Isolation of *Mycoplasma pneumoniae* from the synovial fluid of a hypogammaglobulinaemic patient in a survey of patients with inflammatory polyarthritis

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SUMMARY Mycoplasmas were not isolated from synovial fluid or membrane specimens taken from 41 patients suffering from inflammatory polyarthritis. However, *Mycoplasma pneumoniae* was isolated in two laboratories and on two occasions from the knee joint of a hypogammaglobulinaemic patient with a chronic polyarthritis.

Mycoplasmas cause arthritis in a number of mammalian and avian species and for this reason there have been many attempts to isolate them from the joints of persons suffering from rheumatoid arthritis. Almost without exception these attempts have failed or, in those few instances where mycoplasmas have been isolated, the findings have not been supported by other workers (Stewart *et al.*, 1974; Taylor-Robinson and Taylor, 1976). During a microbiological study of patients with inflammatory polyarthritis, in which specimens from some patients were examined in two separate laboratories, mycoplasma organisms were isolated only from a joint of one patient who was also hypogammaglobulinaemic. The mycoplasma was subsequently identified as *Mycoplasma pneumoniae* and we present details of the microbiological aspects of the study and of this particular patient.

Materials and methods

PATIENTS AND SPECIMENS

Synovial fluid was obtained from sequential patients with rheumatoid arthritis and other forms of inflammatory joint disease attending a routine joint aspiration clinic at the Rheumatism Research Unit, Taplow. These specimens were taken with full sterile precautions and immediately divided, each portion of about 0.5-1.0 ml being placed in a separate bijoux bottle containing 4 ml of

mycoplasma medium. The bottles were placed in an atmosphere of 5% CO₂-95% N₂ immediately, one being transported to the Clinical Research Centre and the other to the Laboratory Animals Centre. Other synovial fluid specimens were taken at routine joint aspirations carried out with similar precautions at Northwick Park Hospital and were studied only in one laboratory. In addition, synovial membrane from patients undergoing surgical synovectomy at Taplow was divided and placed in medium, as described, before transportation.

In all, 32 synovial fluids and 12 synovial membrane specimens from 42 patients were studied. A diagnosis of rheumatoid arthritis was confirmed in 26 of these, the duration of symptoms from onset of disease varying from 3 weeks to 27 years. Of the other patients, 3 were suffering from osteoarthritis, 3 had an acute Reiter's syndrome, 2 had other inflammatory joint diseases, while in 8 no firm diagnosis was established. All specimens were coded and the clinical details were revealed only after the microbiological results became available.

MEDIUM

Mycoplasma medium has been described in detail before (Manchee and Taylor-Robinson, 1968). In brief, it consisted of 70% PPLO broth (Difco), 10% of a 25% solution of yeast extract (DCL), 20% horse serum (Wellcome), penicillin G (1000 U/ml), thallium acetate (0.05%), and phenol red (0.002%). Liquid medium containing 0.1% glucose was adjusted to pH 7.8 and medium containing 0.1% arginine to pH 7.0. Liquid medium was solidified by adding 1.0% Noble agar.

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ISOLATION TECHNIQUE AND IDENTIFICATION

After transport to the respective laboratories, a 0.1 ml volume of the liquid medium containing synovial fluid was placed on agar medium and further ten-fold and 100-fold dilutions of the liquid medium were made in glucose- and arginine-containing media. The caps of the bijoux bottles were kept loose and these bottles and the agar medium were incubated at 37°C in an atmosphere of 5% CO₂-95% N₂. A second specimen from the hypogammaglobulinaemic patient was tested under aerobic conditions only. The liquid media were subcultured to liquid and agar media after 7 and 14 days, or when a colour change was observed. *M. pneumoniae* was suspected when colonies on agar were found to haemadsorb (Manchee and Taylor-Robinson, 1968) and it was identified serologically by the agar-disc growth inhibition technique (Clyde, 1964).

Results

MYCOPLASMA ISOLATION

Specimens from the knee joints of 42 patients were collected, of which 25 were examined in each of the two laboratories. A diagnosis of rheumatoid arthritis had been confirmed in 20 of these 25 patients and in 26 of all patients studied. However, mycoplasmas were not isolated in either laboratory from any of the specimens except in the case discussed below. In this instance the presence of mycoplasma organisms was first detected by a decrease in the pH of the liquid media. This occurred in both laboratories and in one of these the organisms were also recovered from a second specimen of synovial fluid taken 4 weeks after the first. All these isolates were identified as *M. pneumoniae*.

