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Research Article

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The chemotactic indices in three patients with severe connective tissue disease (seropositive rheumatoid arthritis, systemic lupus erythematosus, and polymyositis) returned to normal after 5 days' treatment with 60 mg of prednisolone per day. Incubation of the cells from patients with rheumatoid arthritis with hydrocortisone in vitro failed to alter the chemotactic indices.

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Chemotaxis of Polymorphonuclear Leukocytes from Patients with Rheumatoid Arthritis

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ABSTRACT Using a new in vitro method of measuring the chemotaxis of polymorphonuclear leukocytes from peripheral blood, a chemotactic index has been calculated. The mean chemotactic index of 320 in 24 patients with definite rheumatoid arthritis, was significantly less ($P < 0.0005$) than the mean of 555 in 24 normal controls matched for age and sex.

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Prior incubation of normal cells with purified rheumatoid factor complexes, rheumatoid serum, or macromolecules of iron dextran impaired their chemotaxis. It is suggested that phagocytosis of complexes in vivo is a possible mechanism by which the chemotaxis of the polymorphonuclear leukocytes of patients with rheumatoid arthritis is impaired.

This impairment in chemotaxis may explain the increased incidence of bacterial infection, both during life and as a cause of death in these patients.

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Dr. Baum is Clinical Scholar of The Arthritis Foundation.

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INTRODUCTION

Infection is a frequent cause of death in patients with rheumatoid arthritis (RA).[1] Uddin, Kraus, and Kelly found that the observed incidence of death from infection was about ten times higher than expected in males and about seven times higher than expected in females (1). The percentage of patients with RA dying from infection has changed little over two decades, reported as 23.4% in 1953 (2), 14.7% in 1964 (3), and 19.1% in 1970 (1). To account for these findings, we thought it probable that improvements in morbidity and mortality rates due to infection by the use of antibiotic therapy have been offset by an increased use of corticosteroids.

The reasons for these increased infection rates are not clear. An early study (4) appeared to show inicreased antibody production in these patients, but two subsequent studies (5, 6), using the same *Brucella* antigen, Failed to confirm this finding. These patients also have a normal antibody response to tetanus toxoid (7). Phagocytosis by the polymorphonuclear leukocyte (PMN) is normal in RA (8).

In recent years renewed interest has been shown in the possibility that infective organisms may play a role in the etiology of RA and that the continued presence of these organisms in the joints or elsewhere could ex-

[1] *Abbreviations used in this paper:* ESR, erythrocyte sedimentation rate; PMN, polymorphonuclear leukocyte; RA, rheumatoid arthritis; RF, rheumatoid factor; SLE, systemic lupus erythematosis.

plain some of the immunological changes associated with the disease (9–11).

We have recently developed a simple, reproducible method for studying chemotaxis of PMN's using small volumes of human peripheral blood (12). It therefore seemed appropriate to study this important function of the PMN in the response of the host to invasive agents. This study reports a defect in the chemotaxis of the PMN from patients with RA compared to normal controls and investigates some of the possible mechanisms responsible for this defect.

METHODS

The healthy normal subjects were members of the laboratory staff; residents of the Monroe County Home, Rochester, N. Y.; and, in the case of the children, patients with simple fractures on an orthopedic service. The patients with rheumatoid arthritis were either in- or outpatients seen at Strong Memorial Hospital and the Monroe Community Hospital, Rochester, N. Y. There were 38 patients with definite rheumatoid arthritis according to the diagnostic criteria of the American Rheumatism Association and 8 patients with definite juvenile rheumatoid arthritis. In the initial study, 24 patients (6 males and 18 females) with adult rheumatoid arthritis were compared with 24 normal controls matched for age and sex (Table I). The patients with juvenile rheumatoid arthritis (3 males and 5 females) were also compared with similarly matched normal controls (Table II). The ages of the patients ranged from 6 to 88 yr. Subsequent investigations involved only those patients with adult onset rheumatoid arthritis. They included some of those in the matched series together with a further group of patients drawn from the same sources.

