

Cytology of rheumatoid synovial cells in culture

II. Association of polykaryocytes with rheumatoid and other forms of arthritis

G. S. PANAYI, J. M. K. MACKAY, W. A. NEILL, J. N. McCORMICK, B. P. MARMION, AND J. J. R. DUTHIE

From the Rheumatic Disease Unit, Northern General Hospital, and the Department of Bacteriology, Edinburgh University Medical School, Edinburgh

The first paper (Mackay, Panayi, Neill, Robinson, Smith, Marmion, and Duthie, 1974) in this series describes the cytology of cultures established *in vitro* from the synovial fluids of rheumatoid patients with arthritis (RA) and other arthritides, and drew particular attention to the sequence of formation of polykaryocytes (multinucleate giant cells) in these cultures. Polykaryocyte formation is a feature of tissue reactions to certain viruses, particularly those that mature at nuclear or cytoplasmic membranes (Poste, 1970; Roizman, 1962) and was therefore of much interest when encountered during an investigation of the possible importance of viruses in the aetiology of rheumatoid arthritis. However, our studies of polykaryocytes in cell culture have not so far yielded evidence of virus, although investigations of this possibility continue. This negative finding, plus the sequence of events in culture (Mackay and others, 1974) and the recognition of polykaryocytosis in non-RA material, led to the hypothesis that the reaction was more likely to be a

manifestation of macrophage activation or a cellular immune response to an, as yet, unidentified target cell with consequential recruitment of macrophages into polykaryocytes with epithelioid cells as an intermediate step. Accordingly, the clinical association of, and factors influencing, polykaryocyte formation in synovial cultures from a range of patients have been reviewed and the results are described in this paper. The mechanism of polykaryocytosis in cultures of human peripheral blood ('buffy coat') has also been investigated.

Material and methods

The composition of the clinical groups is shown in Table I. In the rheumatoid group the patients had classical or definite rheumatoid arthritis (RA) according to the American Rheumatism Association criteria (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959).

(1) Synovial fluid cultures

The methods used are described by Mackay and others (1974).

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Address: Prof. B. P. Marmion, Department of Bacteriology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG.

Table I Analysis of predominant cell type in cultures of synovial fluid from rheumatoid, inflammatory, and degenerative arthritis

Diagnosis	No. of patients	No. of cultures	Number of cultures that were predominantly			
			Polykaryocytic	Mononuclear	Fibroblastic	No growth
Rheumatoid arthritis	74	80 (100)	28 (35)	36 (45)	2 (2.5)	14 (17.5)
Other inflammatory arthritides (1)	19	19 (100)	8 (42.1)	9 (47.4)	0	2 (10.5)
Osteoarthritis	4	4	0	0	1	3
Total No. Per cent.	97	103 (100)	36 (35)	45 (43.7)	3 (2.9)	19 (18.4)

() = percentage of total.

(1) Reiter's disease 4; non-specific synovitis 3; Still's disease 3; ankylosing spondylitis 2; other conditions (one patient each): post-rubella arthritis, scleroderma, psoriatic arthropathy, polymyalgia rheumatica, polyarteritis nodosa, agammaglobulinaemic polyarthritis, reticulum cell sarcoma.

(2) Cultures of peripheral white cells

20 ml. of venous blood was collected into 500 i.u. heparin (Evans Medical Ltd.), mixed with 6 ml. Dextran 150 (Fisons Pharmaceuticals Ltd.), and allowed to sediment at 37°C. After 1 hour the leucocyte-rich plasma layer was removed and mixed with an equal volume of tissue culture medium, Eagle's Minimal Essential Medium with 20 per cent. heat-inactivated foetal bovine serum; antibiotics were added to a final concentration of 250 units penicillin/ml. and 100 µg. streptomycin/ml.). This cell suspension was dispensed in 1 ml. volumes into Leighton tubes containing a glass coverslip and cultured at 37°C. in an atmosphere of 5 per cent. v/v CO₂/air mixture with twice-weekly medium changes. At 5, 10, 15, and 20 days, duplicate cultures were terminated and coverslips washed, fixed, and stained with Giemsa.

