Detection by electron microscope of rod-shaped organisms in synovial membrane from a patient with the arthritis of Whipple's disease

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Hawkins, C. F., Farr, M., Morris, C. J., Hoare, A. M., and Williamson, N. (1976). *Annals of the Rheumatic Diseases*, 35, 502-509. **Detection by electron microscope of rod-shaped organisms in synovial membrane from a patient with the arthritis of Whipple's disease.** Rod-shaped organisms identical to those present in the jejunal mucosa have been found in the synovial membrane of a patient with Whipple's disease. These probably caused inflammatory changes which were reflected in an increase of the cellular content and high enzyme levels (acid phosphatase and 5-nucleotidase) of the synovial fluid. Tetracycline was effective in controlling the bowel lesion but only had a temporary effect upon the arthritis. Erythromycin controlled both the bowel lesion and the arthritis.

The occurrence of arthritis in Whipple's disease is well documented (Kelly and Weisiger, 1963; Delcambre and others, 1974), Whipple himself having described the joint symptoms in 1907. Arthritis often precedes gastrointestinal symptoms, sometimes by many years. The most commonly affected joints are the knees, ankles, and wrists, though occasionally the spine, proximal interphalangeal joints, metacarpophalangeal joints, and elbows are affected.

Histological studies of the synovial membrane have been carried out by Caughey and Bywaters (1963) and Delcambre and others (1974), but no electron microscope studies (EM) have been reported. In 1961 Yardley and Hendrix and Cheers and Ashworth described a characteristic rod-shaped organism in the intestinal mucosa and since then numerous EM studies of the jejunum (reviewed by Maizel, Ruffin, and Dobbins, 1970) have confirmed this.

We describe similar organisms in the synovium of a patient with the arthritis of Whipple's disease together with a study of the cytology, enzymology, and acute-phase proteins of the synovial fluid.

Case report

A 59-year-old man, on admission in 1969, gave a 16-year history of migratory arthritis of the knees, ankles, wrists, elbows, and shoulders, and occasional arthralgia in the fingers. 6 months previously he had developed diarrhoea, weight loss, lack of energy, and colicky abdominal discomfort; hitherto his bowels had been normal. The diarrhoea at first cleared up spontaneously but a severe remission occurred 6 weeks before admission. Stools were pale, offensive, and difficult to flush away. He was pale and thin with a distended abdomen. The left knee was painful and swollen, and he suffered from arthralgia in other joints.

He had a hypochromic normocytic anaemia and leucocytosis, Hb 10 g/dl, white cell count $24 \times 10^9/l$ ($24,000/mm^3$) with neutrophilia, polymorphs $22.2 \times 10^9/l$ ($22,200/mm^3$). Serum folate was low $1.3 \mu g/l$, with a normal vitamin B₁₂ $450 ng/l$. There was hypoalbuminaemia, albumin 28 g/l, and a raised serum globulin 39 g/l, with increased α -globulin shown by cellulose acetate electrophoresis. Serum iron was low $1 \mu mol/l$ ($6 \mu g/100 ml$), with a normal iron binding capacity of $52 \mu mol/l$ ($291 \mu g/100 ml$). Malabsorption was proved by xylose excretion of 20% of a 5 g dose in 5 hours and faecal fat excretion of 10 g/day. Jejunal biopsy confirmed Whipple's disease (Figs. 1 and 2). X-rays of the knees showed slight osteoarthrosis only.

Tetracycline 250 mg four times daily was given. Abdominal pain and diarrhoea disappeared and his weight increased by 6 kg. The pain and swelling of the knees at first disappeared but returned when tetracycline was reduced by half. The left knee was aspirated and injected with steroids. 8 months after discharge he was readmitted for assessment. He had no gastrointestinal symptoms but his left knee was painful, stiff, and swollen, and he suffered from occasional arthralgia in other joints.