The method for studying the chemotaxis of PMN's, which has been described in greater detail elsewhere (12), utilized a Sykes-Moore tissue culture chamber[2] which was modified by the design of a new gasket. The chamber was assembled with a 25 mm round cover slip, the gasket, a 25 mm 3 μ Millipore[3] filter, another gasket, a 25 mm cover slip again, and then closed with the screw top. Ports in the side wall of the chamber enabled the compartments above and below the Millipore filter to be filled with various solutions.

PMN's were obtained from peripheral blood. 10 ml of blood were drawn into a heparinized tube, 0.75 ml of a 2% solution of methyl cellulose in normal saline was added, and the tube gently inverted. The methyl cellulose caused the rapid sedimentation of red blood cells and mononuclear leukocytes (13). After standing for 30–45 min, the plasma layer, which then contained a concentration of PMN's 20–40% higher than in the peripheral blood, was removed and diluted with approximately one-half its volume of Hanks's solution. The cells from 4–5 drops of this cell suspension were deposited on a circumscribed area of 3 μ Millipore filter using the Shandon cytocentrifuge.[4] The filter was placed in the Sykes-Moore chamber; and after filling both compartments of the chamber (approximately 0.5 ml each), the chamber was incubated for 3 hr at 37°C. After incubation, the filters were stained by Boyden's method (14), trimmed, and mounted.

Cell viability was studied using the trypan blue dye exclusion method, after centrifugation and after incubation, and better than 99% of the cells from normal and rheumatoid arthritic subjects were found to be viable at both times.

Since in other techniques some of the cells entering the filter may have done so due to inherent motility of the polymorphonuclear leukocyte, as well as chemotatic direction, we eliminated as much of the effects of motility as possible by counting only those cells which had completely penetrated the filter and were present upon the bottom or attraction surface of the membrane. This was done by focusing beyond any cells seen and then counting only those cells which appeared first in focus as the membrane was approached. The same technique was used for counting those cells on the upper or starting side. The number after counting 10 random fields on each side was expressed as a ratio and called the chemotactic index. In an average experiment this method of cell counting resulted in a figure of 90–100 cells on the starting side of the filter. In this way a precise number of cells did not have to be delivered onto the filter surface and obviated procedural delays and the necessity for frequent handling and counting of the cells with possible reductions in cell viability. On the starting side, the cells were counted within the outer circle (1.5 mm diameter) of the photographic reticule of a Leitz microscope.[5] On the opposite (attraction) side of the Millipore filter, the cells were counted within the rectangular photographic reticule (11.5 × 7 mm). The cells were counted with a 10 × ocular and a 25 × objective.

$$\text{Chemotactic index} = \frac{\text{Number of cells (attractant side)}}{\text{Number of cells (starting side)}} \times 1000$$

The following solutions were used in the study:

1. Hanks's solution[6] was prepared without the addition of bicarbonate (pH 6.8).
2. Casein was made up in a concentration of 5 mg/ml in the Hanks's solution.
3. Human complement. This was provided by human serum, type AB, from one of several healthy donors, whose serum was used throughout the study. The serum was separated immediately after being drawn, kept in small portions at −70°C., and thawed just before use. Complement levels were checked in each donor and found to be well within the normal range.
4. Pure hydrocortisone[7] was made up in the Hanks's solution in concentrations to provide 80 and 800 μg/100 ml after mixture with the cell suspensions.
5. Iron dextran (Imferon[8]) was made up in the Hanks's solution in concentrations to provide 1, 10, 100, and 1000 μg/ml elemental iron after mixture with the cell suspensions. Analysis of the iron dextran by analytical ultracentrifugation (kindly performed by Dr. G. Abraham) showed a small amount of a component with an approximate mol wt of 8000 with most of the material present as macromolecules with mol wt of over 1 million.
6. Rheumatoid sera. Sera from three patients with active rheumatoid arthritis were stored at −70°C and used for studies with normal cells. Rheumatoid factor as measured by latex activity was present in dilutions of greater than 1:5120 in each serum and all showed the presence of macromolecular complexes (material larger than 19S) when

