystals may induce synovial inflammation similar to inflammation seen in other crystal induced arthritides.

Key words: synovial fluid, liposomes, articular inflammation.

Birefringent lipid microspherules, appearing as Maltese crosses, have occasionally been observed during synovial fluid analysis.¹ These microspherules are formed by multilayers of phospholipids and water and are variously described as liposomes, smetic mesophases, or lipid liquid crystals.² Recently such microspherules have been reported in the synovial fluid of one patient with otherwise unexplained acute monoarthritis³ and in the synovial fluid and synovial lining cells of another patient with chronic unexplained arthritis.⁴

In this report we describe three patients with acute inflammatory monoarthritis whose synovial fluid was characterised by the presence of abundant intracellular and extracellular strongly birefringent lipid liquid crystals. Transmission electron microscopy identified osmiophilic homogeneous round bodies or lamellated material in phagocytic vacuoles. In addition the lipid composition of the

synovial fluid supernatant in two patients showed an increase in free fatty acids, lysolecithin and phosphatidic acid. All three patients were seen during the first 24 hours of the attack and had normal serum lipid profiles, serum amylase, lipase, and radiographs of the affected joints. In each case the arthritis subsided completely in five to 10 days after treatment with colchicine or non-steroidal anti-inflammatory agents.

Patients and methods

PATIENT 1

The first patient was a 41-year-old black woman who had sarcoidosis of her lungs proved by biopsy, lymph nodes, and skin and bones which required steroid therapy for bone pain and fever. She presented with acute swelling of her left wrist. Over the previous 15 years she had experienced recurrent episodes of similar synovitis of both wrists, which had been rapidly controlled with either oral or intravenous colchicine.

1 ml of cloudy synovial fluid was aspirated from her acutely inflamed left wrist (Table 1). The fluid

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Table 1 *Synovial fluid analyses in three patients with acute arthritis and lipid liquid crystals*

	Patient No. 1		Patient No. 2		Patient No. 3		
Days	1	2	3	10	1	6	8
Appearance	Cloudy	Bloody	Cloudy	Clear	Cloudy	Bloody	Bloody
Amount (ml)	1	40	20	10	50	5	3
Viscosity	Poor	Poor	Poor	Poor	Poor	Poor	Poor
Leucocytes/l	10×10^9	10.4×10^9	24.4×10^9	0.85×10^9	40.5×10^9	27×10^9	12×10^9
Polymorphonuclear cells (%)	75	94	92	40	95	46	28
Lipid crystals	+++	+++	++	+	+++	++	+
Bacterial culture	NG*	NG	NG	NG	NG	NG	NG

*NG=no growth.

showed decreased viscosity with a leucocyte count of 10×10^9 cells/l with 75% polymorphonuclear neutrophils. A fresh drop preparation showed abundant strongly birefringent intracellular and extracellular microspherules (Fig. 1). No other crystals were seen. Other laboratory studies including serum lipid profile, serum lipase and amylase, calcium, phos-

phorus, alkaline phosphatase, and uric acid were within normal limits. Repeated radiographs and ^{99m}Tc pyrophosphate bone scan of her hands after the inflammation subsided were unremarkable. She received oral colchicine 0.6 mg four times a day, and her left wrist synovitis subsided completely within five days.

PATIENT 2

A 21-year-old black woman came to the emergency room complaining of a painful swollen left knee of