His blood count was normal, as were albumin 45 g/l and globulin 32 g/l. Erythrocyte sedimentation rate (ESR) was 22 mm/1 h. Jejunal biopsy showed improvement and the rod-shaped organisms had disappeared. The left knee was again aspirated and injected with corticosteroids. Tetracycline was continued in the original dose, 250 mg four times daily. 3 months later, because the knees and wrists were painful, prednisone 5 mg daily was added to the tetracycline and the arthritis disappeared. During the next 9 months prednisone was reduced to 2 mg daily without relapse. He was admitted again in 1972 for assessment. He remained symptomless for 4 months providing he took 2 mg prednisone daily. As his ESR was normal, he stopped tetracycline and immediately his joint symptoms returned, especially in the knees.

ESR had risen to 33 mm/1 h, haemoglobin was 14.3 g/dl, and the white cell count $13 \times 10^9/l$ ($13\,000/mm^3$), with neutrophilia $11.5 \times 10^9/l$ ($11\,500/mm^3$). Jejunal biopsy was virtually normal and there was no evidence of malabsorption. Prednisone was continued and tetracycline restarted, 250 mg four times daily. During the next 2 years there were exacerbations in the knees which required aspiration and injection with steroids but no relapse of the intestinal lesion. He was reassessed in 1974. Examination showed mild inflammation of the left knee. He had a hypochromic normocytic anaemia (haemoglobin 12.8 g/dl with neutrophilia and leucocytosis ($17 \times 10^9/l$; $17\,000/mm^3$). ESR was 35 mm/1 h. The Rose-Waaler test was negative in the blood. Synovial needle biopsy was carried out. He continued on prednisone 2 mg daily and tetracycline.

In 1975 the arthritis worsened. Three flare-ups of the left knee had occurred, with rupture of the synovial membrane once, releasing fluid into the calf. The arthralgia of the right elbow, neck, feet, and shoulders had also relapsed. Salicylates were added to his treatment. The left knee was aspirated and injected with steroids with improvement. Later the left knee became severely inflamed. Aspiration and synovial biopsy were performed and steroids again injected. An attempt to change the antibiotic was unsuccessful. He developed severe toxic reactions to co-trimoxazole and ampicillin, a course of each being given on two separate occasions, and tetracycline was restarted. He was well for 4 months and then the left knee again flared up. Steroids were not injected into the joint but erythromycin was given orally. He began to improve within a few days and after a month's treatment with erythromycin 250 mg four times daily, a further synovial biopsy was carried out. The antibiotic was continued.

Methods

Repeated jejunal biopsies were carried out using a Crosbie capsule. Sections were processed and stained with haematoxylin and eosin (H and E), periodic acid

Schiff (PAS), toluidine blue, alcian blue, and Gram stains. Biopsies of the synovial membrane were obtained from the left knee on four occasions using the synovial biopsy needle of Williamson and Holt (1966). Specimens were transferred immediately from the biopsy needle into the fixatives. Some were processed for light microscopy and stained in the same way as sections of jejunal biopsies. Small fragments were prepared for electron microscopy by fixing in 2% glutaraldehyde in phosphate buffer containing sucrose, followed by fixation in osmium tetroxide. Fixed tissue was dehydrated in graded alcohols and embedded in Epon. Ultrathin sections were double stained with uranyl acetate and lead citrate and examined, with a Siemens Elmiskop 102 electron microscope at an accelerating voltage of 80 kV.

The left knee was aspirated seven times during the past 3 years, and synovial fluid analysed. Total white cell count, total red cell count, and differential white cell count were determined. 5-Nucleotidase activity of the supernatant fraction was estimated by the method of Persijn and others (1968), and acid phosphatase on whole and supernatant fractions of the fluid. Immunoglobulins

FIG. 1 Jejunal biopsy (15 Oct 1969). Villi are shorter than normal and some are broader and clubbed. Periodic acid-Schiff $\times 27$

FIG. 2 Jejunal biopsy. Tip of a broad villus showing macrophages containing Schiff-positive material. PAS $\times 115$

G, M, A, and D were measured by the method of Fahey and McKelvey (1965). C-reactive protein was estimated by Crockson's method (1968) and haptoglobin by the technique of Ratcliff and Hardwicke (1964). Rose-Waaler titres and glucose were estimated on the supernatant fractions. Smears of synovial fluid were air dried, fixed in 96% ethanol, and stained with Jenner Giesma, Gram, methyl green-pyronin, toluidine blue, PAS, van Gieson, Oil Red O, and H and E.