[2] Bellco Glass, Inc., Vineland, N. J.
[3] Millipore Corporation, Bedford, Mass.
[4] Shandon Scientific Co., Inc., Sewickley, Pa.
[5] E. Leitz, New York.
[6] Difco Laboratories, Detroit, Mich.
[7] Calbiochem, Los Angeles, Calif.
[8] Lakeside Laboratories, Inc., Milwaukee, Wis.

studied by analytical ultracentrifugation (kindly performed by Dr. G. Abraham).

7. Rheumatoid complexes. A patient was found who showed, on ultracentrifugation, large amounts of 22S complexes in his serum. Dr. George Abraham purified these complexes by passage of the patient's serum through a Sephadex G-200 column [9] with neutral buffer. The first peak when ultracentrifuged showed it to be composed of material larger than 19S. This peak was then used as a source of complexes. This material contained 7.7 mg protein/ml. It was diluted 1:1 before being placed in the chamber. Since each compartment has a volume of about 0.5 ml, 1.9 mg of complexes were added to approximately 1000 polymorphonuclear leukocytes (12). Incubation was carried out for 3 hr at 37°C. This peak was dialyzed against 1 N acetic acid to bring it to pH 2.8. There was complete dissociation of the peak. This was demonstrated by recentrifugation showing complete absence of the 22S material with conversion into lighter peaks.

8. Normal sera. Sera from three normal subjects were also stored at −70°C and used for comparative studies.

Previous studies have shown that a mixture containing two parts of the casein solution and one part of the human serum produced a reliable, standard chemotactic attraction; and this mixture was used to fill the lower attractant compartment throughout this study (12). The upper chamber was filled with Hanks's solution. In the various studies of the effects of hydrocortisone, insulin, and rheumatoid and normal sera, the cell suspension was diluted with one-half its volume of the test solutions and incubated at 37°C for 30 min. The final suspension was then handled in the usual way with 4–5 drops being deposited on the Millipore filter using the cytocentrifuge. No attempt was made to wash the cells free of the test solutions. In each experiment using these test materials, a control was simultaneously incubated with Hanks's solution alone and used to establish the basal chemotactic index for the cells.

In the initial study comparing normal controls with patients with rheumatoid arthritis, chambers were set up in duplicate or triplicate. The accuracy of the method, which was found previously (12) to be 7%, was confirmed. Subsequent studies were done mostly with single chambers.

The activity of the rheumatoid disease was assessed on a 0 to 3+ scale on the basis of articular and systemic activity at the time blood was drawn from the chemotactic studies. In most cases blood was also drawn for estimation of the erythrocyte sedimentation rate (ESR: Westergren) and the anti-IgG activity determined by the latex flocculation test in serum inactivated by heating for 30 min at 56°C (15).

Serum levels of the immunoglobulins IgG, IgA, and IgM and the serum complement B_{1c}/B_{1a} values were measured by single radial diffusion in agar and reported in mg per 100 ml of serum. Commercial antibody-agar plates were used (Immunoplates [10]).

A semiquantitative assessment of the concentration of immunoglobulin, IgG, and IgM, rheumatoid factor (RF), and complement B_{1c}/B_{1a} on the surface of the PMN from 25 normal controls and 18 patients with rheumatoid arthritis was made. The cell suspension was further diluted with Hanks's solution and sedimented directly onto microscope slides using the cytocentrifuge. The slides were air dried and stored at 4°C. Immunofluorescent staining for IgG, IgM, and B_{1c}/B_{1a} was carried out using Hyland reagents in dilutions of 1:24, 1:16, and 1:32 respectively. Fluoresceinated human F-II was kindly supplied by Dr. J. H. Vaughan (16). All staining for each component was done at the same time and the amount of fluorescence on the cell surface was graded on a scale of 0 to 3+ by two observers independently.

