Differential effects of therapeutic regimens on specific classes of rheumatoid factor

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SUMMARY This study was performed to define further the relationship of circulating IgM and IgG rheumatoid factor to the articular manifestations of rheumatoid arthritis. Patients with substantial clinical improvement ($>55\%$) or no improvement or worsening were chosen for study. Patients were further selected to include those treated with non-steroidal anti-inflammatory drugs (NSAIDs) alone, or NSAIDs plus D-penicillamine or gold. Significant reductions of IgM ($p<0.001$) and IgG ($p<0.005$) rheumatoid factor were seen only in those treated with gold who improved clinically. These observations suggest that in patients experiencing comparable degrees of clinical improvement the simultaneous alterations of circulating IgG and IgM rheumatoid factor observed are dependent upon the therapeutic regimen employed.

Key words: rheumatoid arthritis, articular manifestations.

Rheumatoid factors of the IgM, IgA, and IgG classes have been detected in the circulation and are produced locally by the synovium of patients with adult onset rheumatoid arthritis (RA).¹⁻⁵ Although the presence of high concentrations of circulating rheumatoid factors has been associated with a poor prognosis,^{1,6,7} the relationship of circulating rheumatoid factors to disease activity, particularly the articular manifestations, remains poorly defined. In particular, the differing effects of various drug therapies on the relationship between serologic and articular manifestations have not been systematically examined. This study examines the relationship between changing articular manifestations and circulating IgM and IgG rheumatoid factors in RA patients treated with non-steroidal anti-inflammatory drugs (NSAIDs) alone, or NSAIDs plus D-penicillamine or gold.

Patients and methods

PATIENTS

Patients (total 144) were examined prospectively in the outpatient clinics of the Medical Center Hospital and the Audie Murphy Veterans Administration

Hospital, San Antonio, TX. The eligibility requirements for this study were: (a) a willingness to participate; (b) definite or classical RA;⁸ (c) treatment with NSAIDs alone, or with NSAIDs plus gold or D-penicillamine (choice determined by patient's physician); (d) patients remained on the drug of interest for at least three months; (e) clear cut improvement or failure to improve on the described regimen (definition below).

In a previous study³ we observed substantial variability of clinical findings when patients improved only modestly (between 20 and 50%). In order to avoid this variability and the attendant uncertainty patients were chosen for this study if their articular index improved more than 55% (75% for those on NSAIDs alone) or if it failed to improve ($<10\%$ improvement or worsening). More stringent criteria were applied to those treated with NSAIDs alone to improve our chances of observing a reduction of rheumatoid factor if this were due to clinical improvement on NSAIDs. Thus although the clinical criteria for inclusion were not strictly comparable, the selection process intentionally minimised the likelihood of observing a serologic trend associated with clinical improvement on NSAIDs alone. We reasoned that if a trend were not observed despite this bias it would be even more credible than if identically stringent criteria were applied to the group on NSAIDs. All patients were

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selected for study before performing the laboratory studies. In summary, the study groups included patients who improved or clearly did not improve on each of three therapeutic regimens.

The follow up evaluations used in this study for those who improved were not obtained at a uniform time after initiating therapy but at a time when comparable and substantial improvement (see criteria above) had occurred. For 'unimproved' subjects sampling was timed to correspond with that performed for the improved group. Thus although the follow up was not obtained at a uniform chronological time, it was obtained at a uniform time with regard to the clinical, therapeutic response. Nonetheless, as shown in Table 1, the mean time at follow up was similar for the three groups.

CLINICAL EVALUATIONS PERFORMED

An articular index was derived as previously described.³ Each swollen joint was multiplied by a weighted factor (f) which was dependent on the relative size of the joint. For the shoulder and knee, f=3; for the elbow and ankle, f=2; for all other joints, f=1. The articular index was derived by adding the number of swollen joints which had each been multiplied by the appropriate weighted factor. The articular index was chosen as the principal measure of clinical activity before initiating this study. For comparison, the joint count and sedimentation rate were included. The joint count was performed by summation of all the joints which were either swollen or tender to palpation or painful on standard range of motion. The erythrocyte sedimentation rate was performed by the Westergren method and reported in millimetres in the first hour.

