Fall in immune complex levels during gold treatment of rheumatoid arthritis

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SUMMARY Prior to starting gold treatment 30 patients with rheumatoid arthritis had an elevated mean level of circulating immune complexes measured by C1q binding activity. Gold treatment led to an improvement in disease reflected by significant falls in erythrocyte sedimentation rate ($p < 0.001$), C-reactive protein ($p < 0.01$), Ritchie articular index ($p < 0.001$), and duration of morning stiffness ($p < 0.05$). Concurrently immune complex levels fell, and this change first reached significance after 3 months' treatment ($p < 0.05$). Serum C1q binding activity was not related to clinical and laboratory measurements of joint inflammation. This suggested to us that there is no direct immunopathological relationship between circulating immune complexes and joint inflammation in rheumatoid arthritis. Serum C1q binding activity was strongly related to IgM-RF levels measured as latex titre ($r = 0.7$, $p < 0.001$). Removal of immune complexes from serum with Sepharose 4B—staph A (staphylococcal protein A) led to a fall in IgM-RF from 2 mg/ml (2 g/l) to 0.4 mg/ml (0.4 g/l). This suggests that the reason for the relationship between C1q BA and IgM-RF is that, on average, 80% of serum IgM-RF exists as part of immune complexes containing IgG.

Numerous studies have documented the presence of raised levels of immune complexes in patients with rheumatoid arthritis, and some authors have suggested that they may be related to the aetiology of the disease.¹ Fewer investigations have been made into the change in levels of immune complexes during effective treatment of rheumatoid arthritis. This might be expected to yield useful information on the temporal relationship between changes in immune complex levels and joint inflammation. If immune complexes are aetiologically related to joint inflammation, circulating levels might be expected to fall prior to or at the same time as improvement in joint inflammation. It also seemed to us that the period following initiation of gold therapy in which disease activity was changing most rapidly would be a particularly good time in which to study the relationship between clinical and serological measurement of disease activity and levels of circulating immune complexes.

Materials and methods

Thirty patients with definite or classical rheumatoid arthritis² were studied. These were 23 females and 7 males, mean age 58.4 years (range 34–76). Twenty-four were seropositive and 6 persistently seronegative for rheumatoid factor. Disease duration ranged from 1 to 48 years, mean 7.6.

PATIENT ASSESSMENT

All patients were treated with intramuscular sodium aurothiomalate (GST), 16 in a high dose schedule and 14 in a low dose schedule. The high dose group received 50 mg GST weekly to a total dose of 1 g and then 50 mg monthly; the low dose group 10 mg GST to a total of 200 mg and then 20 mg monthly. Each patient was assessed monthly by the same observer, who measured Ritchie articular index,³ grip strength in mmHg using a modified sphygmomanometer cuff, time in seconds to walk 10 m, total proximal interphalangeal joint circumference in mm, and duration of morning stiffness in minutes. Blood was taken at the time of each assessment for measurement of erythrocyte sedimentation rate

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(ESR, Westergren), C-reactive protein (CRP), rheumatoid factor (IgM-RF) as latex titre, and C1q binding activity (C1q BA) as a measure of immune complexes. Observations were started immediately before the initiation of gold therapy. Mean follow-up was 8 months, range 4 to 12 months.

C-REACTIVE PROTEIN LEVELS

CRP levels were measured by single radial immunodiffusion in 1% agar in phosphate buffered saline, pH 7.2, containing 1% rabbit anti-CRP (Behringwerke).

LATEX TITRE

IgM-RF levels were measured by latex agglutination in tubes⁴ with sera stored at -20°C . To avoid within-patient variation all sera relating to a particular individual were assayed in the same batch. Known positive and negative sera were included in each assay as controls. Results were expressed as the reciprocal of the serum dilution in the most dilute positive tube. Results of 80 or above were regarded as positive for RF.

THE ^{125}I C1Q BINDING ASSAY

The C1q subcomponent of complement was isolated from pooled normal human serum by the method of Reid *et al.*⁵ and radioiodinated with chloramine T to a specific activity ranging from 0.4 to 0.8 $\mu\text{Ci}/\mu\text{g}$ C1q.⁶ The ^{125}I C1q binding test was performed as described by Zubler *et al.*⁷ Before they were tested the sera were stored at -70°C and were then estimated in batches, so that all sera from each individual patient were included in the same batch. Results were expressed as percentage ^{125}I C1q bound.