[9] Pharmacia Fine Chemicals, Inc., Piscataway, N. J.

[10] Hyland Laboratories, Los Angeles, Calif.

RESULTS

As can be seen in Table I, the mean chemotactic index ±1 SD for the 24 patients with rheumatoid arthritis was 320 ±72 compared to 555 ±52 for the 24 age- and sex-matched normal controls ($P < 0.0005$). The mean chemotactic index ±1 SD for the eight patients with juvenile rheumatoid arthritis (Table II) was 435 ±72 for the matched controls ($P < 0.01$). In Table III are listed details of 22 patients with rheumatoid arthritis including 8 from Table I (same numbers used). The table includes the chemotactic index, latex titer, serum values of the immunoglobulins, and complement B_{1c}/B_{1a} and the semiquantitative assessment of the amount of immunoglobulin (IgG and IgM), complement B_{1c}/B_{1a}, and RF on the surface of the cells.

There was no correlation between the chemotactic index and age or sex in either the patients or controls. In the rheumatoid groups no correlation was noted between the chemotactic index and the activity of the disease (Table I).

There was no correlation between the chemotactic index and the latex activity in the serum of the 38 patients with adult onset rheumatoid arthritis (Table III). There was no correlation between the chemotactic index and the amounts of any of the proteins coating the cells, and no difference between the protein coating compared to normal controls was found. There was no correlation between the chemotactic index and the serum immunoglobulin values. However, the chemotactic index did correlate with the serum complement B_{1c}/B_{1a} value in 18 patients (Table III and Fig. 1). Although the serum complement values are associated with the lowest chemotactic indices (coefficient of correlation +0.678; $P < 0.01$).

A correlation was sought between the chemotactic index of the patients and drugs used in the treatment of the disease (Table I). The patients were either taking no drugs (four cases), salicylates or an equivalent preparation in a dose of up to 5 g per day, corticosteroids in doses of up to 15 mg of prednisolone per day, or both drugs. No correlation was found between the chemotactic index recorded each day. The presalicylate index was 502 and the indices on the four consecutive days during salicylate ingestion were 497, 482, 492, and 506. In three patients with severe connective tissue disease, the chemotactic indices were measured before and after five days' treatment with 60 mg prednisolone per day. These were patients with seropositive rheumatoid arthritis and vas-

TABLE I

Chemotactic Index in Patients with Rheumatoid Arthritis and Normal Controls

Rheumatoid arthritis								Normal	
Subject No.	Age	Sex	Mean chemotactic index	Latex titer	Disease activity 0–3+	Drug therapy: Salicylate	Drug therapy: Prednisolone	Age	Mean chemotactic index
						g/day	*mg/day*		
1	30	F	237	0	0	2	—	27	570
2	41	M	281	5120	+	4	—	42	618
3	47	M	292	5120	++	3	7½	47	484
4	47	F	332	80	++	4*	—	46	554
5	48	F	307	0	+	3	—	48	587
6	50	F	359	5120	+	—	—	52	565
7	57	F	368	80	+	—‡	12½	58	546
8	59	F	290	0	+	3§	—	60	563
9	61	M	305	2560	+	3*	—	61	529
10	62	F	237	1280	++	4	—	62	475
11	63	M	221	2560	++	3	10	63	491
12	64	M	295	640	++	4	—	63	588
13	64	M	300	5120	++	3	7½	64	586
14	65	F	406	640	++	3	10	63	548
15	67	F	315	640	++	3	—	67	595
16	67	F	399	320	+	—	—	67	455
17	68	F	265	0	++	3	—	69	531
18	68	F	384	160	+	4	5	71	507
19	69	F	128	0	+	2	—	71	522
20	74	F	356	5120	+	—*	—	73	657
21	77	F	401	160	+	3	—	75	610
22	77	F	392	5120	++	—	—	76	592
23	81	F	405	5120	++	3	40	80	630
24	88	F	411	0	+	—	—	83	517
Mean	62.3		320					62.0	555
Values (± 1 SD)			±72						±52

