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Correlation of antibody to rheumatoid arthritis associated nuclear antigen and immune complexes to disease activity in patients with rheumatoid arthritis

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SUMMARY

Twenty-seven patients with rheumatoid arthritis (RA) maintained on drug regimens were studied monthly for 6–10 months. Disease activity was assessed and levels of anti-RA associated nuclear antigen (RANA) and immune complexes were determined. Anti-RANA generally paralleled disease activity in 64% of cases. Immune complex levels paralleled disease activity in 56% of cases and paralleled anti-RANA in 52% of patients. Immune complexes paralleled anti-RANA together with disease activity in only 33% of patients. Anti-RANA and/or immune complex levels paralleled disease activity indices in 82% of cases. Significant fluctuations ($\geqslant$four-fold) in anti-RANA were frequently found (65%) and were associated with concordant changes in immune complex levels 50% of the time and with changes in disease activity indices 59% of the time. The data suggest that levels of anti-RANA and immune complexes may be important in RA and warrant further investigation.

INTRODUCTION

Previous investigations demonstrated that sera from patients with seropositive rheumatoid arthritis (RA) frequently (90%) have elevated levels of antibody to RA associated nuclear antigen (RANA) (Alspaugh & Tan, 1975, 1976) and that RANA was associated with Epstein–Barr virus (EBV) (Alspaugh *et al.*, 1978). While further studies showed that EBV specific antibodies are elevated in RA (Henle *et al.*, 1979; Alspaugh *et al.*, 1981; Ferrell *et al.*, 1981), they never fluctuated with disease activity and therefore, there was no evidence to support their role in the pathogenesis of RA (Alspaugh *et al.*, 1981). In contrast, a preliminary study in our laboratory suggested that anti-RANA may change in some patients having RA (Alspaugh *et al.*, 1979).

Previous studies have shown that circulating immune complex like materials are elevated in RA (Nydegger *et al.*, 1977; Gupta *et al.*, 1979; Theofilopoulos & Dixon, 1979) and that there may be a correlation between such immune complex-like material and the disease activity or the presence of extra-articular disease (Gupta *et al.*, 1979; Zubler *et al.*, 1976; Hay *et al.*, 1979). The purpose of this study was to determine if anti-RANA titres and levels of immune complexes in RA patients change in the course of the disease, and whether changes parallel each other and disease activity.

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MATERIALS AND METHODS

Patients. Twenty-seven patients fulfilling the ARA criteria for classical or definite RA (American Rheumatism Association, 1973) were studied monthly prospectively over a period of 6–10 months. Patient data, maintenance drug regimens (Table 1) and levels of Epstein–Barr virus (EBV) specific antibodies and their relationships to disease activity have been reported previously (Alspaugh *et al.*, 1981). Five patients were in an early structural stage, 13 in moderate and 10 were in a severe structural stage. Three patients were in functional class I, 19 in class II, and six in class III (McCarty, 1974). One patient was studied serially retrospectively after the initiation of gold therapy. Twelve patients with osteoarthritis (OA) and 16 apparently normal adults served as controls.

Disease activity. The disease activity was determined as previously described (Alspaugh *et al.*, 1981; McCarty, 1974) and reported as an activity index. The joint counts, joint indices, systemic indices, as well as the individual parameters of the systemic index were each graphed monthly (Lansbury, 1957, 1958) in addition to the activity indices. In this study, the systemic indices paralleled the activity indices for at least two-thirds of the study period in all cases. However, no one parameter of the systemic index always correlated well with the others and justified averaging. The joint counts paralleled the systemic indices for at least two-thirds of the study period in 74% of these cases. Disease indices and other laboratory parameters were compared with anti-RANA levels so that it could be determined if they paralleled anti-RANA levels. Since joint counts were more sensitive than joint indices and they always parallel each other, the joint counts were used for analysis to determine parallelism rather than joint indices. Changes ($\geqslant 10$) in the activity indices from one month to the next were arbitrarily set so that concordance or discordance with anti-RANA changes (four-fold) could be determined.

