Chemotactic effect of joint effusions

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This paper reports the incidence of chemotactic activity of joint effusions obtained from several diseases involving joints. Ward and Zvaifler (1971) found that such activity was present in the majority of effusions in rheumatoid arthritis, but also in some cases of other nonspecified forms of inflammatory arthritis, gout, and osteoarthritis. These authors used rabbit neutrophil polymorphs to assess chemotaxis. We have repeated their observations using human polymorphs from both autologous and homologous sources. This work was conducted independently in two centres, using different methods. The results, which proved to be similar, are reported separately below.

Methods

Joint effusions were obtained from patients as detailed in Tables I and II. Diagnosis of rheumatoid arthritis (RA) was made in accordance with A.R.A. criteria, and of juvenile rheumatoid arthritis following the criteria of Ansell and Bywaters (1959). Other diseases were diagnosed according to the usually accepted criteria. Fluids were mixed with 15 units heparin/ml. No hyaluronidase was used for the Basle cases; it was used in a few of the Taplow cases, when the viscosity of fluids was high. The fluids were centrifuged at 1,800 *g* for 20 mins, to obtain clear supernatants. Some fluids were used fresh, others after storage at -20°C . Tests were conducted with joint fluids undiluted, or diluted 1:1 with Gey's medium.

White blood cells, the source of polymorphs, were obtained from heparinized (10 units/ml) human venous blood. Sedimentation was achieved by adding 1 ml dextran 110 (Fisons) to 10 ml blood, and sedimenting for 1 hour at room temperature. For the Taplow work, Plasmagel (Roger Bellon) was used instead. After centrifugation and washing in Gey's medium, the cells were suspended in Gey's containing 2% human serum albumin to give a leucocyte suspension of $1-1.5 \times 10^6$ polymorphs/ml. For the method used at Taplow, a concentration of $3-4 \times 10^6$ /ml was used.

CHEMOTAXIS

This was assayed by a modified Boyden chamber technique (by J. F. B. and B. S.) (Boyden, 1962; Keller, Borel, Wilkinson, Hess, and Cottier, 1972) and by the method of Maroni, Symon, and Wilkinson (1972) (by G. L.). For the Boyden chamber technique, double filters were used.

Positive controls were supplied by the use of supernatant phase from a 24-hour culture of *Esch. coli*, diluted 1:10 in Gey's medium. Negative controls were Gey's containing 2% human serum albumin. Millipore filters of 3 μm pore size were used. The number of cells having completely moved to the lower aspect of the first filter during 2.5 hrs and the number of cells collected on the second filter (pore size 0.45 μm) were assessed and added. Rabbit neutrophils were obtained from peritoneal exudates, induced by casein injection.

The technique used by G. L. employed plastic tuberculin syringes the lower cut ends of which were sealed with millipore filters of 3 μm pore size. A suspension of white blood cells, volume 0.2 ml, was placed above the filter, and the lower end was inserted into a 10 ml beaker containing joint fluid, a positive control, or a negative control. Three syringes were inserted into each beaker, affording observations in triplicate. For positive controls, normal human serum was activated by *Esch. coli* endotoxin (*Esch. coli* lipopolysaccharide B from *Esch. coli* 0127:B8) and subsequently inactivated by heating at 56° for 30 mins. The negative control, as in the other system, was Gey's medium, containing 2% human serum albumin. Chemotaxis was allowed to proceed for 2 hrs at 37° .

In Tables I and II fluids considered to have given a positive test are designated.

Results and discussion

These are tabulated separately for the Basle (J. F. B. and B. S.) and Taplow (G. L.) series. In the former, 20 out of 25 fluids tested induced polymorph chemotaxis. This was irrespective of diagnostic categories comprising rheumatoid arthritis, osteoarthritis, or Reiter's disease, and of the presence or absence of serum rheumatoid factor. In the Taplow series, 7 fluids of 18 tested induced chemotaxis. It was evident here also that the result was independent of the nature of the disease associated with the effusion. Ward and Zvaifler (1971), using a system with filters of 0.65 μm pore size, reported chemotaxis by fluids from cases of rheumatoid arthritis, inflammatory nonrheumatoid arthritis, gout, and osteoarthritis. It is evident that chemotactic activity remained in spite of the exhibition of various drugs. This does not, of course, imply that these regimens failed to affect the degree of chemotaxis *in vivo*. Direct addition *in vitro* of similar

Table I Chemotactic activity of synovial fluids assessed with autologous (A) or homologous (H) polymorphonuclear leucocytes, using the Boyden chamber technique

