Experimental haemarthrosis produces mild inflammation associated with intracellular Maltese crosses*

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SUMMARY Injection of autologous blood into rabbit joints induces an inflammatory reaction with Maltese cross-like birefringent spherulites. Similar microspherules seen in human joint fluids may be formed by lipids derived from breakdown of erythrocytes and other cells.

Key words: polarised light, synovial fluid, lipid liquid crystals, crystals, electron microscopy, rabbits, arthritis.

Intracellular birefringent liquid microspherules appearing as Maltese crosses have been reported in synovial fluids (SF) of patients with unexplained acute or chronic arthritis.^{1–4} These appeared as multilayered membranous arrays by electron microscopy.³ The origin of the spherulites in such patients was not explained. Since mild inflammation with ultrastructurally similar inclusions has been reported to occur after injections of blood⁵ we proposed that birefringent microspherules might be seen with polarised light. This could suggest membrane lipids of lysed cells as at least one source for the unexplained spherulites.

We have therefore studied experimentally produced haemarthrosis in the rabbit and performed the first sequential light microscopic examinations of their SF.

Subjects and methods

RABBITS

Five mature male albino New Zealand rabbits weighing 3.0–3.5 kg were sedated with ketamine 40 mg/kg and xylazine 5 mg/kg intramuscularly. In each rabbit 0.5 ml of arterial blood was obtained from the animal's own ear and under aseptic conditions

immediately injected without any additives into the right knee. The left knee served as an uninjected control. At two, four, and six days after single injections of blood and one week and two weeks after twice weekly injections animals were autopsied and SF and synovial membranes (SMs) were examined by polarised light, regular light, and electron microscopy.

SYNOVIAL FLUIDS AND MEMBRANES

The SF when present was analysed manually for leucocyte count (with 0.3% saline as a diluent). A wet preparation was immediately examined under polarised light and Wright's stained smears were done for differential leucocyte counts. For lipid stain two or three drops of SF were placed on a slide, mixed with an equal amount of Sudan black B, and the preparation was examined immediately.⁶ The remaining fluid was prepared for transmission electron microscopic study by centrifuging immediately, fixing pellets in half strength Karnovsky's paraformaldehyde-glutaraldehyde fixative, and processing as previously described.^{7–9}

Synovial membrane specimens for light microscopy were fixed in buffered formalin and processed through routine paraffin embedding, sectioned, and examined after staining with haematoxylin and eosin.

Results

SFs of blood injected knees showed leucocyte

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Fig. 1 (A) Maltese cross-like inclusions in a degenerating synovial fluid leucocyte. Wet preparation. Compensated polarised light. (B) Sudan black B positive inclusions in joint fluid leucocyte. Regular light. (C) Other irregular birefringent material in an intact leucocyte. Wet preparation. Compensated polarised light.

counts of $1600\text{--}7100/\text{mm}^3$ ($1.6\text{--}7.1 \times 10^9/\text{l}$), while uninjected control knees had $500\text{--}800$ leucocytes/ mm^3 ($0.5\text{--}0.8 \times 10^9/\text{l}$) (Table 1). Examination of wet smears of SFs from all blood injected joints by compensated polarised light showed intracellular positively birefringent spherulites appearing as Maltese crosses (Fig. 1A) and staining with Sudan black B (Fig. 1B). Other birefringent inclusions were irregular and less clearly Maltese crosses, but probably similar in origin (Fig. 1C). Many of these appeared as Maltese crosses with very careful focusing. Control joints had no birefringent material. Approximately 10–30% of leucocytes from blood injected joints contained Maltese cross-like

spherulites. SFs of joints repeatedly injected with blood had more abundant spherulites than those of once injected joints.

Histology of SMs of blood injected joints showed mild to moderate proliferation of synovial lining cells with infiltration of polymorphonuclear leucocyte (PMN), lymphocyte, and mononuclear cells. At two days PMN infiltration was prominent throughout superficial synovium and at four and six days there were many mononuclear cells and lymphocytes with only a few PMN.

Electron micrographs of SFs and SMs of blood injected joints showed osmiophilic multilayered membranous arrays in PMN, macrophages, and

Fig. 2 Electron micrograph showing membranous whorls in vacuoles of a synovial lining cell after two injections of blood into the joint (rabbit 16).

synovial lining cells (Fig. 2). No similar inclusions were seen in the control joints.

