Immunological reactivity to *Mycoplasma fermentans* in patients with rheumatoid arthritis

Preliminary communication

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Any speculation about the underlying causes of rheumatoid arthritis must take into account the random distribution of the disease which suggests an infectious rather than a genetically determined pattern. In Still's disease, for example, the presenting symptoms strongly resemble an acute bacterial infection.

There is conclusive evidence for an immune response in the synovial membrane of rheumatoid joints. Deposits of gamma globulin, for example, may be demonstrated, as may plasma cells producing gamma globulins and rheumatoid factors (Smiley, Sachs, and Ziff, 1968). Complement becomes fixed in the synovium and the complement level in the synovial fluid is decreased (Lundh, Hedberg, and Laurell, 1970). This may be interpreted as an immune response in the synovial tissue itself, that is, antigen (possibly a micro-organism) located in the joint, stimulating immunologically competent cells in the synovial membranes. In support of this may be cited the histology of the rheumatoid synovial membrane which shows the cellular infiltration to be characteristic of a cell-mediated hypersensitivity reaction.

The isolation of mycoplasmas from the synovial effusions of patients with rheumatoid disease has previously been reported (Williams, 1968), but antibody levels to these organisms, as detected by migration inhibition (MI) and indirect haemagglutination (IHA), do not clearly discriminate rheumatoid patients from controls (Williams, 1967).

Since antiglobulin (rheumatoid factor) titres remain fairly constant over considerable periods of time it is possible to postulate that in rheumatoid joints the antigen is persistent. These antibody levels, however, are found to diminish in response to treatment, for example, with gold or the anti-malarials. Both these substances have been shown

to inhibit the growth of mycoplasmas (Williams, 1971).

In view of the isolation of *M. fermentans* from rheumatoid joints, the lowering of antiglobulin titres by agents known to inhibit the growth of these organisms, and the histopathology of the diseased synovium, we decided to investigate leucocyte migration inhibition with mycoplasma antigens in rheumatoid and control patients, since we interpret this test as being a measure of cell-mediated hypersensitivity.

It seemed likely that such measurements of cell-mediated hypersensitivity in the diseased patients might more accurately reflect their immunological status than measurements of humoral antibody levels. Lymphocyte transformation with denatured IgG (Kačaki, Bullock, and Vaughan, 1969) does not occur and skin tests with aggregated IgG do not give a response with a classical delayed time course (Chamberlain, Shapland, and Roitt, 1970).

Leucocyte migration inhibition does reflect cell-mediated hypersensitivity to bacterial antigens (Bendixen and Søborg, 1969) and also perhaps in other autoimmune diseases (Brostoff, 1970); it seemed relevant therefore to use this test to investigate cell-mediated hypersensitivity to *M. fermentans* in control subjects and patients with rheumatoid arthritis.

Patients, material, and methods

PATIENTS

Seventeen patients attending a routine rheumatology clinic at the Middlesex Hospital with definite or classical rheumatoid arthritis (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959) were studied. Of these, twelve were women. Their ages ranged from 43 to 78 years (mean 59) and the duration of the rheumatoid arthritis was 4 to 28 years (mean 11). A record was made of the patient's current drug therapy, functional state (Steinbrocker, Traeger, and

Batterman, 1949), presence of subcutaneous nodules, and periarticular radiological erosions. Erythrocyte sedimentation rate (Westergren), sheep cell agglutination, and latex tests for rheumatoid factor were performed on all patients.

Normal healthy adults, free from symptoms of rheumatoid arthritis, were selected as controls from members of the staff in the Departments of Rheumatology Research and Immunology at the Middlesex Hospital Medical School. The male:female ratio was 1:1 and the age range 25 to 37 years.

MATERIAL AND METHODS

Preparation of antigens

Mycoplasma antigens were prepared by the methods already described (Williams, Brostoff, and Roitt, 1970). Briefly, the organisms were grown in standard medium supplemented with 20 per cent. serum and 2.5 per cent. yeast extract. Cells were harvested from 36-hr cultures, washed, and broken by osmotic lysis by incubating in 2M glycerol before adding to deionized water. Membranes were collected from broken cells, washed, and sonicated three times before re-suspending to give a concentration of approximately 10 mg./ml. of protein.

Leucocyte migration inhibition

The method of Sjøborg and Bendixen (1969) was followed: 25 ml. heparinized venous blood was allowed to sediment at 37°C. in sterile universal containers until the supernatant was clear of red blood cells. This sediment was then removed and centrifuged at 125 x G. for 5 min. The cell pellet obtained was washed three times in Eagle's tissue culture medium (Burroughs Wellcome). The washed leucocytes were then re-suspended in 9 volumes of medium containing 10 per cent. foetal calf serum, aspirated into capillary tubes which were then sealed and spun at 1,000 r.p.m. for 5 min. The capillary was broken at the fluid-cell interface and the capillary containing the cells was placed in a migration chamber with the antigen. The chamber was sealed with a silicone-greased cover slip. Experiments were performed in triplicate.