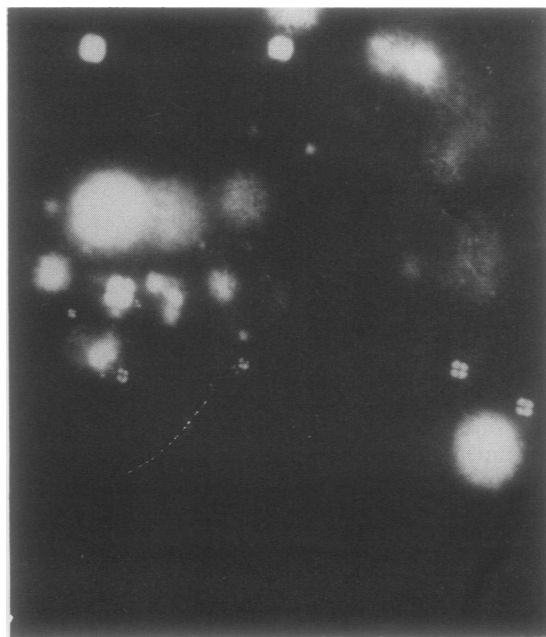


Fig. 1a

Figure 1 *Synovial fluid, fresh preparation of patient No. 1. (a) Abundant intracellular and extracellular doubly birefringent microspherules. Regular polarised light. ($\times 42$). (b) Strongly positively birefringent microspherules and a single larger spherule (arrow). The large line indicates the axis of slow vibration of the compensator. The dark portions of the spherule are parallel to the marked axis. Compensated polarised light. ($\times 60$). (c) A single large spherule. Compensated polarised light. ($\times 200$).*

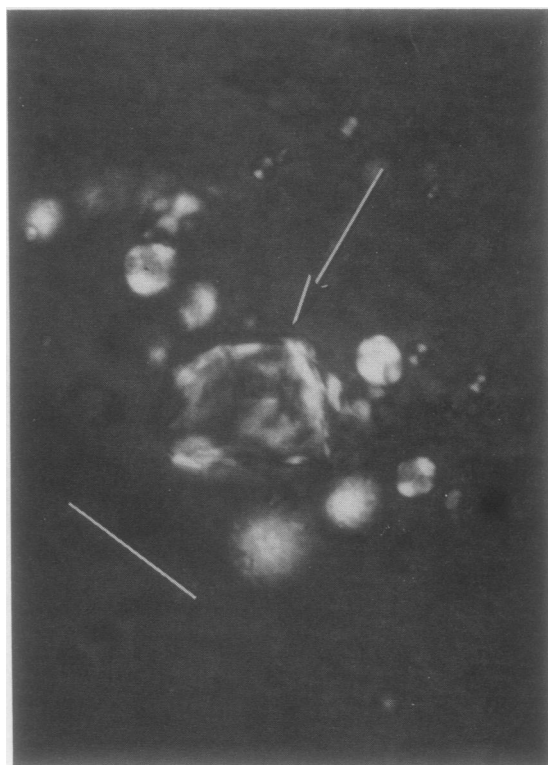


Fig. 1b

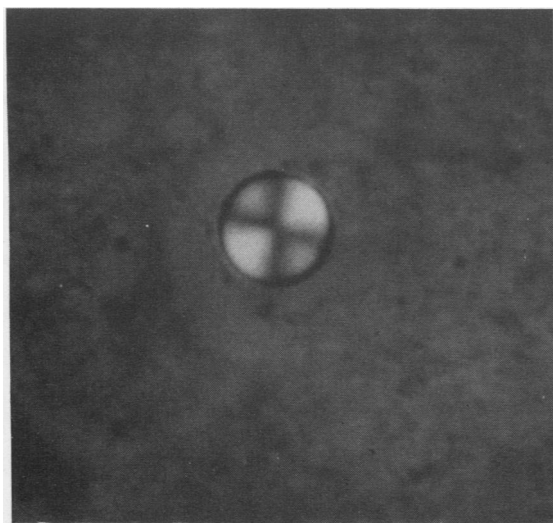


Fig. 1c

24 hours' duration. Three weeks before she had fallen to the floor landing on her right knee but had experienced no pain in either knee subsequent to the fall. Her left knee appeared swollen, warm, and tender with flexion limited to 50°. Synovial fluid was aspirated from her left knee on the second, third, and tenth days of her arthritis (Table 1). The fluid which was initially haemorrhagic became cloudy by the third day with decreased viscosity and leucocyte counts up to 24.4×10^9 cells/l with 92% polymorphonuclear cells. Abundant lipid liquid crystals similar to those observed in the first patient were seen inside and outside of the polymorphonuclear neutrophils. No other crystals were seen. By the 10th day the synovial fluid aspirate was clear and had a total cell count of 0.85×10^9 cells/l with 40% polymorphonuclear cells and few lipid liquid crystals. Bacterial cultures of blood and synovial fluid were negative. Serum lipid profile, amylase, lipase, uric acid, and ^{99m}Tc pyrophosphate bone scan were all unremarkable. Left knee synovitis subsided completely after 14 days of treatment with 75 mg of indomethacin daily.

