Clin. exp. Immunol. (1979) **36**, 423–429.

Inhibition of neutrophil migration by sera from patients with rheumatoid arthritis

A. S. KEMP, P. ROBERTS-THOMSON, S. H. NEOH & SUSAN BROWN *Department of Clinical Immunology, Flinders Medical Centre, Bedford Park, S.A.* 5042, *Australia*

(*Accepted for publication* 3 *January* 1979)

SUMMARY

Sera from patients with rheumatoid arthritis inhibited the migration of human neutrophils in 63% (twenty-two out of thirty-five) of the cases tested. The inhibition was not due to a toxic effect of the serum as it was reversed by a chemotactic stimulus. There was a strong correlation between the degree of inhibition of neutrophil migration and the amount of immune complexes present in the sera as determined by the C1q binding activity. It is suggested that the inhibition of neutrophil migration is due to the presence of circulating immune complexes, and that the capacity of immune complexes to inhibit neutrophil migration *in vitro* may also contribute to the accumulation of neutrophils at sites of immune complex formation *in vivo*.

INTRODUCTION

An increase in the number of neutrophils within the joint space is a feature of inflammation in rheumatoid arthritis (Ropes & Bauer, 1953). A mechanism proposed for this accumulation of neutrophils is chemotactic attraction by complement components activated by the immune complexes (Ward & Zvaifler, 1971) which can be demonstrated in the serum (Zubler *et al.*, 1976) and synovial fluid (Zubler *et al.*, 1976; Haanestad, 1967). Paradoxically, attempts to activate complement by IgM rheumatoid factor-IgG complexes *in vitro* decreased the chemotactic activity of serum (Wagner, Abraham & Baum, 1974). However, in addition to the generation of chemotactic factors, immune complexes might also affect neutrophil migration by a direct interaction with the neutrophil surface. This concept is supported by the finding that IgG complexes in the form of heat-aggregated gammaglobulin can inhibit neutrophil migration in the absence of any chemotactic gradient (Kemp, Roberts-Thomson & Brown, 1979). In this study we explore the possibility that immune complexes found in rheumatoid arthritis may inhibit neutrophil migration and thus contribute to the accumulation of neutrophils at sites of immune complex formation.

PATIENTS AND METHODS

Patients. Sera were collected from forty-five adult patients being bled for routine laboratory tests, who had classical or definite rheumatoid arthritis fulfilling ARA criteria. Control sera were obtained from healthy adult volunteers. All sera were stored at −80°C prior to use.

Neutrophil migration. Neutrophil migration was determined following the method of Wilkinson (1974) utilizing sawn off tuberculin syringes with 3 μ pore size filters (Millipore Corporation, Bedford, Massachusetts) glued to the end as chemotactic chambers.

Preparation of neutrophil suspensions and quantification of migration is described in greater detail elsewhere (Kemp *et al.*, 1979). Briefly, neutrophils from normal group O donors were separated by centrifugation through Ficoll–Hypaque followed by sedimentation of red cells in 2% dextran/phosphate buffered saline. Neutrophils were suspended in 10% serum and

Correspondence: Dr A. S. Kemp, Department of Clinical Immunology, Flinders Medical Centre, Adelaide, Australia 5042.

0099-9104/79/0060-0423 $02.00

Hanks' balanced salt solution (HBSS) at a concentration of $2{\cdot}5 \times 10^6$ ml. The cells were incubated at 37°C for 30 min prior to the transfer of 0·2 ml of the suspension to chemotactic chambers to determine migration with HBSS alone on the attractant side of the filter. Assays were incubated for 60 min at 37°C in an atmosphere of 5% CO_2 in air. In the experiment where equal concentrations of serum were placed on both sides of the filter, 0·1 ml of neutrophil suspension was placed above, and 0·4 ml of medium below the filter as described previously (Kemp *et al.*, 1979).

Filters were read on a Leitz Ortholux microscope with a ×40 objective. Migration was determined as the distance in microns from the top of the filter to the leading front, as measured by the furthest plane containing at least three cells in focus. Three fields were read per filter. Migration was expressed either as the mean distance in microns plus or minus the standard error of the means obtained from each of the triplicate filters, or as a percentage of the migration observed when neutrophils were suspended in 10% fresh autologous serum. Casein ('Hammarsten', Merck AG, Darmstadt) below the filter was used a a chemotactic stimulus at a concentration of 5·0 mg/ml in HBSS.

C1q binding assay. C1q binding was performed as described by Zubler *et al.* (1976). The percentage C1q binding activity for 144 sera from normal blood donors was 12·5±3·3 (mean±s.d.).

