Peripheral leucocyte migration

Inhibited by diphtheroid organisms isolated from patients with rheumatoid arthritis

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The isolation of diphtheroid organisms from rheumatoid synovial membranes and synovial fluids (Duthie, Stewart, Alexander, and Dayhoff, 1967; Clasener and Biersteker, 1969; Stewart, Alexander, and Duthie, 1969) has raised the question of the role of diphtheroid bacilli in the aetiology of rheumatoid arthritis. The view that rheumatoid disease might involve an abnormal cellular immunological response to an infective agent (Walton, 1968) suggested the need to determine whether cellular hypersensitivity to such diphtheroid isolates in subjects with classical rheumatoid arthritis was different from that in non-rheumatoid subjects.

Inhibition of peripheral leucocyte migration by whole bacteria and by bacterial antigens has been taken as an expression *in vitro* of cellular hypersensitivity. This technique has been used to determine whether peripheral leucocytes from rheumatoid and non-rheumatoid subjects differ in their responsiveness to a characteristic diphtheroid strain (SF 16) isolated from rheumatoid synovial fluid, and to a standard corynebacterial strain (*Corynebacterium bovis*: NCTC 3224) which is similar in morphological characteristics to SF 16, but which is not known to be associated with human disease.

Material and methods

Two groups of patients were chosen from persons attending the Department of Rheumatology or that of General Medicine at the West London Hospital.

The sero-positive rheumatoid group comprised twelve subjects with 'classical' rheumatoid arthritis according to the diagnostic criteria of the American Rheumatism

Association (1959); individual patients varied widely in duration of disease and in current clinical disability (Table I, overleaf).

The 'control' group comprised nine individuals, six of whom had gout.

Heparinized peripheral blood was obtained from these 21 patients; leucocyte-rich plasma was separated by sedimentation at 37°C. (in the absence of dextran) for 1 to 3 hours, and the thrice-washed leucocytes were set up in capillary tubes for cell migration by the technique of Bendixen and Søborg (1969), using triplicate sets of culture chambers of capacity 1.1 ml. containing 10 per cent. horse serum—Waymouth's medium. Cell migration was undertaken at 37°C. for 18 hours in the presence and absence of 5-fold serial dilutions of each of the two strains of corynebacteria.

Two strains of diphtheroid bacilli were used:

(1) Strain SF 16 was derived by isolation from rheumatoid synovial membranes as previously reported (Duthie and others, 1967; Stewart and others, 1969), and was similar to 60 per cent. of such isolates in morphological, cultural, and biochemical characteristics (Stewart, unpublished, 1970).

(2) A standard diphtheroid strain, *C. bovis*: NCTC 3224, was obtained from Mr. L. R. Hill of the Central Public Health Laboratory, Colindale; this organism was used because it resembled SF 16 in certain morphological and cultural features (Stewart and others, 1969).

Both strains were grown in supplemented PPLO Difco broth, killed by exposure to 0.1 per cent. formalin, and washed repeatedly in phosphate-saline at pH 7.4 until the washing fluid was free of formalin. Organisms were then suspended in cell migration medium at 1×10^9 , 2×10^8 , and 4×10^7 organisms/ml.; and at each dilution triplicate chambers were set up for measurement of mean leucocyte migration.

Table I *Clinical features of 12 rheumatoid and 9 non-rheumatoid subjects*

Group	Patient no.	Age (yrs)	Sex	Duration of disease (yrs)	Clinical status*	Hb (g./100 ml.)	Erythrocyte sedimentation rate (mm./1 hr)	SCAT †
Rheumatoid arthritis	1	53	F	12	II	11	34	1 : 256
	2	60	M	12	III	13.5	60	1 : 64
	3	56	M	12	III	14.5	70	1 : 64
	4	62	F	3	II	8.2	75	1 : 128
	5	58	M	4	I	14.0	25	1 : 32
	6	78	M	11	II	11.8	20	1 : 64
	7	68	M	19	II	11.2	129	1 : 64
	8	55	F	6 mths	II	13.5	25	1 : 64
	9	20	F	6 mths	II	12.8	15	1 : 32
	10	68	F	24	III	12.7	82	1 : 32
	11	43	F	6	III	12.7	25	1 : 64
	12	43	M	7	II	13.5	51	1 : 128
Other conditions	13	70	M	Past gouty arthritis		14.8	4	—
	14	35	M	Past gouty arthritis		15.0	8	—
	15	45	M	Chronic bronchitis		15.6	3	<1 : 8
	16	56	M	Asbestosis		14.2	5	<1 : 8
	17	61	M	Past gouty arthritis		15.0	6	<1 : 8
	18	59	M	Past gouty arthritis		14.8	6	—
	19	52	F	Myxoedema		13.0	10	<1 : 8
	20	70	M	Past gouty arthritis		15.0	10	<1 : 8
	21	40	M	Acute gout		15.0	29	<1 : 8

* Classes I to IV: Classification of functional impairment (Steinbrocker, Traeger, and Batterman, 1949).

† SCAT: Sheep cell agglutination titre for rheumatoid factor.

Cell migration results were evaluated by subtracting from unity the ratio:

$$\frac{\text{mean cell migration area in the presence of bacteria}}{\text{mean cell migration area in tissue culture medium alone}}$$

This fraction was expressed as percentage inhibition of cell migration which was plotted as a scattergram (Fig. 1).

Statistical evaluation of corresponding groups of results (Table II, opposite) was undertaken (Table III, opposite).