PATIENT 3

A 54-year-old white woman with Laennec's cirrhosis proved by biopsy developed acute painful swelling of her right knee associated with fever and chills. Physical examination showed a temperature of 101.6°F and a warm swollen right knee with flexion limited to 60°. Synovial fluid was aspirated from her right knee on the first, sixth, and eighth days of her arthritis (Table 1). The fluid which was initially

cloudy with decreased viscosity had a leucocyte count of 40.5×10^9 cells/l with 95% polymorphonuclear cells. A fresh drop preparation showed abundant intracellular and extracellular strongly positively birefringent Maltese cross like liquid crystals. No other crystals were seen. On subsequent synovial fluid analysis on day 8 the total cell count was 12×10^9 cells/l with only 28% polymorphonuclear cells and few lipid liquid crystals. Bacterial cultures of both synovial fluid samples and blood showed no bacterial growth. Repeat radiographs of her knees and serum biochemical profile were unremarkable. She was treated with antibiotics for two days followed by indomethacin 100 mg daily, which resulted in complete resolution of her knee synovitis by the 10th day.

SYNOVIAL FLUID STUDIES

Lipid staining

Synovial fluid smears of all three patients were stained with Sudan black B stain for lipids.⁵ 1 ml synovial fluid aliquots from patients 2 and 3 were mixed with 1 ml of 1:1 alcohol:ether at room temperature and observed under compensated polarised light.

Uricase digestion

Uricase digestion of the microspherules was attempted with a single drop of uricase solution of 75 units of activity per 2 ml of distilled water on a glass slide with equal amounts of synovial fluid. The slide was sealed with a coverslip, and individual lipid liquid crystals were observed during the next 24 hours.⁶

Electron microscopic studies

Synovial fluid pellets from patients 2 and 3 were fixed in half strength Karnovsky's glutaraldehyde-paraformaldehyde fixative. After 15 minutes fixation the pellets were minced into 1×1 mm pieces, fixed for four more hours at room temperature, and processed by a previously reported technique.⁷ Specimens were examined in a Zeiss EM10 transmission electron microscope with a 60 kV beam.

Biochemical lipid analysis

Synovial fluids of patients 2 and 3 were available for biochemical lipid analysis. Fluid was centrifuged for 3–5 minutes at 100 g. Total lipids, neutral lipids and phospholipids were measured in accordance with a technique previously reported by the authors in a study of lipid composition of the tissue of normal human knee joints.⁸ Values for total lipids, neutral lipids, and phospholipids from normal pooled synovial fluid from that study were used as controls.⁸

Results

LIPID STAINING, ALCOHOL:ETHER SOLUBILITY AND URICASE DIGESTION

All three synovial fluid specimens showed Sudan black B stained fat droplets. Compensated polarised light showed abundant 2–6 μm birefringent microspherules with a Maltese cross appearance. The strongly positive birefringent microspherules were completely dissolved in a mixture of 1:1 alcohol:ether but were resistant to uricase digestion.

TRANSMISSION ELECTRON MICROSCOPIC STUDIES

Under transmission electron microscopy two predominant types of pertinent intracellular inclusions were observed. Some phagocytic vacuoles contained homogeneous grey lipid droplets (Fig. 2a). Additional homogeneous grey lipid inclusions similar to those described by Zucker-Franklin⁹ were seen in the cytoplasm (Fig. 2b). Other vacuoles had multi-layered lamellated inclusions similar to the arrangement reported by others in lipid liquid crystals.² Some of these inclusions showed a dark dense