Aggregated gammaglobulin. Heat-aggregated gammaglobulin (HAGG) was prepared by aggregation of gammaglobulin, Cohn Fraction II (Commonwealth Serum Laboratories, Melbourne) at 63°C for 20 min and removal of monomer IgG by gel filtration on Sepharose 6B (Pharmacia Fine Chemicals AB, Sweden) (Kemp *et al.*, 1979).

Drugs. D-penicillamine tablets (Dista), sodium aurothiomaleate (May & Baker) and prednisolone sodium phosphate (Glaxo) were dissolved in HBSS containing 10% serum. Two sera containing salicylate were obtained 90 min after an oral dosage of 1·8 g soluble aspirin. Final drug concentrations of gold 1·0 μg/ml, sodium prednisolone 2·0 μg/ml and penicillamine 10 μg/ml correspond to the highest values that would be found in 10% serum if serum concentrations were at the upper limits of those expected in clinical practice (Rubinstein & Dietz, 1973; Leclercq & Copinschi, 1974; Mowat, 1978).

Rheumatoid factor. Rheumatoid factor was assayed by the Rose–Waaler test. Sera with titres of greater than 1/16 were regarded as positive.

Statistical evaluation. Results were evaluated by the Mann–Whitney U test and linear regression analysis by the method of least squares.

RESULTS

Inhibition of neutrophil migration

The migration of neutrophils suspended in 10% control sera or 10% sera obtained from patients with rheumatoid arthritis was compared (Fig. 1). The migration of neutrophils suspended in twenty-four control sera was 107±14% (mean±s.d., $n = 24$). The migration of neutrophils suspended in twenty-two out of thirty-five sera obtained from patients with rheumatoid arthritis was reduced below 2 s.d. of the migration observed in the control sera. The difference between the two groups was significant ($P < 0{\cdot}001$). The addition of HAGG (50 μg/ml) to the autologous sera inhibited migration in all cases tested (Fig. 1).

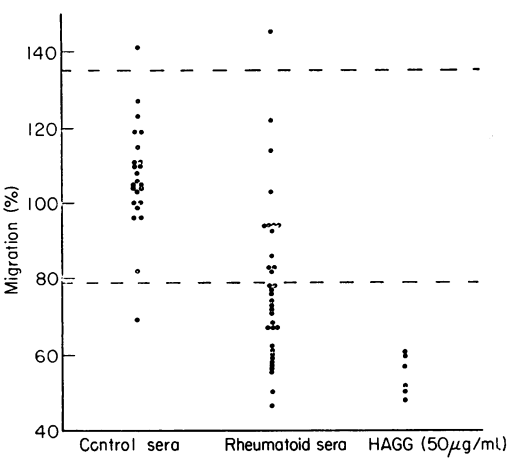

FIG. 1. Migration of neutrophils suspended in 10% normal control sera, 10% sera from patients with rheumatoid arthritis and 10% autologous sera containing 50 μg/ml of heat-aggregated gammaglobulin. In each case, migration is expressed as a percentage of that observed when neutrophils were suspended in 10% fresh autologous sera. 2 s.d. of the migration in control sera are indicated by dotted lines.

Effect of a chemotactic stimulus

The effect of a chemotactic stimulus (casein) on the migration of neutrophils suspended in normal sera and sera from patients with rheumatoid arthritis was examined (Fig. 2). Cells suspended in 10% control sera or sera from rheumatoid arthritis patients which were not inhibitory to migration exhibited minimal chemotactic effect after the addition of casein. The mean increase in migration was 5 μ ($n = 5$) for cells suspended in control sera, and 13 μ ($n = 8$) for cells suspended in sera from rheumatoid arthritis patients which failed to significantly inhibit neutrophil migration. In contrast, neutrophils suspended in sera from patients with rheumatoid arthritis which were inhibitory to migration showed a mean increase in migration of 42 μ ($n = 6$). The migration in inhibitory sera after the addition of a chemotactic stimulus became equivalent to that observed in non-inhibitory patients' sera or control sera.

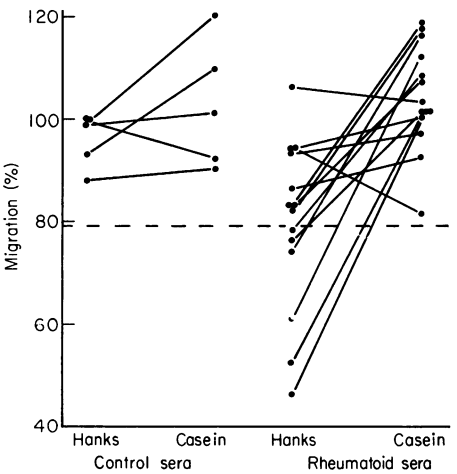

FIG. 2. Migration of neutrophils suspended in 10% normal control sera or 10% sera from patients with rheumatoid arthritis in the presence and absence of the chemotactic stimulus casein. In each case migration is expressed as a percentage of that observed when neutrophils were suspended in 10% fresh autologous serum alone. The dotted line indicates the lower limit of the previously determined 2 s.d. of neutrophil migration in control sera.