CASE REPORT

At the age of 20 years, a white male now aged 55 started to have recurrent attacks of bronchitis, pleurisy, and herpes zoster. In 1956, a diagnosis of hypogammaglobulinaemia was established, and he was treated with weekly intramuscular immunoglobulin injections. These were continued until 1968, when they started producing attacks of light-headedness and difficulty in breathing, and despite attempts to desensitise him the patient was unable to continue the injections. A reduction in the number of attacks of bronchitis was achieved until 1965, when they became more frequent. However, after discontinuing immunoglobulin therapy in 1968, fewer bronchitic episodes were noted, although the patient was now chronically breathless.

In May 1969 the patient noticed the onset of an inflammatory polyarthritis, which spread to affect wrists, ankles, knees, and shoulders, and to a lesser

extent the small joints of the hands and feet and one hip. He was first admitted to the Rheumatism Research Unit, Taplow in June 1970. Examination showed small effusions in both knees, soft tissue swelling around the extensor tendon sheaths of both wrists, and tender metatarsophalangeal joints. In addition, there were bilateral olecranon nodules. The patient looked chronically ill, was breathless at rest, and had nail clubbing, coarse crepitant rales widespread in the chest, moderate hepatomegaly, and widespread lymphadenopathy. Extensive investigation, including biopsy of the synovial membrane of the knee, and culture of blood, sputum, urine, and stool failed to show infection. Tests for rheumatoid factor were repeatedly negative and trace amounts only of IgG, IgM, IgA, and IgD were found in the serum. Percutaneous synovial biopsy of the knee showed no more than mild lining cell hyperplasia. He was treated with anti-inflammatory drugs only.

In February 1972 there was increased swelling of the left ankle, necessitating the use of crutches, and tense swellings around the left ankle and subtalar joints. Ankle joint aspirate was sterile on aerobic and anaerobic bacteriological culture. The following week the patient complained of a cough with expectoration and the left knee had become tender and swollen. Routine bacteriological culture of aspirates of knee and ankles were sterile, as were routine blood cultures. However, the mycoplasma was isolated from the knee synovial fluid which was obviously purulent, containing $40 \times 10^7/l$ erythrocytes and $1 \times 10^7/l$ leucocytes, of which 81% were polymorphonuclear cells and 19% lymphocytes. The joint swellings subsided somewhat on conservative treatment. A month later, when the mycoplasma was again recovered from the same knee, tetracycline therapy was started. Over the next few months the patient continued on antibacterial therapy, either tetracycline or co-trimoxazole (sulphamethoxazole and trimethoprim; Septrin), but there was no major improvement in the polyarthritis.

Discussion

Examination of specimens in separate laboratories was done to try to exclude laboratory contamination as a reason for any isolation that might be made. The failure to isolate mycoplasmas from all but one patient is consistent with the negative findings of most other workers. In view of the isolation of *M. pneumoniae* from synovial fluid in two laboratories, its subsequent recovery from a second clinical specimen, and the fact that it is not carried as a commensal in the throat, there seems no doubt that it was present in the knee joint of the patient.

It is likely that the patient's respiratory infection was caused by *M. pneumoniae* and that the organisms easily gained access to the knee joint as a result of the hypogammaglobulinaemic state. Lack of antibody in the synovial fluid may also have been an important factor in successful isolation of the organisms. As a corollary, we suggest that the possible existence of antibody in the joints of immunologically normal patients may prevent isolation from this site.

Whether or not the mycoplasma was directly responsible for the exacerbation of arthritis is debatable. However, arthralgia and polyarthritis have been found after *M. pneumoniae* respiratory disease (Lambert, 1968; Weinstein and Hall, 1974), and the report by Hernandez *et al.* (1977) is a striking confirmation of this. Previous isolation attempts and serological investigations have not suggested that *M. pneumoniae* is a cause of rheumatoid arthritis (Chanock *et al.*, 1967). However, in view of the reports of *M. pneumoniae*-associated arthritis and the known ability of this mycoplasma to produce a range of sequelae, some autoimmune-mediated (Hayflick, 1972; Murray *et al.*, 1975), we still feel (Taylor-Robinson and Taylor, 1976) that this mycoplasma should be further investigated as a cause of rheumatic disease, particularly seronegative disease. The most direct approach would no doubt be to seek *M. pneumoniae* antigen in the synovial cells of such patients by an immunofluorescence technique.

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