RHEUMATOID FACTORS

Assays specific for IgM rheumatoid factor and IgG rheumatoid factor were performed as previously described.^{2,3,9} Briefly, normal IgG was employed at 50 µg/ml (mg/l) to coat polystyrene wells overnight. For IgM rheumatoid factor, normal pooled human IgG was employed. For IgG rheumatoid factor,

rabbit IgG was used. The importance of employing these specific antigens to ensure maximal sensitivity has been previously reported.¹⁰ Any remaining adherent sites were blocked with 1% bovine serum albumin. Diluted patient sera were then added to the wells and incubated overnight. Before performing the IgG rheumatoid factor radioimmunoassay the sera were first pepsin digested in order to abrogate the effects of IgM and IgA rheumatoid factor.⁹ Individual classes of rheumatoid factor were then quantified with class specific antisera: F(ab')₂ antihuman IgM (µ specific) for IgM rheumatoid factor and F(ab')₂ rabbit antihuman Fab (γ specific) for IgG rheumatoid factor. The upper limits of normal were determined by employing 20 simultaneously examined normal sera and purified polyclonal IgG rheumatoid factor and IgM rheumatoid factor. The upper limit of normal for IgM rheumatoid factor was 2.9 µg/ml and for IgG rheumatoid factor, 29 µg/ml.

CALCULATIONS AND STATISTICS

Percentage change in study variables was determined by the formula:

$$\text{Percentage change} = \frac{\text{final value} - \text{initial value}}{\text{higher value}}$$

A negative percentage change was indicative of improvement and a positive value of worsening. This formula allowed all changes to be compared with the maximal value for each variable.

The significance of differences between group means was determined by Student's *t* test. A paired *t* test was used for matched pairs. Correlation coefficients were determined with Spearman's rank correlation test.

Results

PATIENT DESCRIPTION

Clinical characteristics of the patient groups are presented in Table 1. Therapeutic groups were comparable in number, ranging from 13 to 16

Table 1 Patient description

Group therapy	No	Therapeutic effectiveness subgroup (No)	Mean age (range) (years)	Mean duration (years) of RA (range)	Gender	Mean duration (months) of follow up (range)
NSAIDs only	16	Improved (8) Not improved (8)	57 (37-65) 57 (42-71)	7 (0.3-15) 12 (0.3-20)	6F, 2M 5F, 3M	8.3 (5-13) 8.4 (3-17)
D-Penicillamine	13	Improved (8) Not improved (5)	56 (41-64) 53 (50-59)	15 (8-30) 8 (5-10)	4F, 4M 3F, 2M	11.3 (6-17) 11.4 (6-17)
Gold	15	Improved (9) Not improved (6)	54 (36-65) 54 (40-71)	6 (0.3-20) 9 (2-23)	6F, 3M 4F, 2M	9.3 (5-14) 9.3 (4-15)

patients each. The therapeutic effectiveness subgroups ranged from five to nine patients each. The mean ages of the patients ranged from 53 to 57 years. The mean duration of rheumatoid arthritis before entry in this study was broad, ranging from four months to 30 years. The mean duration of follow up was comparable between the subgroups.

CLINICAL AND LABORATORY CHANGE OF THE SUBGROUPS

Clinical and laboratory data at the initial visit for each subgroup are recorded in Table 2. The initial clinical and laboratory measures of disease activity were generally higher in the patients who improved compared with those who failed to improve. The

only baseline differences which were statistically significant, however, were the articular index of the improved and unimproved patients receiving penicillamine ($p<0.02$) and the ESR of the improved v unimproved subjects receiving gold ($p<0.05$).