Negative correlation between neutrophil migration and C1q binding activity

Neutrophil migration was compared with C1q binding activity in twenty-three sera from patients with rheumatoid arthritis (Fig. 3). There was a highly significant negative correlation ($r = -0{\cdot}79$, $P<0{\cdot}001$) between the neutrophil migration and the C1q binding activity of the sera.

Correlation between neutrophil migration and rheumatoid factor titre

There was no significant correlation ($r = -0{\cdot}28$, $P>0{\cdot}05$, $n = 25$) between neutrophil migration and rheumatoid factor titres (Fig. 4). The presence of rheumatoid factor *per se* did not cause inhibition as five sera with titres of 1/128 or greater did not inhibit neutrophil migration.

Effect of drugs

Neutrophil migration was not inhibited by gold, prednisolone and penicillamine (Table 1). Comparison of migration in sera collected before and 90 min after an oral dose of 1·8 g of soluble aspirin showed that salicylate somewhat increased neutrophil migration (Table 1).

Removal of chemotactic gradient

If the inhibition observed were due to the establishment of a negative chemotactic gradient as a result of complement activation, then it should be reversed with the same concentration of serum placed on both sides of the filter. The inhibitory activity of three rheumatoid sera either above or on both sides of

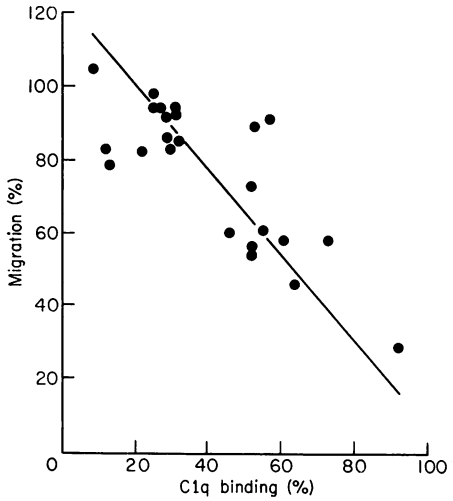

FIG. 3. Correlation between neutrophil migration and the C1q binding activity. $r = -0{\cdot}79$ $P < {\cdot}001$, $n = 23$

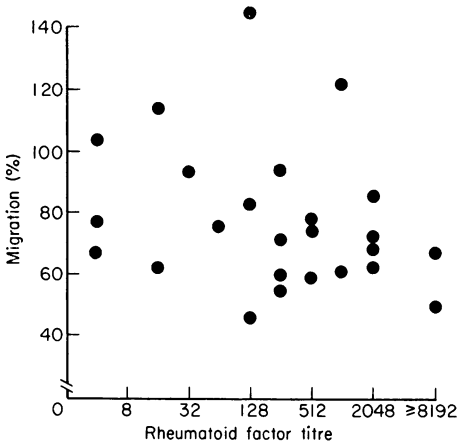

FIG. 4. Correlation between neutrophil migration and rheumatoid factor titre. $r = -0{\cdot}28$, $P > 0{\cdot}05$, $n = 25$.

the filter was examined (Table 2). As observed previously (Kemp *et al.*, 1979), the chemokinetic effect of serum is greater when 10% serum is placed on both sides of the filter. All three sera were inhibitory to migration when equal concentrations were placed on both sides of the filter, thus removing any potential chemotactic gradient.

DISCUSSION

The migration of human neutrophils suspended in serum obtained from patients with rheumatoid arthritis is significantly reduced when compared to the migration of neutrophils suspended in sera obtained from normal controls. The inhibition observed was not due to a toxic effect of the serum on neutrophil migration as neutrophils suspended in inhibitory sera could migrate a normal distance into the filter under the influence of the chemotactic stimulus, casein. Furthermore, the presence of salicylate, gold, penicillamine and prednisolone in concentrations at least equivalent to those to be found in 10% diluted serum *in vitro* did not inhibit neutrophil migration.