ABSORPTION OF RHEUMATOID SERA WITH SEPHAROSE 4B-STAPH A PROTEIN

Fourteen rheumatoid sera were absorbed with staphylococcal protein A (staph A) conjugated to Sepharose 4B (Pharmacia) to remove IgG-containing immune complexes. Sepharose 4B-staph A was extensively washed and an equal quantity (0.2 ml) aliquoted into 2.5 ml plastic tubes (Luckhams PTO944) and 0.6 ml serum added. Tubes were rotated overnight at 4°C . The sera were then spun twice to remove Sepharose and stored at -70°C prior to estimation of C1q binding activity and IgM-RF. An exactly similar procedure was carried out with unconjugated Sepharose 4B (Pharmacia) as a control.

QUANTITATIVE MEASUREMENT OF IgM RHEUMATOID FACTOR IN ABSORBED SERA
Levels of IgM-RF in absorbed sera were measured by an enzyme-linked immunosorbent assay (ELISA).

This assay will be described in detail in a subsequent publication. Briefly, 100 μl DE-52 purified human IgG (7 $\mu\text{g}/\text{ml}$) is added to each well of a microtitre plate and allowed to react overnight at room temperature. Test sera are diluted 1/100 and 100 μl added to wells in duplicate and again reacted overnight at room temperature. 100 μl of alkaline phosphatase conjugated goat antihuman IgM (Miles Yeda) at 1 in 500 dilution is then added to each well for 2 hours. Enzyme substrate is made by dissolving 1 tablet of Sigma phosphatase substrate 104 per 5 ml of ethanolamine buffer, pH 9.0, and 100 μl added to each well. The reaction is stopped after 10 minutes by adding an equal quantity of 3 M sodium hydroxide. Results are read as absorbance at 405 nanometers with a Beckman Micro-elisa minireader.

Serial dilutions of a known seropositive serum are included as a standard. The amount of IgM-RF in the standard has been previously estimated by absorption of the IgM-RF with aggregated IgG followed by measurement of serum IgM before and after absorption by single radial immunodiffusion. A standard curve is constructed from the readings from the serial dilutions of this standard serum and results read as μg IgM-RF per ml.

EVALUATION OF RESULTS

Changes in each parameter over the period of observations were examined for significance by analysis of variance on a computer program (Statistical Package for the Social Sciences, SPSS). Mean values for each month were compared with the initial values by means of an unpaired Student's *t* test. Unpaired testing was used because the length of follow-up varied, so that pairing was not possible.

Correlations between the parameters measured were evaluated by linear regression on an SPSS program. Because of the large number of observations made (170–230), *r* values as low as 0.15 were statistically significant. This level of correlation obviously has no meaning, and compensation for the large number of individual observations and the large numbers of parameters correlated (9) was therefore made by selecting an *r* value of 0.4 or greater as being meaningful.

Results

At the beginning of treatment this group of patients with rheumatoid arthritis had active disease; mean ESR was 46 mm/h, CRP 40 $\mu\text{g}/\text{ml}$ (40 mg/l), Ritchie articular index (RI) 9.5, and duration of morning stiffness (DMS) 60 minutes. During gold treatment there was an improvement in disease activity. This was reflected by significant falls in

ESR ($p < 0.001$), CRP ($p < 0.01$), RI ($p < 0.001$), and DMS ($p < 0.05$). There was also a fall in mean latex titre from ≈ 300 to 2500, but this was not statistically significant. Changes in grip strength, walking time, and total proximal interphalangeal (PIP) joint size were not significant.

At the time of maximum disease activity before the start of gold treatment patients had raised levels of circulating immune complexes. Mean C1q BA was 34%, which is approximately twice the upper limit of normal (mean ± 2 SD = 15%). Individual levels ranged from 4.8 to 70.1%. During treatment there was a fall in immune complex levels which was

statistically significant by analysis of variance ($p < 0.01$).