The mean changes between the initial and final observations for the clinical and serologic variables are presented in Figs 1–3. For the NSAID subgroup that improved (Fig. 1) amelioration of the clinical parameters ranged from 86% ($p<0.001$) for the articular index and 61% ($p<0.002$) for the joint count to 32% ($p=NS$) for the erythrocyte sedimentation rate. Reductions of rheumatoid factor concentrations in this subgroup were modest: only 19% for IgM RF and 25% for IgG RF. In the

Table 2 Initial clinical and laboratory values for the different therapeutic groups and subgroups

	Articular index	Joint count	ESR (mm/1st h)	IgM RF (μ g/ml)	IgG RF (μ g/ml)
NSAIDs					
Improved	29 $\pm$ 14* (16–50)	25 $\pm$ 10 (18–42)	60 $\pm$ 43 (17–139)	50 $\pm$ 38 (11–135)	1388 $\pm$ 1408 (112–3110)
Unimproved	18 $\pm$ 11 (7–35)	22 $\pm$ 12 (10–41)	48 $\pm$ 29 (4–97)	37 $\pm$ 38 (0–109)	346 $\pm$ 528 (13–1584)
D-Penicillamine					
Improved	34 $\pm$ 12 (18–52)	27 $\pm$ 11 (18–52)	63 $\pm$ 29 (27–98)	37 $\pm$ 15 (20–62)	818 $\pm$ 832 (218–2708)
Unimproved	14 $\pm$ 10 (5–31)	13 $\pm$ 11 (5–32)	42 $\pm$ 30 (10–75)	36 $\pm$ 11 (22–46)	615 $\pm$ 567 (154–1524)
Gold					
Improved	32 $\pm$ 12 (17–59)	28 $\pm$ 11 (17–46)	66 $\pm$ 26 (27–103)	43 $\pm$ 8 (33–55)	596 $\pm$ 599 (112–1970)
Unimproved	19 $\pm$ 12 (0–33)	18 $\pm$ 10 (4–28)	37 $\pm$ 17 (14–52)	37 $\pm$ 19 (8–51)	490 $\pm$ 356 (127–1156)

*Values are mean $\pm$ SD; the values in the parentheses represent the ranges.

Fig. 1 Change of clinical and laboratory parameters in patients treated with NSAIDs alone. The values for the patients who improved are presented in the left of the figure and for those who did not improve on the right. The values are presented as the mean $\pm$ SEM. The abbreviations employed are: AI=articular index; JC=joint count; ESR=erythrocyte sedimentation rate; IgM RF=IgM rheumatoid factor; IgG RF=IgG rheumatoid factor.

Fig. 2 Change of clinical and laboratory parameters in patients treated with NSAIDs plus D-penicillamine. See Fig. 1 for details.

Fig. 3 Change of clinical and laboratory parameters in patients treated with NSAIDs plus gold. See Fig. 1 for details.

unimproved subgroup deterioration of clinical activity ranged from 19% for the articular index to 6% for the joint count. In this subgroup IgM RF increased 9%, while the IgG RF increased a mean of 37%. Neither class of rheumatoid factor changed significantly in either subgroup.

In the groups treated with NSAIDs plus D-penicillamine (Fig. 2) improvement was somewhat less and deterioration generally somewhat worse than noted for those on NSAIDs alone. In the subgroup that improved the articular index and joint count both decreased 71% ($p<0.001$), while the ESR decreased 31% ($p<0.005$). In the subgroup that failed to improve the clinical manifestations deteriorated by 40–44%. In neither the improved nor unimproved subgroups did the IgM RF or the IgG RF change significantly, with mean changes of 11% or less.

The changes noted in the patients treated with gold (Fig. 3) were somewhat different. Clinical improvement ranged from a mean of 83% ($p<0.001$) for the articular index and 74% ($p<0.001$) for the joint count to 57% ($p<0.005$) for the erythrocyte sedimentation rate. In this subgroup the mean reduction of IgM RF and IgG RF were comparable to the clinical changes, 59% ($p<0.001$) and 70% ($p<0.005$) respectively. In those patients who failed to improve on gold all parameters deteriorated modestly, with the exception of IgM rheumatoid factor which decreased slightly.