FIGURE *Inhibition of leucocyte migration by bovine (*C. bovis*) and synovial (*SF 16*) corynebacteria, at corresponding bacterial concentrations, using peripheral leucocytes from rheumatoid and control subjects*

Table II Inhibition of leucocyte migration by diphtheroids in rheumatoid and non-rheumatoid subjects

Diagnosis	Patient no.	Percentage inhibition of leucocyte migration* by:					
		10 ⁹ org./ml.		2 × 10 ⁸ org./ml.		4 × 10 ⁷ org./ml.	
		SF 16	C. bovis	SF 16	C. bovis	SF 16	C. bovis
Sero-positive rheumatoid arthritis	1	82	68	60	54	4	-10
	2	79	89	30	65	8	36
	3	76	63	67	47	7	14
	4	73	73	62	43	47	38
	5	70	29	53	53	34	40
	6	68	65	39	50	18	21
	7	60	62	42	45	25	12
	8	56	51	60	26	19	24
	9	51	43	22	23	2	11
	10	48	7	20	33	16	29
	11	47	63	33	17	-26	-2
	12	46	58	20	23	15	-21
	Mean ±S.D.	63 ±13·8	56 ±22·3	42 ±11·4	40 ±8·4	14 ±18·8	16 ±14·9
Other conditions	13	80	63	29	42	42	29
	14	71	62	31	19	17	17
	15	68	50	45	58	30	27
	16	55	70	14	64	28	32
	17	49	58	24	31	6	11
	18	64	48	28	18	36	38
	19	61	74	48	72	21	53
	20	72	55	56	33	50	38
	21	57	42	43	48	40	14
	Mean ±S.D.	64 ±10·9	58 ±10·4	35 ±13·4	43 ±19·3	30 ±13·7	29 ±13·4

* Values are means of triplicate observations

Table III Statistical analysis (Student's 't' test) of percentage inhibition of leucocyte migration of coryne-bacteria

Organisms and concentration in migration test	Percentage inhibition of leucocyte in migration		
	Rheumatoid (Nos 1-12) Mean ± S.D.	Non-rheumatoid (Nos 13-21) Mean ± S.D.	P-value difference (t-test)
10 ⁹ /ml. { SF 16 C. bovis	63 ± 13·8 56 ± 12·3	64 ± 10·9 58 ± 10·4	0·85 0·8
P-value for difference (t-test)	0·45	0·2	—
2 × 10 ⁸ /ml. { SF 16 C. bovis	42 ± 11·4 40 ± 8·4	35 ± 13·4 43 ± 19·3	0·2 0·7
P-value for difference (t-test)	0·7	0·25	—
4 × 10 ⁷ /ml. { SF 16 C. bovis	14 ± 18·8 16 ± 14·9	30 ± 13·7 29 ± 13·4	0·05 0·05
P-value for difference (t-test)	0·8	0·8	—

Results

The addition of both diphtheroid bacilli to leucocyte culture chambers caused inhibition of cell migration. The magnitude of this effect was related to the concentration of bacilli present in the tissue culture

medium (Table II). At any one bacillary concentration, the degree of inhibition of cell migration caused by the two organisms varied from patient to patient; however, for each of the two groups of patients there was no significant difference between the migration effects of the standard diphtheroid

strain (*C. bovis*) and the diphtheroid synovial isolate (SF 16) at any of the three bacillary concentrations (Table III). Similarly, there was much overlap between the migration inhibition produced by both the diphtheroids in the rheumatoid and non-rheumatoid groups; a low level of significance for the difference between these results was observed only at the lowest concentration of bacteria (Table III). Although the control group appeared slightly more reactive than the rheumatoid group at these low diphtheroid concentrations ($P = 0.05$) there was no significant difference in the reactivity of one or other group to both organisms ($P = 0.8$; Table III).

Discussion

The ability of particulate antigen to inhibit the migration of human peripheral leucocytes is being increasingly used as a cellular index of clinical hypersensitivity. Although it is not yet clear whether this test correlates with immediate or delayed hypersensitivity or with the presence of some forms of circulating antibody (Nerup, Andersen, and Bendixen, 1969), inhibition of cell migration by particulate and soluble antigens can be demonstrated in autoimmunity (Dumonde, 1970), transplantation immunity (Falk, Thorsby, Möller, and Möller, 1970), bacterial infections (Søborg, 1967), and clinical allergic diseases (Brostoff and Roitt, 1969). The results we have obtained by this technique reveal that there is little difference, if any, between the migration responses of rheumatoid and non-rheumatoid subjects to two different strains of diphtheroid organisms. One of these strains (SF 16) was isolated from rheumatoid synovia, but the other (*C. bovis*), a standard type culture, was originally derived in 1916 from a veterinary source (milk) and is not known to be associated with human disease (L. R. Hill, 1970, personal communication).

The possible role of diphtheroids as aetiological agents in rheumatoid arthritis has been viewed in

terms of exogenous antigens, as local adjuvants which alter immune tolerance, or as organisms existing within synovial cells as protoplasts or L-forms, and thereby circumventing the host's immunological defence mechanisms (Stewart and others, 1969). The present results indicate that neither cellular energy nor cellular hypersensitivity to synovial diphtheroid organisms is playing a major role in the immunopathology of established rheumatoid disease. It is suggested that more extensive studies of the immunological response to corynebacterial antigens will be required in a variety of clinical circumstances in order to provide further evidence bearing upon the immunological role of diphtheroids in rheumatoid disease.

Summary

Two strains of diphtheroid bacilli were examined for their ability to inhibit the migration of peripheral leucocytes from twelve rheumatoid and nine non-rheumatoid subjects. One of the diphtheroid strains had been isolated from synovial fluid in rheumatoid arthritis; the other diphtheroid strain was of bovine origin, and unassociated with human disease. No difference was detected between the ability of synovial or bovine diphtheroids to inhibit leucocyte migration. The studies do not support a specific immunological role of synovial diphtheroids in the pathogenesis of rheumatoid arthritis.

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