* Indomethacin (Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, Pa.) 100 mg/day.
‡ Phenylbutazone (Geigy Pharmaceuticals, Ardsley, N. Y.) 300 mg/day.
§ Gold sodium thiosulfate 50 mg/wk.

culitis, systemic lupus erythematosus, and polymyositis and were considered to be ill enough to warrant large dose corticosteroid therapy. The chemotactic index improved in each case (Fig. 2) and was accompanied by substantial clinical improvement. The chemotactic indices after treatment were within the normal range. An attempt to reproduce this effect of corticosteroids in vivo by incubating the PMN's from nine patients with hydrocortisone in vitro was unsuccessful. The mean chemotactic indices of these patients, who all had active disease despite drug therapy which included low dose corticosteroids in four cases, did not change from basal values after incubation with hydrocortisone in concentrations of 80 and 800 μg/100 ml (Fig. 3). The higher concentration of hydrocortisone represents a 40- to 80-fold increase on normal plasma cortisol values and is in excess of that which would be achieved by the administration of 60 mg of prednisolone per day to patients.

Although there was no evidence of a correlation between the chemotactic index and the latex titer or serum immunoglobulin levels, studies were undertaken to determine whether some component of rheumatoid serum could be responsible for the defect in chemotaxis. A correlation between chemotactic indices and the serum complement B_{1c}/B_{1a} has been noted in this study but a low complement value is unlikely to be the mechanism directly responsible for the defect in chemotaxis, as normal serum is used to provide an excess of complement in the chambers. Accordingly, the PMN's from seven normal controls were incubated with sera from three patients with high titre seropositive rheumatoid

TABLE II

Chemotactic Index in Patients with Juvenile Rheumatoid Arthritis and Normal Controls

Juvenile rheumatoid arthritis						Normal	
Subject No.	Sex	Age	Mean chemotactic index	Drug therapy: Salicylate	Drug therapy: Prednisolone	Age	Mean chemotactic index
				g/day	*mg/day*		
1	F	6	523	—	15	8	508
2	F	7	384	3	—	10	530
3	M	9	439	3	—	10	560
4	M	13	506	—	—	12	614
5	F	13	415	3	—	13	432
6	F	14	245	3	—	14	540
7	F	15	568	3	—	14	677
8	M	16	401	—	—	16	561
Mean		11.6	435			12.1	553
Values (± 1 SD)			±100				±72

arthritis who were known to have 22S complexes present in their serum by analytical ultracentrifugation. The chemotactic indices as a percentage of normal basal values are shown in Table IV. A reduction in chemotaxis was demonstrated for each serum being most marked in patient 3 (SH). Control studies using three normal sera did not show any change from basal values.

Fluorescent studies with anti-IgG and anti-IgM showed staining of numerous inclusions in the normal cells. The phagocytosis of these complexes from the rheumatoid sera was presumed to be the mechanism responsible for the induction of a defect in chemotaxis. In order to establish this effect as being due to complex ingestion, purified rheumatoid complexes were incubated with normal cells in four separate experiments (Table V). A constant, but variable degree of inhibition of chemotaxis was seen.

In an attempt to correlate the impairment of the chemotactic index with a definitive concentration of macromolecules presented for phagocytosis, the cells from six normal controls were incubated with 10-fold increases in concentration of iron dextran known to contain macromolecules with a mol wt of over 1 million. The mean chemotactic index expressed as a percentage of the normal basal value progressively fell as the concentration of iron dextran was increased (Fig. 4). Phase microscopy studies confirmed that the iron dextran macromolecules were freely ingested by the PMN.

DISCUSSION

In the system used in this study, chemotaxis of the PMN occurred in response to the standardized interaction of various components of complement and casein (17, 18). We have previously shown the method to be accurate and reproducible in 48 normal controls (12). The results of the present study, therefore, indicate a relative deficiency in the chemotaxis of the PMN from patients with rheumatoid arthritis when compared to normal controls. The results show also a similar, but less striking, deficiency in the chemotaxis of the PMN from patients with juvenile rheumatoid arthritis.