Antibody detection. All antibody determinations on each patient were performed at the same time. Rheumatoid factor levels were determined by the Rheumaton test (Wampole Laboratories, Cranbury, New Jersey, USA). Anti-RANA was determined by the double immunodiffusion method, using one lot of WI-L2 extract as previously described (Alspaugh & Tan, 1975). Immunodiffusion was used for these studies rather than the more sensitive indirect immunofluorescent test (Alspaugh & Tan, 1976) because it is presently the only specific test for the detection of anti-RANA. Changes in anti-RANA were considered significant only if they increased or decreased

Table 1. Comparison of levels of anti-RANA, rheumatoid factor, immune complexes and disease activity in patients with RA on different drug therapies

Group	Drug therapies	Number of patients	Frequency	Geometric mean titres		Means	
			anti-RANA	anti-RANA	RF*	IC† (μg AHG/ml)	AI‡
	NSAID§						
I	Aspirin	4	4/4	21	287	356	28
II	Carprofen¶	10	9/10	4·4	181	373	41
III	Gold + NSAID	4	3/4	7·7	233	248	55
IV	Prednisone** + NSAID	1	1/1	6·4	80	279	36
V	Gold + prednisone* + NSAID	9	9/9	5·3	210	196	44
	Total	28	26/28 (93%)				

*RF = rheumatoid factor; †IC = immune complexes; ‡AI = activity index; §NSAID = non-steroidal anti-inflammatory drug; ¶Carprofen = drug under investigation (Hoffmann-LaRoche);**prednisone = low dose (< 10 mg daily).

There was a statistical difference in immune complex levels when comparing groups II and V ($P = 0{\cdot}005$). There was no statistical difference in the activity index when comparing groups II and V.

equal to or greater than four-fold from one month to the next. Double immunodiffusion was also used for the detection of anti-SS-A, -SS-B, -Scl-70, -Sm, -ribonucleoprotein (RNP) (Scopelitis, Biundo & Alspaugh, 1980).

Immunoglobulin levels. IgG, IgA and IgM levels in sera from each patient were determined at the same time by radial immunodiffusion (Meloy Laboratories, Springfield, Virginia, USA).

Method of immune complex detection. All serum samples were tested blindly by the Raji cell radioimmunoassay for levels of immune complexes as described previously (Gupta *et al.*, 1978). The amount of immune complex like materials in each serum tested was expressed in terms of μg aggregated human globulin (AHG)/ml equivalents (μg AHG eq/ml). Immune complex levels of 55 μg AHG eq/ml were considered elevated based on results of 100 normal sera tested. The mean value was 27·3 μg AHG eq/ml (mean + 2 s.d. = 55 μg AHG eq/ml). A significant variation in immune complexes from one month to the next was arbitrarily set at 55 μg AHG eq/ml so that comparisons with other parameters of disease activity could be made. The Raji cell assay was the only test performed for immune complex levels since it has been previously shown to correlate well with the monoclonal rheumatoid factor test and the C1q binding test in RA (Gupta *et al.*, 1979).

Complement determinations. Complement levels were determined by both a haemolytic assay and radial immunodiffusion. All sera from each patient were tested at the same time. In the first method C3 was detected by radial immunodiffusion (Meloy Laboratories). Plasma levels of complement were determined by a haemolytic CH_{50} test using rabbit (IgM) antibody to sheep red blood cell membrane to sensitize cells (Kabat & Mayer, 1961). Plasma in EDTA was obtained monthly from apparently normal individuals and stored at −70°C along with patient samples to determine the normal range (80–125 units).

Biometrics. Geometric mean titres of antibody levels were calculated by logarithmic transformation of the geometric mean (Snedecor & Cochran, 1978a). We tested the hypothesis that there was no difference between mean scores of different groups by the Student's *t*-test (Snedecor & Cochran, 1978b).

RESULTS

Anti-RANA generally paralleled the activity indices, systemic indices and/or the joint counts for at least two-thirds of the study period in 64% of patients. This 64% could be broken down into: (1) cases where anti-RANA paralleled both the systemic indices and joint counts (30%); (2) paralleled the systemic indices alone (26%); (3) paralleled the joint counts (4%) and (4) paralleled the activity indices better than either of the other indices (4%). Thus, anti-RANA followed the systemic index most frequently. Figs 1 & 2 are examples of 64% of the cases where anti-RANA generally paralleled disease activity. Immune complexes paralleled the activity indices, systemic indices and/or joint counts in 56% of the patients. This 56% could be broken down into: (1) cases that paralleled both the systemic and joint indices (26%); (2) paralleled the systemic indices (19%) and (3) cases that more closely paralleled the joint counts (11%). Thus, immune complexes paralleled the systemic index most frequently. Anti-RANA and immune complexes together paralleled disease activity in only 33% of patients (Figs 1 & 2). However, anti-RANA and/or immune complex levels paralleled disease indices in 82% of patients.