Two pieces of evidence suggest that the inhibition of migration is due to the presence of immune complexes in the serum of patients with rheumatoid arthritis. Firstly, the addition of artificially prepared

TABLE 1. Effect of drugs on neutrophil migration

Drug	Migration (μ) (mean ± s.e.)
Experiment (i)*	
Nil	59 ± 2
Gold 1·0 μg/ml	57 ± 4
Sodium prednisolone 2·0 μg/ml	59 ± 1
Penicillamine 10 μg/ml	55 ± 2
Experiment (ii)†	
Autologous serum	66 ± 2
Autologous serum + salicylate	76 ± 3
Homologous serum	69 ± 2
Homologous serum + salicylate	84 ± 3

* Neutrophils suspended in HBSS containing 10% autologous serum plus the concentration of drug indicated.

† Neutrophils suspended in HBSS containing 10% autologous or homologous serum taken before and 90 min after ingestion of 1·8 g of soluble aspirin. In both cases, the salicylatec oncentration after aspirin ingestion was 138 μg/ml.

TABLE 2. Removal of chemotactic gradient

	Migration (μ) (mean ± s.e.)	
Serum*	Serum above	Serum both sides
Autologous	45 ± 3	89 ± 1
Rheumatoid a	29 ± 3 (64)†	65 ± 3 (73)
Rheumatoid b	24 ± 0 (53)	31 ± 2 (35)
Rheumatoid c	29 ± 1 (64)	51 ± 3 (57)

* Neutrophils were pre-incubated in normal or rheumatoid serum for 30 min at 37°C and then 0·1 ml volumes were placed above the filter with either HBSS, or HBSS and 10% serum below the filter and incubated for 40 min.

† The migration of neutrophils suspended in rheumatoid sera expressed as a percentage of the autologous control is shown in parentheses.

complexes in the form of HAGG to normal sera inhibits the migration of neutrophils, and this inhibitory effect can also be reversed by the chemotactic stimulus casein (Kemp *et al.*, 1979). Secondly, there was a strong correlation between the inhibition of migration and the C1q binding activity of the sera. C1q binding activity in rheumatoid sera is considered to indicate the presence of immune complexes (Zubler *et al.*, 1976) and the majority of patients with rheumatoid arthritis have evidence of circulating immune complexes as measured with the C1q binding assay (Zubler *et al.*, 1976). Neutrophil migration inhibition (Kemp *et al.*, 1979) and the C1q binding assay (Zubler *et al.*, 1976) have an equivalent sensitivity in the detection of HAGG (30–50 μg of HAGG/ml in the presence of normal serum). Thus, if both assays do detect circulating immune complexes it might be expected that a correlation could be demonstrated, as in the present experiments.

The mechanism by which rheumatoid sera can inhibit neutrophil migration is not known. Inhibition

was not due to the formation of a negative chemotactic gradient as a result of complement activation, as the inhibitory activity was still apparent when equal concentrations of rheumatoid serum were placed on both sides of the filter. In contrast, the inhibition produced by activated complement components is removed if equal concentrations are placed on both sides of the filter thus removing any potential gradient (Kemp *et al.*, 1979). It has been suggested that phagocytosis of immune complexes (Mowat & Baum, 1971) may cause the reduced chemotactic index observed after incubation of neutrophils in rheumatoid sera (Mowat & Baum, 1971; Beeuwkes & Bijlsma, 1974). In the present system, inhibition mediated by HAGG was reduced by washing cells after pre-incubation with HAGG, and pre-incubation with HAGG was equally effective at 4°C or 37°C (Kemp *et al.*, 1979). These two findings suggest that a surface interaction between neutrophil and aggregate, rather than phagocytosis, is important in the inhibition observed. Furthermore, neutrophils suspended in all rheumatoid factor positive sera can be shown to phagocytose IgG (Cats, Lafeber & Klein, 1975), yet not all rheumatoid factor positive sera were inhibitory to migration. By analogy with the observed effects of HAGG on neutrophil random migration (Kemp *et al.*, 1979), we suggest that inhibition is mediated by an interaction of immune complexes and the neutrophil surface which interferes with the chemokinetic activity of serum.

There was a strong correlation between the amount of immune complexes in serum and the degree of inhibition of neutrophil migration. This reduction in motility could contribute to the reduced chemotactic index observed after incubation of neutrophils in rheumatoid sera (Mowat & Baum, 1971; Beeuwkes & Bijlsma, 1974), and indicates a biological effect of immune complexes on neutrophils, in addition to the activation of the hexose monophosphate shunt (Henson & Oades, 1975), release of superoxide anion (Johnston & Lehmeyer, 1976) and lysosomal enzymes (Henson, Johnson & Spiegelberg, 1972), and the reduced phagocytic activity (Turner, Schumacher & Myers, 1973; MacLennan *et al.*, 1973) described previously. This effect of immune complexes suggests one factor which could promote the accumulation of neutrophils at sites of immune complex formation *in vivo*. Interaction of immune complexes and neutrophils, attracted by chemotactic stimuli, would reduce motility and thus diminish the numbers of cells leaving the site. However, the relative contributions of chemotactic stimuli and inhibitory influences to the accumulation of neutrophils *in vivo* are not yet clearly defined (Keller, Hess & Cottier, 1975).