The area covered by the migrating cells was measured at 17 hrs. Inhibition was considered to be significant when the area of migration in the presence of antigen was less than 80 per cent. of that seen in the control chambers lacking antigen.

Results

In this study, the leucocyte migration inhibition test in the presence of *Mycoplasma fermentans* was positive in ten of the seventeen rheumatoid patients examined (Figure). When those receiving sodium aurothiomalate (Myocrisin) as part of their therapy are excluded, nine of the twelve remaining patients (75 per cent.) gave positive results in this test. Of the five rheumatoid patients receiving myocrisin, only one was positive in this test and four had migration inhibition test results which were comparable with the normal group.

FIGURE Migration of leucocytes from rheumatoid patients and healthy persons in the presence of *Mycoplasma fermentans* antigen.

There was no correlation of migration inhibition with other therapy, sex, age duration of disease, functional grade, erythrocyte sedimentation rate, sheep cell agglutination, and latex titres, or the presence of subcutaneous nodules or radiological periarticular erosions (Table, opposite).

Discussion

As the normal controls were younger than the rheumatoid patients our results must be interpreted with caution. However, similar control results have been obtained with older osteoarthritic patients (Williams and others, 1970).

Although there is an overlap of the rheumatoid with the control group in the leucocyte migration inhibition test when the groups are taken as a whole, the separation of the groups becomes much sharper when those rheumatoid patients who were on gold therapy are excluded.

In four out of five of these patients on myocrisin results of this test were in the normal range. This information, though needing confirmation and amplification, is in accord with the known beneficial effect of gold on the clinical course of rheumatoid disease (Empire Rheumatism Council Sub-committee, 1961), the effect of gold in inhibiting growth of mycoplasmas *in vitro*, and its effect in lowering titres of rheumatoid factor.

The leucocyte migration inhibition test has shown (Williams and others, 1970) that rheumatoid arthritic patients may be clearly differentiated from those with osteoarthritis and from normal healthy controls. It is becoming clear that this distinction is due to a state of cell-mediated (delayed-type) hypersensitivity to antigens of *Mycoplasma fermentans*.

The results reported above confirm an earlier observation (Williams and others, 1970) and are of particular interest in that patients undergoing a

Table *Clinical particulars of seventeen patients*

Patient no.	Sex	Age (yrs)	Duration of arthritis (yrs)	Functional grade	Investigations					Therapy*					Migration inhibition (per cent.)
					ESR	SCAT	Latex	Nodules	Erosions	Gold	Phenylbutazone	Indomethacin	Fenamates	Salicylates	
1	F	53	20	III	14	1:64	1:80	+	+				+	+	75
2	F	43	5	I	34	1:512	1:320	+	—		+				49
3	F	46	4	I	18	—ve	—ve	—	—	+					86
4	F	53	23	II	10	1:256	1:160	—	+	+			+		102
5	F	67	4	III	66	1:64	1:80	—	—						92
6	F	44	5	I	34	1:32	1:80	—	+					+	74
7	F	60	15	I	34	1:64	1:160	—	+	+				+	91
8	F	78	10	III	55	—ve	1:80	—	+					+	73
9	F	72	12	III	51	—ve	—ve	—	+					+	71
10	M	66	28	II	33	1:128	1:1280	—	+					+	53
11	M	62	8	III	56	1:32	+ve	—	+	+	+				109
12	F	46	15	II	12	1:64	1:80	—	+					+	92
13	F	53	7	II	23	—ve	—ve	+	+			+		+	82
14	F	74	7	IV	31	—ve	—ve	—	+	+			+		66
15	M	61	5	I	54	—ve	—ve	—	+		+				52
16	M	62	8	III	40	+ve	+ve	—	+			+			80
17	M	62	7	I	36	+ve	+ve	+	+		+			+	68

*No patient on steroids or antimalarials.

recognized course of myocrisin therapy were included in the experimental group. While the total number of patients investigated in this study is small, it nevertheless seems to us most encouraging that treatment with gold, a substance which strongly inhibits the growth of *Mycoplasma fermentans* *in vitro*, should so radically influence the cell-mediated immune response of these patients to *Mycoplasma fermentans* antigens.

These investigations are being continued and extended in an attempt fully to examine the mechanism of the beneficial effects of gold on the

clinical course of rheumatoid disease.

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