We were able to study one patient retrospectively, who had been treated with various NSAIDs and steroids without improvement and then placed on gold (Fig. 3). The significant reduction in anti-RANA appeared to be correlated with reduction in disease activity as a result of therapy and remained at normal levels during remission.

Rheumatoid factor was found in 89% of patients but rarely changed with anti-RANA or disease activity (Fig. 3).

Anti-RANA showed at least one significant change of titre in 17 of 26 patients (65%). Rheumatoid factor showed at least one significant change only in seven of 25 (28%) of patients. However, anti-RANA often fluctuated significantly from one month to the next. In fact, there were five patients who had two significant changes, one who had three and one who had four changes within the study. Anti-RANA frequently made concordant changes with immune complexes (50%)

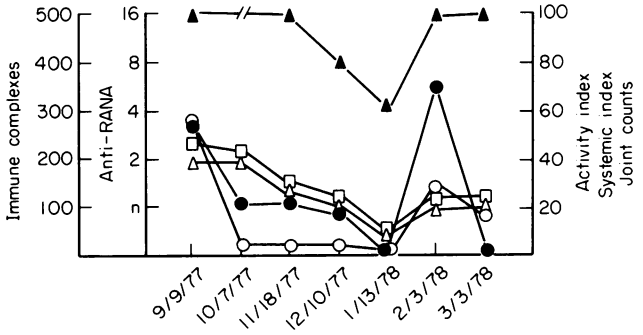

Fig. 1. Patient J.B. is a Caucasian male with seropositive RA, being maintained on gold and low dose steroids. Anti-RANA generally paralleled all of the indices, but possibly followed the systemic and activity indices most closely. Immune complexes also paralleled disease indices and anti-RANA. ● = immune complexes; ▲ = anti-RANA; □ = activity index; △ = systemic index; ○ = joint counts.

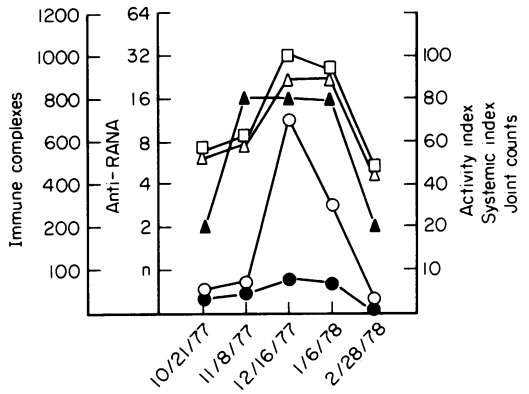

Fig. 2. Patient J.P. is a Caucasian female with seropositive RA, being maintained on gold and low dose steroids. In this case, anti-RANA and immune complex levels generally paralleled all the disease indices but paralleled the systemic and activity indices more closely. For key see Fig. 1.

and activity indices (59%), but anti-RANA, disease activity and immune complexes only made concordant changes together 37% of the time. Only one of the 12 osteoarthritis patients and one of the 16 apparently normal individuals had anti-RANA antibody in undiluted sera and this did not fluctuate within the detection level of this test.

Only three of 22 patients (14%) tested for C3 and two of 11 (18%) tested for CH_{50} levels showed large abrupt changes from one month to the next. All were associated with increased immune complex levels.

Fifty-seven percent of patients had elevated immunoglobulin levels at least once within the study, but noteable changes were detected in only seven patients. In two cases changes in IgG appeared to correlate with anti-RANA changes.

There was no apparent relationship of drug therapy to the number of significant changes in antibody titre or concordance or discordance with immune complexes or disease activity.

The 28 patients were divided into groups on the basis of drug regimens and compared for parameters of disease activity. Table 1 shows the frequency of elevated anti-RANA, geometric mean titres of anti-RANA and rheumatoid factor, and immune complex and activity index means. The frequency of anti-RANA was defined by whether it was found at least once within a serial set, and the means of immune complexes and activity indices were calculated over a 6 month period.