PATTERN OF FALL IN DISEASE MEASUREMENTS
In a comparison of mean levels of immune complexes for each successive month of treatment with initial values by unpaired Student's t test the fall in immune complex levels first reached significance at 3 months (Fig. 1). However, the level at 4 months was not significantly different from the initial value. Levels were more consistently lowered from 5 months onwards, but even then the levels at 7 and 9 months were not significantly lower than initial values.

This reflects the rather small quantitative change in immune complex levels and the fact that they plateau at levels at the limit of significance. This pattern differs from the steeper and more sustained falls in RI and CRP. The fall in these measurements first reached significance at 2 and 3 months respectively. After this they fell further and reached a plateau at levels consistently significantly different from their starting values ($p < 0.001$ at 4 months for both measurements). These differences are clearly seen in Fig. 1.

CORRELATIONS BETWEEN DISEASE MEASUREMENTS

Table 1 shows the correlation between measurements which showed significant falls during treatment as well as latex titre. In general, these correlations fell into 2 groups. The first group consisted of serologic and clinical measurements reflecting joint inflammation, namely, ESR, CRP, RI, and DMS. ESR and CRP were strongly related and both correlate with the clinical measurements to a similar degree.

The second group consisted of C1q BA and latex titre. These 2 measurements were strongly related ($r = 0.7$, $p \leq 0.001$). Latex titre correlated with DMS ($r = 0.41$, $p < 0.001$), but C1q BA was only weakly related to measurements of disease activity (Table 1).

RELATIONSHIP BETWEEN C1Q BINDING ACTIVITY AND IGM RHEUMATOID FACTOR

In order to study the strong relationship between IgM-RF measured as latex titre and C1q BA we absorbed 14 rheumatoid sera with staph A to remove IgG containing immune complexes. On analysis 6 sera had C1q BA $> 15\%$ after absorption with Sepharose 4B ($46\% \pm 7\%$ mean $\pm$ SEM). C1q BA fell significantly ($p < 0.001$, paired Student's t test) with staph A absorption to $13.7\% \pm 3.5\%$ (mean $\pm$ SEM) which is within the normal range (Fig. 2). There was a corresponding 80% fall in the

Fig. 1 Differences in the time and extent of falls in serum immune complex levels and measurements reflecting inflammatory disease activity during gold treatment of rheumatoid arthritis. Immune complex levels measured as C1q binding activity (C1q BA) fall initially and then plateau. The fall is first significant after 3 months of gold treatment ($p = 0.04$) by unpaired Student's t test. Levels at 5 months ($p = 0.03$), 6 months ($p = 0.02$), and 8 months ($p = 0.01$) are also significantly less than the starting value (indicated by stars). The falls in Ritchie articular index (RI) and C-reactive protein (CRP) reach significance at 2 months ($p = 0.03$) and 3 months ($p = 0.003$) of gold treatment respectively (indicated by stars). At 4 months $p < 0.001$ for both of these measurements. ESR falls steadily to reach significance at 6 months ($p = 0.007$).

Table 1 Correlation between clinical and serological measurements made in 30 patients with rheumatoid arthritis during gold treatment

	ESR	CRP	RF	C1q BA
CRP	0.66*			
RF	0.09	0.03		
C1q BA	0.23	0.09	0.7*	
RI	0.42†	0.46†	0.36	0.23
DMS	0.46†	0.44†	0.41†	0.28

Serial measurements of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), IgM rheumatoid factor by latex titre (RF), C1q binding activity (C1q BA), Ritchie articular index (RI), and duration of morning stiffness (DMS) were made monthly in 30 patients with rheumatoid arthritis. The figure shows correlations between measurements as *r* values derived by linear regression. To correct for the large number of correlations made an *r* value of 0.4 was taken to represent a meaningful relationship, indicated by * ($p < 0.001$). The 2 highest correlations are shown by † ($p < 0.001$).