Results

GASTROINTESTINAL FINDINGS

When the condition was first diagnosed (1969) a

section of jejunal mucosa showed the villi to be shorter than normal with many being broader and clubbed (Fig. 1). The lamina propria was slightly oedematous and occasional collections of polymorphs were seen. Large numbers of macrophages contained material which stained positively with PAS (Fig. 2) showing faint metachromasy with toluidine blue and which stained negatively with alcian blue. On electron microscopy typical rod shaped organisms were present (Fig. 3). The most recent biopsy of the jejunum showed a normal appearance both on light and electron microscopy (Fig. 4).

FIG. 3 *Jejunal biopsy. Electron micrograph showing transverse and longitudinal sections of Whipple bacteria with well preserved trilaminar cell walls. The majority of the bacteria are within heterophagic vacuoles and some are undergoing lysosomal lysis.*

FIG. 4 *Jejunal biopsy after tetracycline. Electron micrograph showing that the structure of the jejunal epithelial cells is within normal limits. There is no separation of intercellular membranes due to oedema, and microvilli are numerous and of normal length. The lamina propria was free of organisms.*

FIG. 5 Synovial needle biopsy (21 July 75). This villus shows considerable synovial hypertrophy with infiltration of round cells, mainly lymphocytes with some plasma cells. There are areas of fibrinoid necrosis but no lymphoid follicles. $\times 65$

SYNOVIAL MEMBRANE HISTOLOGY

Needle biopsies of the synovial membrane on three occasions showed a villus with considerable synovial hypertrophy with round cell infiltration due mainly to lymphocytes and occasional plasma cells. There were considerable lymphocytic infiltrations around the blood vessels and scattered areas of fibrinoid necrosis (Fig. 5).

Studies through the electron microscope were performed on all three biopsies. Three blocks were made of each and 50 sections examined. Much of the embedded material consisted of fibrillar collagen of normal structure. However, within this collagen matrix there were collections of macrophages, the majority of which contained bacterial material, consisting largely of fragments of cell walls within heterophagic vacuoles.

In some macrophages transverse sections of rod-shaped organisms, identical to those present in the jejunal biopsy, are seen with trilaminar cell walls and a central core containing a pale granular material. The diameters of the bacteria varied between 0.15 and 0.3 μm . (Figs. 6, 7, and 8).

FIG. 6 Synovial needle biopsy. Electron micrograph of a cell (? macrophage) containing many heterophagic vacuoles some with recognizable bacteria (arrows). The majority, however, contain cell wall fragments and electron dense granular material

FIG. 7 Synovial needle biopsy. Electron micrograph of macrophages containing debris-filled vacuoles and poorly preserved bacteria. Some bacteria show the laminated cell wall seen in well preserved Whipple's bacteria (arrowed)

These rod-shaped organisms were present in every section of the three synovial biopsies taken on separate occasions while the patient was receiving tetracycline 250 mg four times daily. After taking erythromycin 250 mg four times daily for one month, the organisms disappeared from all the sections (Fig. 9) as had happened in the jejunal biopsy after tetracycline. Similar EM studies have been done on 10 synovial biopsies from patients with other forms of polyarthritis, including those of gastrointestinal origin, but nothing resembling these appearances has been found.

SYNOVIAL FLUID ANALYSIS

Synovial fluid findings during the past 3 years are given in the Table. Changes in the total and differential white cell count, 5-nucleotidase, acid phosphatase (whole fraction), and acute-phase proteins indicated an inflammatory arthropathy. The Rose-Waaler titre was consistently negative in the synovial fluid. The components of the synovial fluid have been compared with the activity of the condition judged by the ESR during the past 3 years. Synovial white cell counts and differential cell count, together with synovial glucose and enzymes 5-nucleotidase and acid phosphatase, followed very closely changes in

the ESR during this time. Some of these components are shown in Fig. 10.