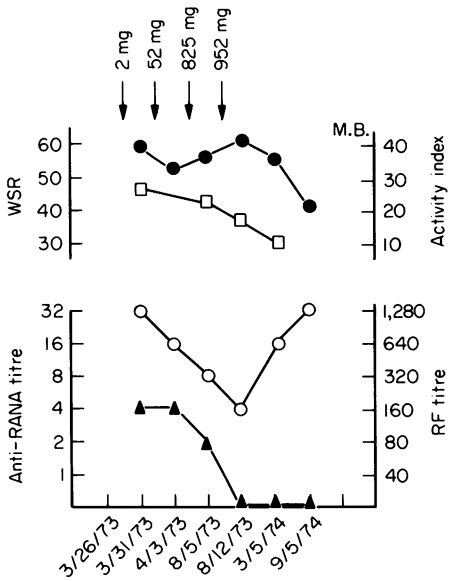

Fig. 3. Patient M.B. is a Caucasian female with seropositive RA patient who was placed on increasing doses of gold. As the gold therapy was continued, the activity index improved and the WSR decreased. At the same time, anti-RANA fell from a titre of four to negative and remained negative for over a year. Thus, anti-RANA appeared to correlate with the disease activity and was at normal levels during remission. Rheumatoid factor activity fell initially, but then rose four-fold, while other parameters were decreased. ▲ = anti-RANA titre; ● = WSR; ○ = RF titre; □ = activity index.

Anti-RANA levels were similar in all groups, with the exception of the aspirin group. Immune complexes were highest in patients taking Carprofen (Group II) and were significantly decreased in patients on combined therapy (Group V). The activity indices were similar in these groups.

Anti-SS-A antibody was found in two patients, but did not appear to change with disease activity or immune complexes.

DISCUSSION

Anti-RANA generally paralleled disease indices in 64% of cases. When significant changes in anti-RANA were compared to other parameters, anti-RANA changed concordantly with disease indices in 59% of cases. This frequency of changes with the disease activity seems quite reasonable if one considers that increased disease activity in RA may be a result of both cellular and humoral immunity and that they often act independently of each other. In addition, we are using a relatively gross detection method for anti-RANA.

Only a few longitudinal studies have attempted to correlate the disease activity with levels of circulating immune complexes (Weisman & Zvaifler, 1975; Farrell *et al.*, 1979; Goldman *et al.*, 1979; Abel *et al.*, 1980). In a recent report (Abel *et al.*, 1980), a parallel improvement in clinical response and fall in levels of rheumatoid factor and immune complexes in five patients with severe rheumatoid vasculitis was described. In this study, we have observed that levels of immune complexes increased in flares and have fallen in serial samples from some patients with improvement in clinical activity. Similar observations on the levels of immune complexes with the therapy have been made in other diseases (Huston *et al.*, 1978; Epstein *et al.*, 1979).

Immune complexes correlated with both anti-RANA and disease activity in only 33–37% of cases in this study depending on the way it was analysed. The correlation between the levels of

anti-RANA and levels of immune complexes is difficult to interpret since the former is an antibody that only in part may be associated with the antigen–antibody complexes and we lack a sensitive assay method to detect this antigen presently. If antibodies are binding RANA, its detection would be masked.

Studies on the role of EBV and anti-RANA in RA have led to evidence which indicates there may be a T cell defect in patients with RA (Bardwick *et al.*, 1980; Depper, Bluestein & Zvaifler, 1981; Tosato, Steinberg & Blaise, 1981); and, it has been suggested that B cells may be persistently activated due to lack of T cell regulation. However, a simple T cell defect may not fully explain why anti-RANA levels are generally normal in systemic lupus erythematosus (SLE), progressive systemic sclerosis (PSS) and OA, and elevated in RA and EBV specific antibodies in RA, SLE and PSS are elevated but do not change while anti-RANA levels in RA change. Therefore, it is still possible that there is also a B cell defect in RA with regard to EBV infection.

The word 'associated' was used in the naming of RANA to distinguish it from EBV specific antigens as while RANA was associated with EBV infection, it is still possible that it is a cellular antigen greatly increased in B cells after EBV infection. A recent article suggests that RANA may be found in low concentration in rabbit thymus extract (Venables *et al.*, 1982), but we have not been able to demonstrate this in early studies (Alspaugh & Tan, 1976) or more recent studies (Alspaugh, 1981). However, if RANA is not an EBV specific antigen, this could explain how RANA and subsequently anti-RANA may change independently of EBV specific antibodies. Alternatively, RANA may be similar to EB nuclear antigen (EBNA) but represent a mutant strain of EBV.