Fig. 2b

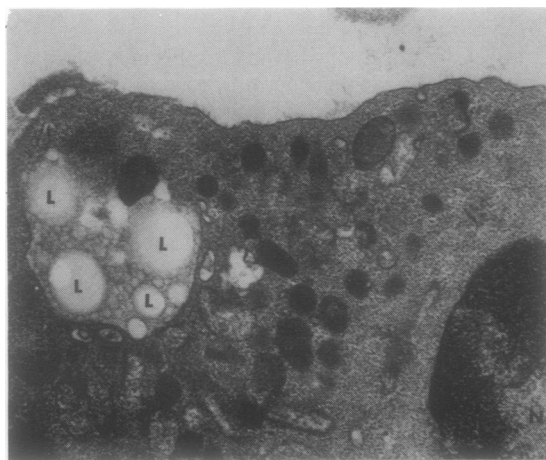


Fig. 2a

Fig. 2 *Electron micrographs of synovial fluid cells with intracellular lipid inclusions. (a) Homogeneous pale lipid droplets (L) in a vacuole of a polymorphonuclear leucocyte. N=nucleus. Patient 2. ($\times 20\,000$). (b) Single grey lipid (L) inclusions in a mononuclear cell. N=nucleus. Patient 2. ($\times 30\,200$). (c) Large multilamellated inclusion (MLI) with a dense centre (D) surrounded by smaller lamellated inclusions (arrows) and an isolated denser body (D) all in a synovial cell vacuole. Patient 3. ($\times 28\,700$). (d) Homogeneous lipid droplets (L) with membranous arrays at their margins (arrows) and multilamellated arrays in vacuoles of synovial mononuclear cell. Patient 3. ($\times 18\,150$).*

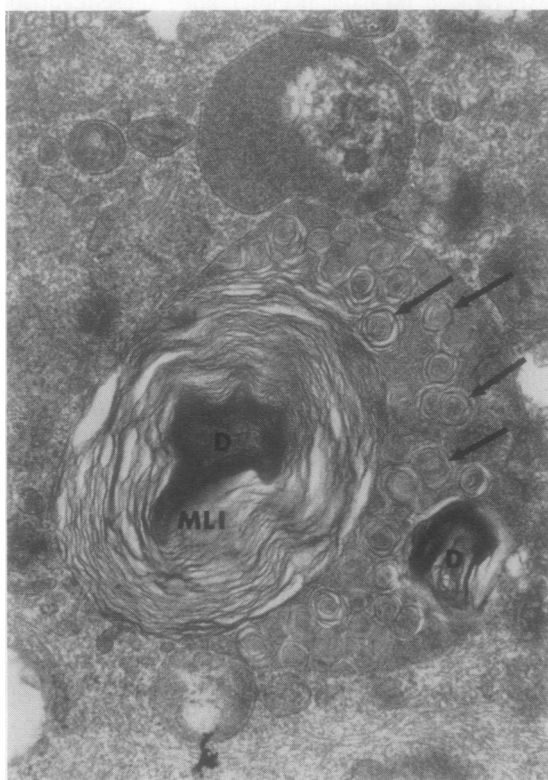


Fig. 2c



Fig. 2d

centre and were surrounded by numerous smaller but identical lamellated inclusions (Fig. 2c). In some areas a suggestion of transition from homogeneous to membranous inclusions was seen, with small membranous arrays at the margin of a homogeneous deposit (Fig. 2d). These inclusions resembled particles which have been described in foamy mouse macrophages in tissue culture.¹⁰ No viral particles or solid crystals were observed in either of the two synovial fluids studied.

BIOCHEMICAL LIPID STUDIES

Table 2 compares the synovial fluid concentrations of total lipids, neutral lipids, and phospholipids of patients 2 and 3 with normal synovial fluid pooled from cadavers from a previous study using a similar analysis.⁸ There was a slight but insignificant increase in the percentage of total lipids of 9.8 mg and 8.8 mg compared with 5 mg in the normal fluid. Increased concentrations of fatty acids, lysolecithin and phosphatidic acid and a decrease in lecithin concentration were observed in our patients compared with normal controls.