The defect in chemotaxis was not related to drug therapy and could not be correlated to the activity of the rheumatoid disease. However, the activity of the disease is difficult to assess including as it does in this and other studies a number of general features relating

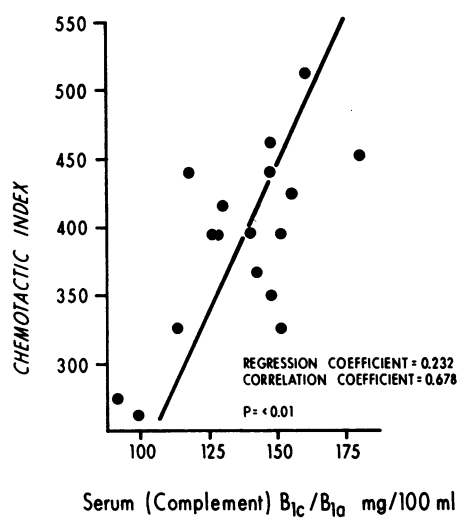

FIGURE 1 Chemotactic index and serum (complement) B_{1c}/B_{1a} values in 17 patients with rheumatoid arthritis.

TABLE III

Patients with Rheumatoid Arthritis Comparison of Chemotactic Indices, Serum Immunoglobulin and Complement B_{1c}/B_{1a} Values and the Protein Coating on the Cell

Case No.	Age	Sex	Serum values: Latex titer	IgG	IgA	IgM	B_{1c}/B_{1a}	Cell coating 0–3+: IgG	IgM	B_{1c}/B_{1a}	RF	Chemotactic index
				mg/100 ml								
4	47	F	80	2359	180	79	180	+	++	—	—	453
6	50	F	5120	—	—	—	—	+++	+	++	—	359
7	57	F	80	—	—	—	—	++	++	—	++	368
10	62	F	1280	2970	120	490	99	++	++	+	++	262
14	65	F	640	407	108	125	143	++	++	++	+++	367
20	74	F	5120	—	—	—	—	0	++	+	—	415
21	77	F	160	935	132	200	127	++	+++	+	+++	395
24	88	F	0	1170	112	51	147	+	++	++	+++	350
25	18	F	2560	—	—	—	—	++	++	+	++	424
26	38	F	5120	1172	144	187	130	+	++	++	+++	417
27	44	F	320	1210	132	210	91	+	++	+	++	275
28	47	F	320	1430	100	450	151	++	++	++	—	395
29	57	F	2560	776	157	125	127	++	++	++	+++	395
30	57	F	5120	1375	234	120	160	0	++	++	++	513
31	58	F	40	1133	67	47	147	+	++	+	+	441
32	59	F	640	1210	110	37	155	++	++	++	++	425
33	60	F	1280	—	—	—	—	++	++	+	++	402
34	65	F	1280	1100	132	153	151	++	++	++	+++	326
35	68	F	5120	1870	174	160	140	++	++	++	+	393
36	69	F	640	2277	156	240	114	++	++	++	++	326
37	71	F	5120	814	110	105	117	++	++	+	+	440
38	75	F	5120	1007	80	310	147	++	++	++	+	463

to the number of joints involved, the degree of articular and systemic involvement, and the ESR. The chemotactic index could only be correlated to the serum complement B_{1c}/B_{1a} value and it may be that in rheumatoid arthritis this is a relatively important measure of disease activity. The serum complement values were within the normal range as has been noted by others (19). However, Mongan, Cass, Jacox, and Vaughan (20) have shown that those patients with severe rheumatoid arthritis and particularly those with vasculitis tend to have the lowest complement values. No detailed examination for the presence of vasculitic lesions was made in our patients.