Table Synovial fluid findings during the past 3 years

Components	Mean	Range
Total white cell count 10 ⁹ /l (per mm ³)	20.89 (20 890)	1.6-36 (1600-36 000)
% polymorph count	86.3	68-95
Absolute polymorph count 10 ⁹ /l (per mm ³)	18.84 (18 840)	1.36-38.52 (1360-38 520)
% lymphocyte count	5.4	0-20
% monocyte count	8.3	4-17
Acid phosphatase		
Whole (KA units)	5.1	2.1-7.1
Supernatant (KA units)	1.6	1.2-1.9
5-nucleotidase (IU/l)	50.3	3-107
Glucose mmol/l (mg/100 ml)	5.2 (94)	4.9-5.6 (88-100)
IgG (g/l)	7.97	3.2-18.0
IgM (g/l)	0.4	0.2-0.7
IgA (g/l)	2.75	1.92-4.20
IgD (g/l)	0	0
C-reactive protein (g/l)	0.68	0.37-0.99
Haptoglobin (g/l)	0.85	0.82-0.88

FIG. 8 *Synovial needle biopsy (15 Oct 1975) Electron micrograph of cell cytoplasm containing poorly preserved but complete bacteria. The inner layers of the trilaminar cell wall are not well seen, but the prominent intermediate and outer cell wall laminations are clearly visible*

Synovial fluid smears stained by PAS reagent showed much Schiff-positive material in the cytoplasm of both polymorphonuclear and synovial cells similar to that observed in other inflammatory arthropathies (Williamson and Holt, 1968). Gram's stain showed occasional small Gram-positive inclusions in the cytoplasm of some synovial cells, but these were not easily recognized as bacterial. With van Gieson's stain small fragments of cartilage were seen suggesting mild osteoarthrotic change. This was in keeping with x-ray changes.

Attempts were made to culture synovial membrane and fluid according to the technique of Clancy and others (1975) but without success. These workers claimed to have isolated the aetiological agent from a lymph node taken from a patient with Whipple's disease.

Discussion

Histological studies of the synovial membrane showed a nonspecific arthropathy as described by Caughey and Bywaters (1963), though Delcambre and others (1974) had found inflammatory changes similar to those present in the synovial membrane in septic arthritis. Electron microscopy of the synovial membrane showed rod-shaped organisms identical to those present in our patient's jejunal

mucosa and as previously described in jejunal biopsies from patients with Whipple's disease (Roberts and others, 1970; Lamberty and others, 1974; Maizel and others, 1970; and Pages, Baldet, and Marty, 1971). The most typical feature was the presence of a trilaminar cell wall showing a very prominent clear space. The presence of bacterial cells within intracellular membrane-bound heterophagic vacuoles indicated a dynamic process of bacterial degradation as noted previously (Lamberty and others, 1974). Also the bacterial cell walls appeared to be partially resistant to lysosomal degradation and many vacuoles were completely filled with these structures. The last stage of breakdown resulted in vacuoles being filled with a granular material.

Synovial fluid findings confirmed an inflammatory arthropathy. The continual high white cell count was good evidence for this and the high polymorph count indicated acute activity. However, Caughey and Bywaters (1963) found that the most striking feature was a high percentage of mononuclear cells with a smaller number of polymorphs; this suggested a more chronic type of inflammation which was also reported by Delcambre and others (1974). Raised enzyme levels of 5-nucleotidase and acid phosphatase also indicated an inflammatory process (Farr and

FIG. 9 Synovial needle biopsy after erythromycin. Electron micrograph showing that the rod-shaped organisms had disappeared. Vacuoles contain a pale granular material only

FIG. 10 Serial study of components of synovial fluid compared with the ESR (during the past 3 years)

others, 1973; Caygill and Pitkeathly, 1966). Levels of acute phase proteins in this patient's fluid were again consistent with levels which we have found in inflammatory arthropathies. Synovial fluid immunoglobulins were not raised.

Tetracycline cured the bowel lesion and this was confirmed by histological and electron microscope studies. However, it only had a temporary effect on the joints. Rod-shaped organisms were present in three biopsies taken at different times while tetracycline was given. However, erythromycin not only controlled the bowel lesion but also cured the arthropathy. The arthralgia disappeared and the swollen, painful knees returned to normal. Further EM studies of the synovial membrane (Fig. 9) showed that the rod-shaped organisms had disappeared.

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