Results**CULTURES OF SYNOVIAL FLUIDS**

Three principal cell types occurred in these monolayer cultures; *viz.* macrophages, polykaryocytes, and fibroblasts (Mackay and others, 1974). Cultures were designated 'polykaryocytic', 'mononuclear', or 'fibroblastic' according to the cell type predominating (see Figs 1 to 3, overleaf). No attempt was made to quantitate in detail the different cell types present in a culture and a particular designation does not exclude the presence of small numbers of the other cell types. The assessment of the various proportions of cells in culture was made at the times stated after seeding of the cultures. Cells did not grow from some synovial fluids; this was unlikely to be due to variation in the media or sera as other cultures seeded at the same time grew satisfactorily.

SYNOVIAL FLUID CULTURES FROM DIFFERENT FORMS OF ARTHRITIS

A total of 103 cultures of synovial fluids from 97 patients was examined (Table I). The proportion of

cultures that were either polykaryocytic or mononuclear in character was similar whether they came from patients with rheumatoid arthritis or from patients with other inflammatory arthritides. The numbers of attempted cultures from degenerative conditions is small and only one grew producing fibroblasts but no polykaryocytes.

In the group with inflammatory arthritis other than RA, polykaryocytes were obtained from the effusions of three patients with Reiter's disease, two with non-specific synovitis of the knees of many years' duration, and one each from cases of scleroderma, ankylosing spondylitis, and reticulum cell sarcoma. Polykaryocytes in cultures of synovial fluids from these conditions have not been described before. The mean time of appearance of the polykaryocytes was 10.7 ± 5.4 days (Mean \pm 1 SD) in the group with rheumatoid arthritis, and 12.3 ± 4.5 days in those with other forms of arthritis.

Table II summarizes an analysis of the predominant cell type in culture in relation to the clinical diagnosis, age, sex, duration of disease, results of tests for rheumatoid factor, and erythrocyte sedimentation rate (ESR). It should be noted that only 72 of the 74 RA patients mentioned in Table I have been included in this analysis because complete details were not available on two patients. In patients with RA there were no very striking differences between those with polykaryocytic cultures and those with mononuclear cultures or no growth. Polykaryocyte cultures were less common among those with a high ESR or with rheumatoid factor (RF), but the differences were not statistically significant. Similarly, polykaryocytic and mononuclear cultures were evenly divided between patients with other forms of arthritis irrespective of age, sex, duration of disease, or the ESR.

The possible relationship between the type of cell culture obtained from the RA synovial fluids and the

Table II Clinical and laboratory details of patients with rheumatoid and other inflammatory arthritis in relation to the predominant cell type found in the synovial fluid culture

Diagnosis	No. of patients	Result of culture	Age* (yrs)	Sex		Duration* of disease	ESR*	Rheumatoid factor	
				M	F			+	0
Rheumatoid arthritis	26	Polykaryocytic	55.2 ± 13.4 (23-74)	18	8	12.1 ± 10.0 (1-43)	36.2 ± 21.5 (1-90)	16	10
	33	Mononuclear	59.5 ± 11.2 (39-85)	19	14	10.5 ± 5.8 (1-24)	51.9 ± 24.6 (22-100)	23	10
	13	No growth	62.7 ± 9.0 (42-75)	5	8	13.9 ± 14.9 (1-55)	42.7 ± 15.9 (17-66)	6	7
Other inflammatory arthritides	8	Polykaryocytic	35.3 ± 10.1 (21-48)	6	2	6.6 ± 6.7 (1-21)	37.3 ± 34.0 (5-105)	0	8
	9	Mononuclear	36.3 ± 16.8 (19-71)	5	4	5.4 ± 5.1 (1-17)	47.1 ± 38.1 (3-105)	0	9

+ Results expressed as: mean \pm 1 standard deviation of the mean; (range of observations)

patient's medication at the time of aspiration of the joint was also examined (Table III). All patients were receiving aspirin. Out of a total sample of 73, two patients had received indomethacin and three gold therapy (not shown in Table III). The numbers of patients receiving corticosteroids or phenylbutazone were evenly distributed between groups yielding different cell types (Table III). However, the proportion of donors yielding polykaryocytic cultures while taking chloroquine phosphate (250 mg. daily) was about half that expected from the proportion in the sample as a whole, a difference unlikely to arise by chance alone ($0.01 < P < 0.05$).