Discussion

LIPID LIQUID CRYSTAL CONCEPT

We describe three patients who developed an acute monoarthritis in association with the presence in the synovial fluid of positively birefringent microspherules which had features of lipid liquid crystals under compensated polarised light.

Liquid crystals represent states of matter having characteristics of both liquids and crystalline solids.^{2 11 12} They have some order in their molecular arrangement and also some degree of fluidity. Those molecules which tend to form liquid crystals

Table 2 Lipids in the synovial fluid of two patients with acute monoarthritis associated with lipid crystals

	Patient No. 2	Patient No. 3	Normal pooled synovial fluid ^a
Total lipids (mg %)	9.8	8.8	5 ± 0.8*
Neutral lipids (%)	75.5	72.7	65.5 ± 5.1
Triglycerides (%)	19.0	12.7	15.7 ± 2.1
Mono-, diglycerides (%)	2.8	2.8	2.5 ± 1
Free fatty acids (%)	8.9	8.7	1.7 ± 0.7 p < 0.001
Free and esterified cholesterol (%)	43.5	47.5	45.1 ± 5.3
Total phospholipids (%)	24.5	26.3	32.5 ± 3.7
Lecithin (%)	1.4	1.7	14.8 ± 3.7 p < 0.001
Cephalin (%)	3.7	4.8	6.7 ± 1.8
Phosphatidyl serine (%)	2.8	3.5	4.3 ± 2.5
Lysolecithin (%)	1.4	1.7	0.6 ± 0.2 p < 0.01
Phosphatidic acid (%)	2.8	3.1	0.9 ± 0.4 p < 0.01
Sphingomyelin (%)	3.5	4.5	4.5 ± 1

*Values are mean ± SD.

are longer than they are wide and have a polar or aromatic part on the molecule. The shape and polar interaction permit the molecules to align in partially ordered arrays described as 'smetic' or layered arrays. This is similar to the arrangement seen in liposomes and is in contrast to nematic arrays, in which the molecules are aligned side by side but not in mandatory layers.^{11 12} Liquid crystals can be formed in vitro in two known ways: (a) molecules when heated may not melt directly to isotropic liquid but may instead pass through an intermediate state of mesophase or liquid crystal (thermotropic liquid crystal); (b) molecules can be partially liquefied by the action of a solvent producing lyotropic liquid crystals. Lyotropic crystals can also undergo additional thermotropic transition.²

A liquid crystalline order of molecules has been described in healthy and pathological biological systems, such as in normal cell organelles, myelin bile, and lipoproteins; and in arteriosclerotic plaques, and lipid storage diseases.¹¹

LIPID LIQUID CRYSTALS AND ARTHRITIS

It is not unusual to find cholesterol crystals and birefringent lipid microspherules in chronically inflamed joints and bursae of patients with rheumatoid arthritis.^{1 13} They are usually associated with increased synovial fluid lipid concentration and probably are a result of marked cell membrane damage and phospholipid release. The three patients in this report and the one described by Weinstein³ differ in that each patient developed acute monoarthritis which was associated with synovial fluid lipid liquid crystals. All these four patients experienced acute onset of monoarticular synovitis associated with florid signs of acute inflammation, synovial fluid leucocytosis, and abundant extracellular and/or intracellular positively birefringent microspherules. Arthritis subsided in all patients within a week after the administration of non-steroidal anti-inflammatory agents. The clinical presentation and course are similar to those seen in patients with acute gout or other solid crystal induced arthritis and therefore introduces the possibility that lipid liquid crystals might induce inflammation as has been proposed for solid crystals. The decrease in numbers of liquid crystal as the attacks subside supports their role as an aetiologic factor producing inflammation.