While recent studies from this laboratory have suggested that EBV may not be the initiating event in RA (Alspaugh, 1981), it may still play a role in the pathogenesis of RA by amplification and perpetuation of the disease through antibody production which leads to immune complex formation resulting in inflammation. However, further studies using a sensitive assay are necessary to quantitate RANA, anti-RANA, immune complexes and dissociated immune complexes in the course of disease.

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REFERENCES

ABEL, T., ANDREROS, B.S., CUNNINGHAM, P.H., BRUNNER, C.M., DAVIS, J.S. & HORWITZ, D.A. (1980) Rheumatoid vasculitis: effect of cyclophosphamide on the clinical course and levels of circulating immune complexes. *Ann. Intern. Med.* **93,** 407.

ALSPAUGH, M.A. (1981) A search for rheumatoid arthritis-associated nuclear antigen in tissues and cells from patients with rheumatoid arthritis. *Clin. Res.* **29,** 780A.

ALSPAUGH, M.A., HENLE, G., LENNETTE, E.T. & HENLE, W. (1981) Elevated levels of antibodies to Epstein–Barr virus antigens in sera and synovial fluids of patients with rheumatoid arthritis. *J. clin. Invest.* **67,** 1134.

ALSPAUGH, M.A., JENSEN, F.C., RABIN, H. & TAN, E.M. (1978) Epstein–Barr virus transformed lymphocytes. Induction of nuclear antigen reactive with antibody in rheumatoid arthritis. *J. exp. Med.* **147,** 1018.

ALSPAUGH, M.A., ROSENSTOCK, D., BIUNDO, J.J., HENLE, W. & GUPTA, R.C. (1979) Correlation of disease activity in rheumatoid arthritis, serial rheumatoid arthritis precipitin levels, antibodies to Epstein–Barr virus antigens and their relationships with complement and immune complexes. *Arthrit. Rheum.* **22,** 588.

ALSPAUGH, M.A. & TAN, E.M. (1975) Antibodies to cellular antigens in Sjögren's syndrome. *J. clin. Invest.* **55,** 1067.

ALSPAUGH, M.A. & TAN, E.M. (1976) Serum antibody in rheumatoid arthritis reactive with a cell associated antigen. Demonstration by precipitation and immunofluorescence. *Arthrit. Rheum.* **19,** 711.

AMERICAN RHEUMATISM ASSOCIATION OF CRITERIA FOR DIAGNOSES AND CLASSIFICATION OF RHEUMATIC DISEASES: PRIMER ON RHEUMATIC DISEASES (1973) *JAMA.* **224,** 137.

BARDWICK, P.A., BLUESTEIN, H.G., ZVAIFLER, N.J., DEPPER, J.M. & SEEGMILLER, J.O. (1980) Altered regulation of Epstein–Barr virus induced lymphoblast proliferation in rheumatoid arthritis lymphoid cells. *Arthrit. Rheum.* **23,** 626.

DEPPER, J.M., BLUESTEIN, H.G. & ZVAIFLER, N.J. (1981) Impaired regulation of Epstein–Barr virus-induced lymphocyte proliferation in rheumatoid

arthritis is due to a T cell defect. *J. Immunol.* **127,** 1899.

EPSTEIN, O., DE VILLIERS, D., JAIN, S., POTTER, B.J., THOMAS, H. & SHERLOCK, S. (1979) Reduction of immune complexes and immunoglobulins induced by d-penicillamine in primary biliary cirrhosis. *N. Engl. J. Med.* **300,** 274.

FARRELL, J.B., PERSON, D.A., ROSSEN, R.D. & LIDSKY, M.D. (1979) Role of immune complexes in rheumatoid polyarteritis. *Ann. Rheum. Dis.* **39,** 390.

FERRELL, P.B., AITCHESON, C.T., PEARSON, G.R. & TAN, E.M. (1981) Seroepidemiological study of relationships between Epstein–Barr virus and rheumatoid arthritis. *J. clin. Invest.* **67,** 681.

GOLDMAN, J.A., CASEY, H.L., MCILWAIN, H., KIRBY, J., WILSON, C.A. & MILLER, S.B. (1979) Limited plasmapheresis in rheumatoid arthritis with vasculitis. *Arthrit. Rheum.* **22,** 1146.