Case no.	Diagnosis	Treatment	Chemotactic assay with human PMN cells*		
			Controls (1)	Controls (2)	Synovial fluid
1	RA sero -	Phenylbutazone; gold	0	(6)†	46 H‡
			3	45	109 H‡
2	RA sero -	Phenylbutazone; gold	0	44	0 A
3	RA sero -	None	0	NT	136 H‡
4	RA sero -	D-penicillamine	0	NT	79 H‡
5	RA sero +	Ibuprofen; gold; phenylbutazone; steroid	3	45	53 H‡
6	RA sero +	Ibuprofen; gold	0	49	109 A‡
7	RA sero +	Ibuprofen; steroid; phenylbutazone	0	60	2 A
8	RA sero +		3	45	9 H
9	RA sero +	Yttrium ⁹⁰	0	18	34 A‡
		D-penicillamine	0	(6)†	52 H‡
			3	45	72 H‡
10	Osteoarthritis	Phenylbutazone	0	21	17 A‡
11	Osteoarthritis		3	45	48 H‡
12	Osteoarthritis	Indomethacin; nil for 7 days before aspiration	0	(6)†	10 H
			3	45	11 H
13	Osteoarthritis	Indomethacin	0	19	30 H‡
14	Reiter's disease	Phenylbutazone	0	(6)†	55 H‡
		Phenylbutazone	0	(6)†	66 H‡
15	Reiter's disease	Phenylbutazone	0	(6)†	46 H‡
			3	45	20 H‡
16	Reiter's disease	Phenylbutazone	3	45	106 H‡
			0	(6)†	24 H‡
17	Reiter's disease		3	45	95 H‡
			0	NT	76 H‡

* Figures in the 3 columns denote average number of cells counted per high-power microscopical field. Controls (1): Gey's solution 2% HSA; Controls (2): supernatants from *Esch. coli* culture, 10% dilution in Gey's solution; synovial fluids: used undiluted.

† Culture supernatant diluted 20×, resulting in low value. ‡ Positive test.

NT: not tested.

Rheumatoid factor (sero +, sero -) was measured by latex test.

Table II Chemotactic activity of synovial fluids, assessed with homologous polymorphonuclear leucocytes, using a micromethod

Case no.	Diagnosis	Treatment	Chemotaxis		
			Control	Control*	Synovial fluid
18	RA sero -	Salicylate	10	107	4
19	RA sero +	Salicylate	21	400	42
20	RA sero +	Salicylate; phenylbutazone	13	450	17
21	RA sero +	Salicylate; indomethacin	5	780	9
			12	450	7
22	RA sero -	Ibuprofen	21	400	18
			1	320	13
23	RA sero -	Salicylate	1	320	87†
24	RA sero -	Salicylate	5	227	6
			27	365	96†
25	RA sero +	Salicylate	21	400	48
26	RA sero -	Salicylate	12	450	15
			1	320	18
27	RA sero +	Gold	27	365	129†
28	RA sero -	Salicylate	17	252	120†
29	RA infectious arthrodesis sero -	Salicylate; indomethacin	13	450	112†
30	Intermittent hydrarthrosis sero -	Nil	5	380	352†
31	Osteoarthritis sero -	Nil	4	367	48†

* Controls: Endotoxin-activated human serum.

For other explanatory notes, see Table I.

† Positive test.

drugs to chemotactic systems has been shown not to affect the migratory behaviour of polymorphs (Borel, 1973). Table I shows that both homologous and autologous polymorphs can respond to joint fluid by chemotaxis. This result was not unexpected, but it shows that polymorphs from seropositive rheumatoid patients are able to migrate to an inflammatory focus. Mowat and Baum (1971) showed that cells from rheumatoid patients responded to a chemotactic stimulus to a lesser degree than the cells of normal controls. Their results suggested that this might be caused by the *in vivo* uptake of complexes by these cells. In a few experiments we have used the cells from joint exudates as the migratory test population using *Esch. coli* supernatant as stimulus. The cells behaved normally; the prevalence of immune complex inclusions within cells of an inflammatory joint exudate has been extensively documented (Hollander, McCarty, Astorga, and Castro-Murillo, 1965).

The results we report were obtained with human polymorphs. Others (Ward and Zvaifler, 1971) have employed rabbit cells. In preliminary experiments of our own, using rabbit polymorphonuclear cells, we obtained inconsistent results. Undiluted synovial fluids, which had proved strongly chemotactic for human cells, induced chemotaxis in cells of some rabbits while they were inactive for the cells of some other rabbits. The results were also inconsistent between individual experiments. The situation was further complicated by the finding that progressive

dilution of joint fluid in some cases led to increased rabbit-cell migration, which was never found with human cells.

The nature of chemotactic factors in joint fluid has been investigated by Ward and Zvaifler (1971). They have adduced evidence for the presence of the complement-derived factor C3 β and the cleavage product C5a as mediators of activity in rheumatoid effusions, while nonrheumatoid fluids contained a chemotactic factor (C3a) derived from C3. In an earlier paper (Zvaifler, 1969), a fast-moving breakdown product of C3 had, however, been found to be correlated with rheumatoid arthritis and the presence of rheumatoid factor. Both Borel, Sorkin, and Loewi (1971) and Wagner, Abraham, and Baum (1972) have shown that rheumatoid factor can cause the generation of chemotaxis in the presence of serum. This might, therefore, provide a positive mechanism in some joint fluids, via a complement sequence.

Summary

Joint fluids from patients with several diseases associated with arthritis were tested for polymorph chemotaxis. A high proportion was found to be positive, using two different systems in two centres. No relationship to serum rheumatoid factor, type of disease, or drug therapy was indicated by the findings.

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