Fig. 2 Absorption of immune complexes with *Staph A* protein leads to a significant fall in serum IgM rheumatoid factor. This figure shows mean levels ($\pm$ standard error of the mean) of C1q binding activity (●) and IgM rheumatoid factor (■) for 6 rheumatoid sera absorbed with sepharose 4B or staphylococcal protein A (staph A) linked to sepharose 4B. Absorption of sera with staph A produced a significant fall ($p < 0.001$ paired Student's *t* test) in serum C1q binding activity. Concomitantly there was a significant fall in IgM rheumatoid factor levels ($p < 0.05$).

mean level of IgM-RF measured by ELISA from 2 mg/ml to 0.4 mg/ml (2 to 0.4 g/l) ($p < 0.05$). Individual falls varied from 39% to 97% of the control absorbed value. The 8 sera with no immune complexes in control sera showed no significant change in C1q BA or IgM RF (data not shown).

Discussion

We have shown that immune complex levels measured as C1q BA fell significantly as disease activity improved during the early phase of gold treatment. This is in agreement with Nineham *et al.*⁸ who also showed a fall in C1q binding immune complexes during gold treatment of rheumatoid arthritis but using a solid-phase technique. Falls in immune complex levels have also been reported during penicillamine therapy⁹ and during the treatment of rheumatoid vasculitis with cytotoxic agents.¹⁰ It would thus seem that effective treatment of rheumatoid arthritis with 'second-line' agents is associated with a fall in levels of circulating immune complexes.

The fall in levels of immune complexes first becomes significant after there is already evidence of clinical improvement in joint inflammation (RI, Fig. 1). The fall in immune complex levels is neither as steep nor as sustained as the falls in RI and CRP, which are generally thought to be good indicators of inflammatory disease activity. These differences favour the concept that circulating immune complex levels are a secondary phenomenon rather than being directly linked to inflammation in joints in rheumatoid arthritis.

If immune complexes were of aetiological significance in the pathogenesis of joint inflammation, one might expect to see a correlation between levels of complexes and clinical and serological measurements of joint inflammation. We did not find evidence for such a relationship (Table 1). This does not preclude an aetiological role for immune complexes in joint inflammation. Firstly, we might be measuring the wrong type of immune complexes, as it is known that different assays give different results.¹¹⁻¹³ Secondly, we measured serum immune complexes and not those in synovial fluid.

Some previous authors have shown a relationship between C1q binding immune complexes and some features of rheumatoid arthritis. For example, Halla *et al.*¹³ found that patients with morning stiffness greater than 2 hours had significantly higher levels of C1q BA than those with morning stiffness less than 2 hours. There was a similar finding in patients with ESRs greater than 50. Others have not found any relationship between serum C1q BA and ESR,¹⁴

or disease stage and functional class.¹²⁻¹⁴ In general, high levels of immune complexes tend to correlate more with extra-articular features of rheumatoid arthritis, either measured as C1q BA¹²⁻¹⁴ or using C1q bound to solid-phase.⁸⁻¹⁵

We have found a strong relationship between levels of C1q BA and IgM RF. Some previous authors have not found such a relationship.¹²⁻¹⁴ However, Lawley *et al.*¹⁶, investigating patients with Sjögren's syndrome, including patients with rheumatoid arthritis, found a highly significant relationship ($r = 0.55$, $p < 0.0005$) as did Haslam *et al.*¹⁷ in patients with fibrosing alveolitis, with or without rheumatoid arthritis ($r = 0.67$, $p < 0.001$).

This strong relationship implies that IgM-RF plays a significant part in the immune complexes measured as C1q binding activity. We have shown that absorption of sera with staph A is capable of removing nearly all immune complexes measured by C1q binding activity, suggesting that all these complexes contain IgG. This is in agreement with Zubler *et al.*¹⁴, who absorbed with rabbit anti-human IgG and Lawley *et al.*¹⁶ using staph A. Concomitantly there is a fall in the mean level of IgM-RF of 80%. If it is assumed that staph A specifically adsorbs IgG this result suggests that on average 80% of IgM-RF is involved in immune complexes containing IgG.