We thank Dr R. Geddes for allowing us to collect sera from his patients and Barbara McManus for typing the manuscript.

REFERENCES

Beeuwkes, H. & Bijlsma, A. (1974) Reduced chemotaxis of polymorphonuclear leukocytes in sera from patients with rheumatoid arthritis. *Antonie van Leeuwenhoek*, **40**, 233.

Cats, A., Lafeber, G.J. & Klein, F. (1975) Immunoglobulin phagocytosis by granulocytes from sera and synovial fluids in various rheumatoid and non-rheumatoid diseases. *Ann. rheum. Dis.* **34**, 146.

Haanestad, K. (1967) Presence of aggregated γG-globulin in certain rheumatoid synovial effusions. *Clin. exp. Immunol.* **2**, 511.

Henson, P.M., Johnson, H.B. & Spiegelberg, H.L. (1972) The release of granule enzymes from human neutrophils stimulated by aggregated immunoglobulins of different classes and subclasses. *J. Immunol.* **109**, 1182.

Henson, P.M. & Oades, Z.G. (1975) Stimulation of human neutrophils by soluble and insoluble immunoglobulin aggregates. Secretion of granule constituents and increased oxidation of glucose. *J. clin. Invest.* **56**, 1053.

Johnston, R.B. Jr. & Lehmeyer, J.E. (1976) Elaboration of toxic oxygen by-products by neutrophils in a model of immune complex disease. *J. clin. Invest.* **57**, 836.

Keller, H.U., Hess, M.W. & Cottier, H. (1975) Physiology of chemotaxis and random motility. *Semin. Hematol.* **12**, 47.

Kemp, A.S., Roberts-Thomson, P. & Brown, S. (1979). Inhibition of human neutrophil migration by aggregated gammaglobulin. *Clin. exp. Immunol.* **36**, 334.

Leclerq, R. & Copinschi, G. (1974) Patterns of plasma levels of prednisolone after oral administration in man. *J. Pharmacokinet. Biopharm.* **2**, 175.

MacLennan, I.C., Howard, A., Gotch, F.M. & Quie P.G. (1973) Effector activating determinants on IgG, I. The distribution and factors influencing the display of complement, neutrophil and cytotoxic B-cell determinants on human IgG sub-classes. *Immunology*, **25**, 459.

Mowat, A.G. (1978) Neutrophil chemotaxis in rheumatoid arthritis. Effect of D-penicillamine, gold salts and Levamisole. *Ann. rheum. Dis.* **37**, 1.

Mowat, A.G. & Baum, J. (1971) Chemotaxis of polymorphonuclear leukocytes from patients with rheumatoid arthritis. *J. clin. Invest.* **50**, 2541.

Ropes, M.W. & Bauer, W. (1953) *Synovial Fluid Changes in Joint Disease*, p. 53. Harvard University Press, Cambridge.

Rubinstein, H.M. & Dietz, A.A. (1973) Serum gold. II. Levels in rheumatoid arthritis. *Ann. rheum. Dis.* **32**, 128.

Turner, R.A., Schumacher, R. & Myers, A.R. (1973) Phagocytic function of polymorphonuclear leukocytes in rheumatic disease. *J. clin. Invest.* **52**, 1632.

Wagner, T., Abraham, G. & Baum, J. (1974) The roles of IgG, IgM rheumatoid factor, and their complexes in the induction of polymorphonuclear leukocyte chemotactic

factor from complement. *J. clin. Invest.* **53**, 1503.

WARD, P.A. & ZVAIFLER, N.J. (1971) Complement-derived leukotactic factors in inflammatory synovial fluids of humans. *J. clin. Invest.* **50**, 606.

WILKINSON, P. (1974) *Chemotaxis and Inflammation*, p. 168. Churchill Livingstone, Edinburgh and London.

ZUBLER, R.H., NYDEGGER, U., PERRIN, L.H., FEHR, K., MCCORMACK, J., LAMBERT, P.H. & MIESCHER, P.A. (1976) Circulating and intra-articular immune complexes in patients with rheumatoid arthritis. Correlation of ^{125}I-Clq binding activity with clinical and biological features of the disease. *J. clin. Invest.* **57**, 1308.