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 microspheres 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 membranes by electron microscopy. 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 microspheres 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 zylazine 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
counts of 1600–7100/mm³ (1.6–7.1×10⁹/l), while uninjected control knees had 500–800 leucocytes/mm³ (0.5–0.8×10⁹/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
Table 1  Synovial fluid findings after injection of blood into the right knee with the left as an uninjected control

<table>
<thead>
<tr>
<th>Rabbit No</th>
<th>Right knee</th>
<th>Left knee</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WBC/mm³*</td>
<td>PMN (%)</td>
</tr>
<tr>
<td>19</td>
<td>7100</td>
<td>24</td>
</tr>
<tr>
<td>20</td>
<td>2025</td>
<td>8</td>
</tr>
<tr>
<td>21</td>
<td>1600</td>
<td>4</td>
</tr>
<tr>
<td>16</td>
<td>Two blood injections with three days’ interval. Rabbit killed three days after the last injection</td>
<td>2650</td>
</tr>
<tr>
<td>26</td>
<td>Four blood injections with three days’ interval. Rabbit killed three days after the last injection</td>
<td>4250</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td>4</td>
</tr>
</tbody>
</table>

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 crossettes.

*SI conversion: WBC/mm³x10⁹=WBC/l.
†1+ = occasional Maltese cross; 3+ = cells with Maltese crossettes in every high power field; 2+ = intermediate.

Discussion

Experimentally produced haemarthrosis...
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.

References
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