Table III *Drug therapy and cell type in cultures of synovial fluids in 73 patients with rheumatoid arthritis*

Type of culture	No. of patients	Number given		
		Chloroquine	Steroids	Phenylbutazone
Polykaryocytic	26	4	7	4
Mononuclear	33	15	8	6
No growth	14	3	2	3

CULTURES OF WHITE CELLS FROM PERIPHERAL BLOOD

The synovial fluid cultures from RA and other forms of arthritis developed polykaryocytes in about the same proportion and at about the same time. As indicated in Table I, too few fluids from osteoarthritis were available to determine the 'spontaneous' rate of the phenomenon in joint fluids in non-inflammatory conditions.

For these reasons, and also because the mononuclear cells presumably reach the joints from the blood, cultures of peripheral blood were set up from twenty patients with RA, from eight with ankylosing spondylitis, and from fourteen with osteoarthritis (Table IV). Peripheral blood cultures from the RA patients exhibited a higher proportion—(70 per

cent.)—with polykaryocytes and in a substantial proportion these developed within 10 days of establishing the culture (Table IV). Similar cultures from patients with osteoarthritis, on the other hand, exhibited polykaryocytes much less frequently (21.4 per cent.) and none of those positive developed in less than 10 days; comparison of the speed of development of polykaryocytes in RA and osteoarthritis showed that this difference was unlikely to arise by chance alone ($P = 0.014$ by Fisher's exact test). The results with cultures from ankylosing spondylitis were intermediate between the RA and osteoarthritis groups and did not achieve significance ($P = 0.37$).

Discussion

The results of the present study show that synovial fluids from patients with RA frequently develop polykaryocytes when grown *in vitro*. This agrees with previous experience (Lackington, 1959; Bartfeld, 1965; Palmer, 1971). It has now been shown that synovial fluids from forms of arthritis other than RA behave in a similar fashion. Thus polykaryocytosis *in vitro* may indicate a common response in inflammatory joint disease rather than a specific feature of aetiological significance.

Because of the inherent difficulties in obtaining normal synovial fluids in sufficient amounts for cell culture, cultures of peripheral white blood cells were used, as it is known that such cultures give rise to polykaryocytes (Lewis and Lewis, 1926; Berman and Stulberg, 1962). Cultures of peripheral blood from patients with RA developed polykaryocytes similar to those seen in synovial fluid cultures and these ap-

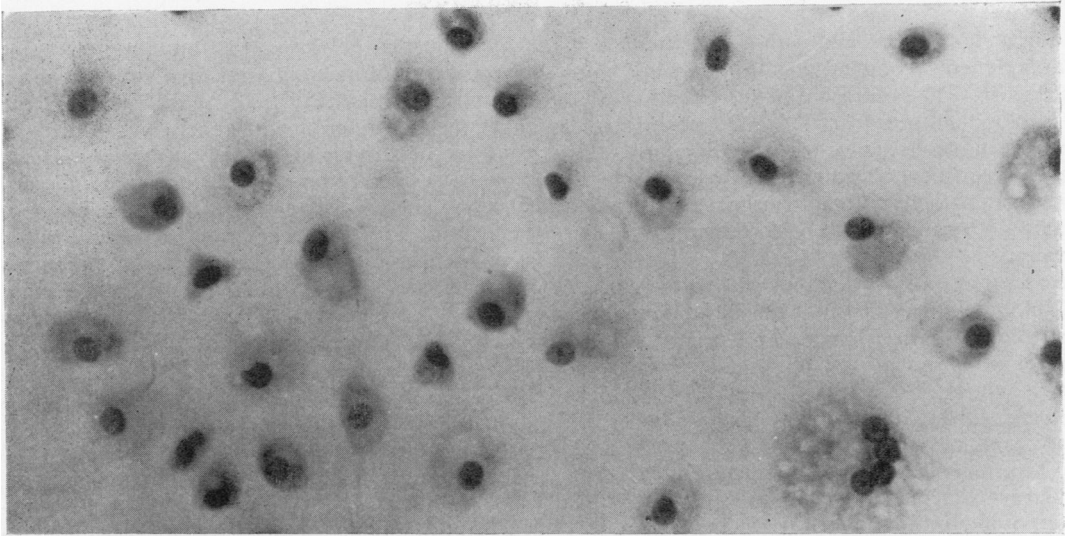
FIG. 1 'Mononuclear culture'. Cells resembling macrophages seen by light microscopy in cultures of peripheral blood. Giemsa. $\times 380$