However, we cannot completely exclude the alternative possibility that these liquid crystals are only a result of acute injury of the cell membrane by an inflammatory process induced by some other as yet unidentified phlogistic agent. Partially digested platelets which are rich in phospholipids have been reported to show multilamellated structures like

those seen by transmission electron microscopy in patients 2 and 3.¹⁴

The double birefringent lipid droplets or lipid liquid crystals observed in the three patients described here are different than those non-birefringent ones observed in joint effusions from patients with joint trauma, pancreatic induced fat necrosis, and aseptic bone necrosis. In these conditions non-birefringent neutral fat droplets can escape from the underlying fatty synovium or bone marrow.^{8 15}

ELECTRON MICROSCOPIC STUDIES

Most of the lipid deposits observed in patients 2 and 3 displayed arrays of concentric layers similar to those described in lipid liquid crystals and cholesteryl ester lipid droplets.^{10 11} Some of these multilamellated inclusions (Fig. 2c) were also similar to those myelin-like figures observed in the tissue of patients with Fabry's disease.¹⁶

Other less numerous homogeneous grey droplet-like inclusions were also seen (Fig. 2b) and were identical to the type described by Schlesinger *et al.* in synovial fluid macrophages of patients with chronic polyarthritis.⁴ The finding of these two types of inclusion bodies is in agreement with the studies of McGookey and Anderson who have described the formation of cholesteryl ester lipid droplets in tissue cultured mouse macrophages.¹⁰ They have shown that cholesteryl esters are initially sequestered in homogeneous neutral lipid droplets. Subsequently, focal membranous cholesterol containing arrays accumulate in the homogeneous deposits. These arrays are first at the margin of the droplet; when cholesterol concentration increases the droplet turns into a multilamellated myelin-like structure, as was seen in the two patients described here.¹⁰ This observation suggests that at least some local intracellular formation of inclusions is possible. The appearance of some droplets and arrays in membrane bounded vacuoles (Figs 2a and 2c) implies that the liquid crystals can also be phagocytised.

LIPID LIQUID CRYSTALS AND INFLAMMATION

At the present time there is no direct evidence that lipid liquid crystals can induce inflammation in joints or elsewhere. Impure preparations of lipids¹⁷ or fatty acids have produced mild inflammation after intra-articular injection. Low density lipoproteins can be shown to be cytotoxic to fibroblasts in tissue cultures.¹⁸ Lipid liquid crystals or liposomes have been used as carriers of synthetic corticosteroids for intra-articular injections but to date no postinjection inflammation has been reported.¹⁹ It seems possible that the ability of these microspherules to induce

inflammation might vary with the nature of the phospholipids contained in the liquid crystals.

In a study of gout negatively birefringent urate spherulites, different from solid crystals and possibly analogous to liquid crystals, have been described in one synovial fluid with acute arthritis as the only form of urate.⁶ We have observed them in another patient in association with typical strongly negatively birefringent monosodium urate crystals. These spherulites can also be observed during synthetic preparation of monosodium urate crystals at a lower temperature.⁶ Uric acid and calcium phosphate crystalline spherulites like those discussed here are part of the normal physiology of kidney and gastrointestinal tract in birds and insects (unpublished data).

As with gout and calcium pyrophosphate deposition disease levels of the soluble materials in the synovial fluid were not useful in the evaluation of our patients. The increases in some lipids in our patients compared with normal pooled synovial fluid will need to be compared further with other inflammatory fluids.

Because the mechanisms of many musculoskeletal manifestations of several types of hyperlipidaemias, such as polyarthralgias, episodic arthritis, and fibrositis, are poorly understood, the possible role of lipid liquid crystals like those reported here deserves to be investigated. Arthralgia and arthritis which is exacerbated by warm weather have been observed in patients with Fabry's disease.²⁰ Synovium or other tissues examined in these patients with Fabry's disease have shown glycolipid inclusions with features of lipid liquid crystals.^{16 20}

Whether the sarcoidosis and cirrhosis seen in patients 1 and 3 could be related in any way to the lipid liquid crystals can be only speculation at this time. The minor trauma in patient 2 of course can be considered as a possible factor in release of lipids into joint fluid.

Confirmation of the possible phlogistic role of the liquid crystals will require in-vitro and in-vivo experiments similar to those previously conducted with the solid crystals of urate, apatite, and calcium pyrophosphate. However, because the ability of these molecules to display a crystalline arrangement is temperature dependent, their synthesis and handling will require more sophisticated techniques than those used in the study of solid crystals in joint fluid.²¹

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