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 Clq binding activity. Gold treatment led to an improvement in disease reflected by significant falls in erythocyte 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 Clq 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 Clq 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 Clq 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 aetio logically 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.

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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 sphygmanometer 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
Complexes. Observations

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 immuno-
diffusion 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 tubes with sera stored at −20°C. To avoid
within-patient variation all sera relating to a par-
ticular 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 125I C1Q BINDING ASSAY

The C1q subcomponent of complement was isolated
from pooled normal human serum by the method of
Reid et al. and radioiodinated with chloramine T
to a specific activity ranging from 0.4 to 0.8 μCi/μg
C1q. The 125I C1q binding test was performed as
described by Zubler et al. 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 125I 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
PT0944) 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 μg/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 μg/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 Clq 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 < 0·01) 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 Clq BA and latex titre. These 2 measurements were strongly related (r = 0·7, p < 0·001). Latex titre correlated with DMS (r = 0·41, p < 0·001), but Clq BA was only weakly related to measurements of disease activity (Table 1).

**Relationship between Clq binding activity and IgM rheumatoid factor**

In order to study the strong relationship between IgM-RF measured as latex titre and Clq BA we absorbed 14 rheumatoid sera with staph A to remove IgG containing immune complexes. On analysis 6 sera had Clq BA > 15% after absorption with Sepharose 4B (46% ± 7% mean ± SEM). Clq BA fell significantly (p < 0·001, paired Student's t test) with staph A absorption to 13·7% ± 3·5% (mean ± SEM) which is within the normal range (Fig. 2). There was a corresponding 80% fall in the
produced a factor. This during reactive protein (CRP), IgM rheumatoid factor by latex titre (RF), measurements of erythrocyte sedimentation rate (ESR), C-

Serial measurements of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), IgM rheumatoid factor by latex titre (RF), Clq binding activity (Clq 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).

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 Clq BA or IgM RF (data not shown).

Discussion

We have shown that immune complex levels measured as Clq BA fell significantly as disease activity improved during the early phase of gold treatment. This is in agreement with Nineham et al. who also showed a fall in Clq 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 therapy and during the treatment of rheumatoid vasculitis with cytotoxic agents.

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 of 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.

Secondly, we measured serum immune complexes and not those in synovial fluid.

Some previous authors have shown a relationship between Clq binding immune complexes and some features of rheumatoid arthritis. For example, Halla et al. found that patients with morning stiffness greater than 2 hours had significantly higher levels of Clq 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 Clq BA and ESR.
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or disease stage and functional class. In general, high levels of immune complexes tend to correlate more with extra-articular features of rheumatoid arthritis, either measured as Clq BA or using Clq bound to solid-phase.

We have found a strong relationship between levels of Clq BA and IgM RF. Some previous authors have not found such a relationship. However, Lawley et al., investigating patients with Sjögren’s syndrome, including patients with rheumatoid arthritis, found a highly significant relationship (r = 0.55, p < 0.005) as did Haslam et al., 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 Clq binding activity. We have shown that absorption of sera with staph A is capable of removing nearly all immune complexes measured by Clq binding activity, suggesting that all these complexes contain IgG. This is in agreement with Zubler et al., who absorbed with rabbit anti-human IgG and Lawley et al., 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 Clq 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 Clq 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 Clq BA is due to the fact that a significant proportion of IgM-RF is involved in immune complexes.

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References

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