FIG. 2 'Polykaryocytic' culture. Well-developed polykaryocyte formation in culture of peripheral blood; note presence of mononuclear macrophages. Giemsa. $\times 380$

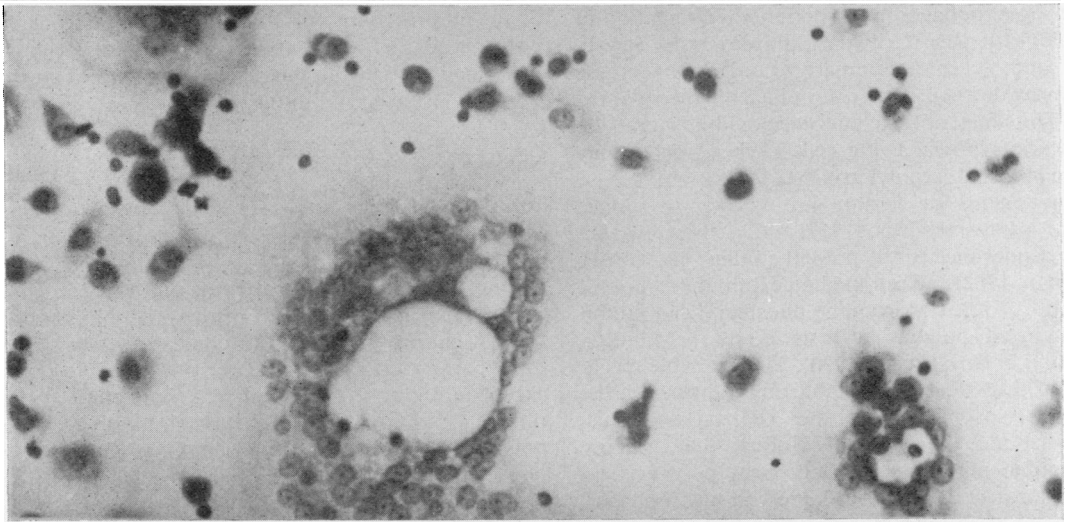
FIG. 3 'Fibroblastic' culture. Predominantly fibroblastic culture of synovial fluid. Giemsa. $\times 380$

Table IV *Polykaryocyte formation in cultures of peripheral white cells from patients suffering from rheumatoid arthritis or other diseases of joints*

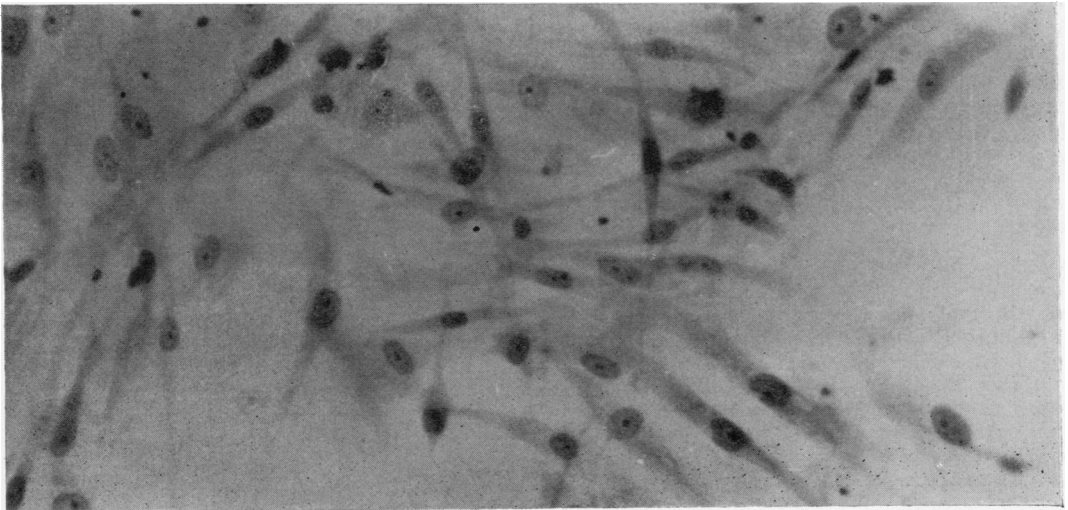
Clinical category	No. of cultures	Result of culture:			Proportion of cultures with polykaryocytes
		No. developing polykaryocytes by:		Cells other than polykaryocytes	
		< 10 days	> 10 days		
Rheumatoid arthritis	20	8	6	6	14/20 (70)
Ankylosing spondylitis	8	0	4	4	4/8 (50)
Osteoarthritis	14	0	3	11	3/14 (21.4)