Discussion

Different types of synovial lipid bodies have been reported in a variety of disorders.^{1-3 10-16} Cholesterol crystals have been seen in rheumatoid, osteoarthritic, and other chronic effusions.¹²⁻¹⁶ Extracellular and intracellular non-birefringent lipid globules have been reported in traumatic arthritis^{10 11} and rarely in aseptic necrosis¹⁷ and rheumatoid arthritis.¹⁴

Birefringent lipid microspherules appearing as Maltese crosses have occasionally been observed without comment during synovial fluid analysis.¹⁸ Recently such microspherules have been reported in the synovial fluid of one patient with otherwise unexplained acute monoarthritis,¹ in the synovial fluid and synovial lining cells of a patient with chronic unexplained arthritis,² and in the synovial fluids of three other patients with unexplained acute monoarthritis.³ SFs in all these case reports were described as having erythrocyte (red blood cell (RBC)) counts from 1350/mm³ (1.35×10⁹/l) to grossly bloody fluid. These findings suggested to us that RBC should be considered as one likely source of Maltese cross-like spherulites. Our SFs of blood injected rabbit joints showed birefringent spherulites similar to those of human cases,¹⁻³ thus supporting this possibility.

Electron microscopic findings of SFs and SMs in the blood injected rabbit joints showed whorled membranous arrays similar to those myelin-like figures described in SF in one human case report³ and in liposome injected rabbit joints.¹⁹ Roy and Ghadially had previously shown similar myelinoid (membranous) bodies in the synovial cells of rabbit joints after intra-articular injection of autologous blood.⁵ It was suggested that these membranous structures might represent hydrated lipidic residues derived from the cholesterol rich erythrocyte membrane.²⁰ Roy and Ghadially, however, only examined SMs, not SFs, in blood injected rabbit joints and had not carried out studies with polarised light.

Our studies and the literature noted thus suggest that erythrocyte membranes can be the source of Maltese cross-like birefringent inclusions in synovial fluid cells. Whether these are an important cause of the inflammation seen after blood injection or human haemarthrosis remains to be determined. Other elements in the blood could also contribute to the inflammatory reaction. Most patients with Maltese crosses described in joint fluids have not recalled joint trauma as the source of the erythro-

Table 1 Synovial fluid findings after injection of blood into the right knee with the left as an uninjected control

Rabbit No		Right knee					Left knee						
		WBC/mm ³ *	PMN (%)	Lymph (%)	Mono (%)	SLC (%)	Intracellular MX†	WBC/mm ³ *	PMN (%)	Lymph (%)	Mono (%)	SLC (%)	Intracellular MX†
19	Two days after single injection of blood	7100	24	32	28	16	3+		ND				—
20	Four days after single injection of blood	2025	8	76	4	12	+		ND				—
21	Six days after single injection of blood	1600	4	24	8	64	+	750	4	16	6	74	—
16	Two blood injections with three days' interval. Rabbit killed three days after the last injection	2650	ND				3+	500	ND				—
26	Four blood injections with three days' interval. Rabbit killed three days after the last injection	4250	13	34	10	43	3+	550	4	12		84	—

Abbreviations: ND=not done owing to insufficient amount of synovial fluid; WBC=leucocyte count; PMN=polymorphonuclear leucocytes; Lymph=lymphocytes; Mono=monocytes; SLC=synovial lining cells; MX=Maltese crosses.

*SI conversion: WBC/mm³ × 10⁶=WBC/l.

†++=occasional Maltese cross; 3+=cells with Maltese crosses in every high power field; 2+=intermediate.

cytes. Some RBC are common in many joint fluids;²¹ RBC may arise from subtle mechanical trauma or can escape from congested vessels in inflammatory arthritis. Leucocyte membranes might also be a source of membrane lipids in effusions without RBC. Small numbers of Maltese cross-like spherulites certainly may occur secondarily and be coincidental findings in a variety of known joint diseases. We frequently see rare birefringent spherulites in chronic inflammatory arthritis. Large numbers of such spherulites, however, from RBC or other origins may very well contribute to the joint inflammation as was seen in these rabbits.

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