It is interesting that there was a wide individual variation in the amount of IgM-RF apparently complexed in this way (39%–97%). This might be due to a difference in the affinity of IgM-RF or variations in the relative quantities of IgG-containing complexes and IgM-RF in the circulation. The variation in the relative levels of IgM-RF and immune complexes in serum is exemplified by 9 patients who at some stage of follow-up were seronegative for IgM-RF but had C1q binding immune complexes, and 4 patients (including 1 of the above) who were seropositive for IgM-RF but had no circulating immune complexes detected. The fact that both situations could be seen in the same patient on different occasions emphasises the dynamic nature of the relationship.

In conclusion this study suggests that (1) the fall in serum levels of immune complexes measured as C1q binding activity occurs as a secondary phenomenon consequent upon an improvement in disease activity induced by gold; (2) the strong relationship between IgM-RF and C1q BA is due to the fact that a significant proportion of IgM-RF is involved in immune complexes.

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References

- Theofilopoulos A N, Dixon F J. The Biology and Detection of Immune Complexes. *Adv Immunol* 1979; **28**: 89–220.
- Ropes M W, Bennet G A, Cobb S, Jacox R, Jessar R A. Diagnostic criteria for rheumatoid arthritis. *Ann Rheum Dis* 1959; **18**: 49–53.
- Ritchie D R, Boyle J A, McInnes J M, *et al.* Clinical Studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. *Q J Med* 1968; **37**: 393–406.
- Singer J M. Standardisation of the latex test for rheumatoid arthritis serology. *Bull Rheum Dis* 1973; **24**: 762–9.
- Reid K B M, Lowe D M, Porter R R. Isolation and characterisation of C1q, a subcomponent of the first component of complement, from human and rabbit sera. *Biochem J* 1972; **130**: 749–63.
- McConahey P J, Dixon F J. A method of trace iodination of proteins for immunologic studies. *Int Arch Allergy* 1966; **29**: 185–9.
- Zubler R H, Lange G, Lambert P H, Miescher P A (1976). Detection of immune complexes in unheated sera by a modified ¹²⁵I C1q binding test. *J Immunol* 1976; **116**: 232–5.
- Nineham L J, Hay F C, Male D K, Roitt I M, Young A, Perumal R. Immune complexes in rheumatoid arthritis: correlations with clinical features and effects of gold. *Protides of Biological Fluids* 1978; **26**: 179–82.
- Mohammed I, Barraclough D, Holborow E J, Ansell B M. Effect of penicillamine therapy and circulating immune complexes in rheumatoid arthritis. *Ann Rheum Dis* 1976; **35**: 458–62.
- Abel T, Andrews B S, Cunningham P H, Brunner C, Davis J S IV, Horwitz D A. Rheumatoid vasculitis: effect of cyclophosphamide on the clinical course and levels of circulating immune complexes. *Arthritis Rheum* 1980; **23**: 646 (abstract).
- Lambert P H, *et al.* A WHO collaborative study for the evaluation of eighteen methods for detecting immune complexes in serum. *J Clin Lab Immunol* 1978; **1**: 1–15.
- Gupta R C, McDuffie F C, Huston K A, *et al.* Comparison of 3 immunoassays for immune complexes in rheumatoid arthritis. *Arthritis Rheum* 1979; **22**: 433–9.
- Halla J T, Volanakis J E, Schrohenloher R E. Immune complexes in rheumatoid arthritis sera and synovial fluids: a comparison of three methods. *Arthritis Rheum* 1979; **22**: 440–7.
- Zubler R H, Nydegger U, Perrin L H, *et al.* Circulating and intra-articular immune complexes in patients with rheumatoid arthritis. *J Clin Invest* 1976; **57**: 1308–19.
- Hay F C, Nineham L J, Perumal R, Roitt I M. Intra-articular and circulating immune complexes and anti-globulins (IgG and IgM) in rheumatoid arthritis: correlation with clinical features. *Ann Rheum Dis* 1979; **38**: 1–7.
- Lawley T J, Moutsopoulos H M, Katz S I, Theofilopoulos A N, Chused T M, Frank M. Demonstration of circulating immune complexes in Sjögren's syndrome. *J Immunol* 1979; **123**: 1382–7.
- Haslam P L, Thompson B, Mohammed I, *et al.* Circulating immune complexes in patients with cryptogenic fibrosing alveolitis. *Clin Exp Immunol* 1979; **37**: 381–90.

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