(1)



(2)



(3)

peared in a shorter time and with greater frequency than in peripheral blood cultures from patients with osteoarthritis. The occurrence of such a differential effect in blood cultures from RA and osteoarthritis has not been described previously. Similarly, the increased frequency of polykaryocytosis in blood cultures from patients with ankylosing spondylitis as compared with patients with osteoarthritis is a new observation.

The occurrence of polykaryocytosis in both peripheral blood cultures and in synovial fluid cultures suggests that joint macrophages may originate from peripheral blood monocytes. There is evidence from studies in animals (Ebert and Florey, 1939; Aronson and Elberg, 1962; Gillman and Wright, 1966) that circulating monocytes enter inflammatory lesions and fuse to form polykaryocytes. Evidence on the origin of human synovial macrophages is not available. It is, however, of interest that there are increased numbers of early macrophages in the peripheral circulation of patients with RA (Crowther, Fairley, and Sewell, 1967) and other inflammatory conditions; these could provide a source of macrophages in the inflamed joint. This does not exclude the possibility that the phagocytic synovial living cells (Type A cells) may also be involved in polykaryocyte formation.

Many agents or factors are known to induce polykaryocytosis (Sutton, 1967) and, of these, two are of direct relevance to the present studies; *viz.* viruses (Roizman, 1962) and cell-mediated immune responses (Galindo, 1972). Chloroquine phosphate can inhibit virus-induced polykaryocytosis *in vivo* (Mallucci, 1966) and *in vitro* (Poste, 1970). The probable mechanism of this effect is the stabilizing influence of the drug on lysosomal membranes (Bertini and Bari, 1970). It does not logically follow that because chloroquine phosphate inhibits virus polykaryocytosis in a range of cells, not merely in macrophages, that its action in inhibiting polykaryocyte formation in RA implies a virus cytopathic effect in this condition. Direct electron microscopy of RA polykaryocytes has not revealed virions or virus material, and the occurrence of the phenomenon in a wide variety of inflammatory states is also against the possibility of virus polykaryocytosis.

The view that polykaryocyte formation represents the end-result of macrophage activation by a variety of stimuli acting either directly on the macrophage or indirectly *via* lymphokines or other mechanisms activated by the combination of antigen and sensitized lymphocytes fits better with current immunological concepts. There is evidence that the inflammation in rheumatoid synovial membrane is the result of immunological mechanisms which lead to the release of inflammatory agents, and in particular, lysosomal enzymes (Chayen and Bitensky, 1971). Clinical trials have shown that chloroquine may cause a decrease in circulating rheumatoid factor (Popert, Meijers, Sharp, and Bier, 1961). Also lymphocytes from RA patients receiving chloroquine show decreased lymphoblastic transformation by phytohaemagglutinin (Panayi, Neill, Duthie, and McCormick, 1973; Neill, Panayi, Duthie, and Prescott, 1973). Hence the suppression of immune responses by chloroquine phosphate may account for the decreased incidence of polykaryocytes in the synovial fluid cultures of those RA patients receiving the drug at the time when the fluid was aspirated from the joint.

Summary

103 synovial fluids were examined *in vitro* for the occurrence of polykaryocytes. The proportion of cultures showing these cells was similar in fluids from patients with rheumatoid arthritis and other inflammatory arthritides. Analysis of the predominant cell type in culture in relation to clinical diagnosis and other parameters revealed no very striking differences except in relation to the patient's medication at the time of aspiration. The proportion of donors yielding polykaryocytic cultures while taking chloroquine phosphate was about half that expected from the proportion of the sample as a whole.

Cultures of peripheral blood white cells revealed that a higher proportion of cultures from patients with rheumatoid arthritis (70 per cent.) developed polykaryocytes than of similar cultures from patients with osteoarthritis (21.4 per cent.). The development of polykaryocytes also took place earlier in cultures from patients with rheumatoid arthritis.

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