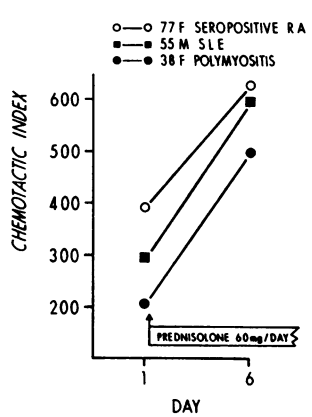

FIGURE 2 Effect of prednisolone on chemotactic indices in connective tissue diseases.

Although a number of the patients were taking up to 15 mg of prednisolone per day, this was not associated with more normal chemotactic indices. However, the administration of 60 mg of prednisolone per day to three patients with more severe connective tissue disease was associated with a return of the chemotactic index to normal values and was accompanied by an improvement in the activity of the disease. Since the incubation of the PMN's from patients with rheumatoid arthritis in substantial concentrations of hydrocortisone in vitro failed to improve the chemotactic indices, it appears that the in vivo effect of corticosteroids may have resulted simply from more prolonged contact of the cells with an elevated plasma cortisol concentration or from an indirect metabolic effect reflected as well in an improvement in disease activity. There are many metabolic abnormalities in patients with active rheumatoid arthritis, some of which can be quickly corrected by the administration of larger doses of corticosteroids (21).

Although a direct effect of corticosteroids on the PMN cannot be excluded, it seems less likely in view of the fact that low dose corticosteroid therapy in vivo was ineffective and since we have been able to correct a similar defect in chemotaxis of the PMN from patients with diabetes mellitus by incubating the cells with insulin and glucose (22). An attempt was made to see if the incubation of PMN from rheumatoid patients with insulin and glucose resulted in a similar improvement in chemotaxis but no change was found. The concentrations of insulin used were 100 μ and 10 mU/ml, which were known to be effective in correcting the impairment in PMN chemotaxis in diabetic subjects but which had no effect on normal cells (22).

Our failure to alter the chemotaxis of the PMN with hydrocortisone in vitro is in apparent disagreement with the findings of others. Ward (23) has shown that corticosteroids inhibit the chemotaxis of normal rabbit PMN. This may reflect a species difference but is more likely to be related to the concentrations of corticosteroids Ward employed which were some 15 times our highest concentration (0.14 mg/ml vs. 0.008 mg/ml). Ketchel, Favour, and Sturgis (24) found inhibition of migration of human PMN's after overnight incubation with hydrocortisone in concentrations greater than 1000 μg/100 ml. Dose differences cannot be implicated to explain these findings, but the longer incubation period employed by these authors (24) may explain the different findings. In addition, Ketchel et al. (24) were measuring random leukocyte migration rather than directed migration (chemotaxis).

From our results it appears that one mechanism which could be responsible for the defect in chemotaxis in patients with rheumatoid arthritis is the prior ingestion of complexes. It is known that leukocytes from the serum and synovial fluid of these patients contain IgG and IgM, probably as complexes (16, 25). Quantitation of this mechanism was provided by the experiment in which the incubation of the cells with varying concentrations of iron dextran produced proportional impairment in chemotaxis. It is not clear how the ingestion of macromolecules by the PMN impairs chemotaxis. It is possible that a cell that is full of phagocytosed material loses the response to chemotactic stimuli; but it appears more likely that phagocytosis, an energy-dependent activity, so depletes the limited energy sources of the cells that chemotactic activity, which is also energy dependent, is limited (26–28).

It is possible that the lowered complement values in those patients with the lowest chemotactic indices reflects the utilization of complement in some way in complex formation. However, it is suggested from our results

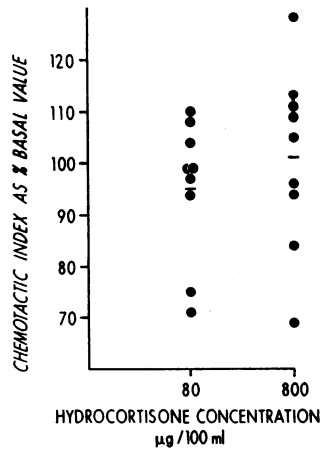

FIGURE 3 Chemotaxis of rheumatoid polymorphonuclear leukocytes after incubation with hydrocortisone.