GUPTA, R.C., MCDUFFIE, F.C., HUSTON, K.A., TAPPEINER, G., MEURER, M., JORDON, R.E., LUTHRA, H.S., HUNDER, G.G., & ILSTRUP, D. (1979) Comparison of three immunoassays for immune complexes. *Arthrit. Rheum.* **22,** 433.

GUPTA, R.C., MCDUFFIE, F.C., TAPPEINER, G. & JORDON, R.E. (1978) Binding of soluble immune complexes to Raji lymphocytes: role of receptors for complement components, Clq and C3-C3b. *Immunology*, **34,** 751.

HAY, F.C., NINEHAM, L.S., PERUMAL, R. & ROITT, I.M. (1979) Intra-articular and circulating immune complexes and antiglobulins (IgG and IgM) in rheumatoid arthritis; correlation with clinical features. *Ann. Rheum. Dis.* **38,** 1.

HENLE, G., LENNETTE, E.T., ALSPAUGH, M.A. & HENLE, W. (1979) Rheumatoid factor as the cause of positive reactions in tests for Epstein–Barr virus and specific IgM antibodies. *Clin. exp. Immunol.* **36,** 415.

HUSTON, K.A., GUPTA, R.C., DONADIO, J.V., MCDUFFIE, F.C. & ILSTRUP, D.M. (1978) Circulating immune complexes in systemic lupus erythematosus. *J. Rheum.* **5,** 423.

KABAT, E.A. & MAYER, M.M. (1961) Complement and complement fixation. In *Experimental Immunochemistry* 2nd edn. (ed. by E.A. Kabat & M.M. Mayer) p. 149. Charles C. Thomas Company, Springfield.

LANSBURY, J. (1957) Numerical method of evaluating the status of rheumatoid arthritis. *Ann. Rheum. Dis.* **16,** 101.

LANSBURY, J. (1958) Report of a three-year study on the systemic and articular indexes in rheumatoid arthritis. Theoretical and clinical considerations. *Arthrit. Rheum.* **1,** 505.

MCCARTY, D.J. (1974) Methods for evaluating rheumatoid arthritis. In *Arthritis and Allied Conditions: A Textbook of Rheumatology* 8th edn. (ed. by J.L. Hollander & D.J. McCarty) p. 419. Lea and Febiger, Philadelphia.

NYDEGGER, V.E., ZUBLER, R., GABAY, G., JOLIAT, G., KARAGEVREKIS, C., LAMBERT, P.H. & MIESCHER, P.A. (1977) Circulating complement breakdown products in patients with rheumatoid arthritis: correlation between plasma C3d, circulating immune complexes and clinical activity. *J. clin. Invest.* **59,** 862.

SCOPELITIS, E., BIUNDO, J.J. & ALSPAUGH, M.A. (1980) Anti-SS-A antibody and other antinuclear antibodies in systemic lupus erythematosus. *Arthrit. Rheum.* **23,** 287.

SNEDECOR, G.W. & COCHRAN, W.C. (1978a) Two way classifications. In *Statistical Methods* 9th edn., p. 299–338. Iowa University Press, Iowa City.

SNEDECOR, G.W. & COCHRAN, W.C. (1978b) The comparison of two samples. In *Statistical Methods* 9th edn., p. 91–119. Iowa University Press, Iowa City.

THEOFILOPOULOS, A.N. & DIXON, F.J. (1979) The biology and detection of immune complexes. *Adv. Immunol.* **28,** 89.

TOSATO, G., STEINBERG, A.D. & BLAISE, R.M. (1981) Defective EBV-specific suppressor T-cell function in rheumatoid arthritis. *N. Engl. J. Med.* **305,** 1238.

VENABLES, P.J.W., ROFFE, L.M., ERHARDT, C.C., MAINI, R.N., EDWARDS, J.M.B. & PORTER, A.D. (1982) Titers of antibodies to RANA in rheumatoid arthritis and normal sera. *Arthrit. Rheum.* **24,** 1459.

WEISMAN, M. & ZVAIFLER, N. (1975) Cryoimmunoglobulinemia in rheumatoid arthritis: significance in serum of patients with rheumatoid vasculitis. *J. clin. Invest.* **56,** 725.

ZUBLER, R.H., NYDEGGER, V., PERRIN, L.H., FEHR, K., MCCORMICK, J., LAMBERT, P.H. & MIESCHER, P.A. (1976) Circulating and intra-articular immune complexes in patients with rheumatoid arthritis. *J. clin. Invest.* **57,** 1308.