TABLE IV

Results of the Incubation of Normal Polymorphonuclear Leukocytes with Rheumatoid and Normal Sera

Cells from normal subject	Basal chemotactic index	Chemotactic index as % basal value					
		Rheumatoid sera			Normal sera		
		1	2	3	1	2	3
1	550	60	65	38	—	—	—
2	578	79	77	49	—	—	—
3	537	84	80	39	—	—	—
4	500	88	70	77	—	—	—
5	489	61	79	48	—	—	—
6	525	79	75	45	99	97	101
7	540	77	73	43	99	98	102
8	516	—	—	—	95	90	99
9	465	—	—	—	104	104	104
Mean % basal value		75	74	48	99	97	102

TABLE V

Chemotactic Index after Incubation with Complexes

Cells from normal subjects	Compartment contents on starting side*		
	Hanks's solution	Hanks's solution and complexes	
1	390	127	
	450	326	
		310	
2	486	412	
	419	220	
		309	
3	344	236	
	260	196	
		372	
4	580	96	
	585	32	
Average	439	240	$P = 0.02$

* Casein and complement on attractant side for all chambers.

that the decreased chemotaxis involves more than phagocytosis of available complexes as the chemotactic index could not be correlated with the serum level of RF as measured by latex activity, and abnormal chemostaxis was noted in patients both with adult onset and juvenile rheumatoid arthritis who were seronegative by the usual tests. The possibility that RF and complexes were actually present in those patients which could be detected by more sensitive techniques (29) was not eliminated.

Support for this concept is found in several studies in which the serum complement value, whether measured as B_{1c}/B_{1a} or $C'H_{50}$, in the serum and joint fluid of patients showed only weak or no correlation to the latex

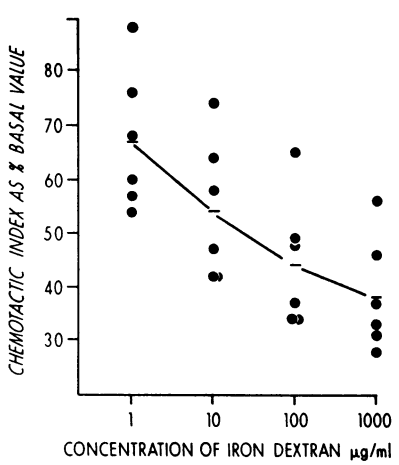

FIGURE 4 Chemotaxis of normal polymorphonuclear leukocytes after incubation with iron dextran.

titer in the serum or joint fluid (19, 20, 30) and no correlation could be demonstrated in this series. Previous workers (31) have suggested from studies of neutrophil function in patients with multiple myeloma or macroglobulinaemia that the noted impairment in adhesion and phagocytosis may be due to a protein coating on the cells. Our studies on the amount of coating on the PMN of IgG, IgM, RF, and complement B_{1c}/B_{1a} showed no significant differences from normal cells and could not be correlated with the chemotactic indices.

If these results represent a real impairment of PMN function, it could explain the increased incidence of infection in patients with rheumatoid arthritis and, at present, is the only reported abnormality in PMN function which could be responsible. In preliminary studies with Dr. Tom Hoffman, we have confirmed the work of Brandt and Hedburg (8) that phagocytosis by the PMN is normal in patients with rheumatoid arthritis. An intriguing possibility is the consideration that decreased chemotaxis in the cells of these patients reflects a more basic manifestation of the disease. In several studies (32, 33) it has been noted that patients with rheumatoid arthritis had an increased rate of pulmonary infection prior to the development of the rheumatoid disease. This could be due to an inherent defect in the PMN and raises the possibility that this defect could make the patient more susceptible to an infectious agent or agents which cause rheumatoid arthritis.

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