Table 3 Relationship between change of clinical and laboratory variables

	Clinical variable	Laboratory variable	
		IgG RF	IgM RF
NSAIDs	Articular index	0.68*	0.25
		$p_c>0.02†$	$p_c=NS$
	Joint count	0.45	0.16
D-Penicillamine	ESR	$p_c=NS$	$p_c=NS$
		0.52	0.40
		$p_c=NS$	$p_c=NS$
D-Penicillamine	Articular index	0.25	0.32
		$p_c=NS$	$p_c=NS$
	Joint count	0.07	0.45
Gold	ESR	$p_c=NS$	$p_c=NS$
		0.21	0.74
		$p_c=NS$	$p_c<0.02$
Gold	Articular index	0.79	0.71
		$p_c<0.004$	$p_c<0.01$
	Joint count	0.76	0.66
Gold	ESR	$p_c<0.01$	$p_c<0.04$
		0.80	0.79
		$p_c<0.004$	$p_c<0.004$

*Correlation coefficient determined by Spearman's rank correlation test.

†Significance of the correlation coefficient corrected (p_c) for the number of laboratory variables examined.

RELATIONSHIP BETWEEN CHANGE OF CLINICAL VARIABLES AND CHANGE OF RF

To examine the relationship between the change of the clinical variables and the change of IgM and IgG rheumatoid factor data both for those patients who improved and those who failed to improve were combined within each of the three therapeutic groups (Table 3). These relationships must be evaluated with caution since the data were obtained from the two extremes of therapeutic responsiveness. Nonetheless, change of RF correlated poorly with change of clinical activity in those treated with NSAID alone or with D-penicillamine. In contrast, IgM RF, and to a greater extent IgG RF, correlated significantly with change of clinical activity in those treated with gold.

Discussion

This study examined the relationship between the articular manifestations of rheumatoid arthritis and concentrations of circulating IgM and IgG RFs. Only in those patients treated with gold was a significant reduction of both classes of rheumatoid factors observed in patients preselected on the basis of clinical improvement. Although rheumatoid factors decreased in the patients treated with NSAIDs alone or with NSAIDs plus D-penicillamine, these changes were not significant even in the face of highly significant clinical improvement which was comparable to the improvement of those treated with gold.

In previous studies rheumatoid factor reduction has been associated with certain forms of therapy. One study¹¹ observed an increase of IgM rheumatoid factor after cessation of NSAIDs for two weeks. After reinstitution of NSAIDs for six weeks a significant reduction of circulating IgM RF was noted. No objective measurement of disease activity was made in this study. In our study, even in the group of patients preselected on the basis of dramatic clinical improvement (81%), the reduction of both IgG and IgM RFs failed to achieve statistical significance. However, our patients were taking a variety of NSAIDs and this therapy was not routinely altered at the time of entry. The previous study¹¹ employed a single agent and therapy was modified at the onset of the study. Differences of design probably account for the discrepancies between these two studies.

Patients treated with D-penicillamine have often,^{12–14} but not uniformly,^{15, 16} experienced significant reductions of circulating rheumatoid factor during therapy. Although most studies employing agglutination techniques have noted a reduction of rheumatoid factor with D-penicillamine therapy, reduction has not always been significant even in the

face of significant clinical improvement^{15 16} and has often been associated with doses of D-penicillamine which are highly toxic. The study by Wernick *et al.*¹² employing radioimmunoassays noted a significant reduction of circulating IgM RF in those treated with D-penicillamine. This study¹² included all patients regardless of clinical response. In our patients who improved no significant reduction of either IgG or IgM RF was observed, despite a mean 71% clinical improvement. Wernick *et al.*¹² noted significant reduction of IgM RF by nine months regardless of the dose employed (250 or 750 mg/day). Our patients were followed up for a mean of 11.3 months. Wernick *et al.*¹² did note, however, that the reduction of IgM RF was greater and occurred sooner in those on the higher dose (750 mg/day). The majority of our patients received a maximally tolerated dose (mean 412 ± 71 mg/day), and none received less than 250 mg/day.

The most dramatic modulation of serologic variables noted in this study was in those who improved clinically during treatment with gold. Despite the fact that the clinical amelioration was comparable with that of the other two therapeutic subgroups, only in those treated with gold were significant reductions of IgM and IgG RF noted. Previous studies have not examined the effects of class specific radioimmunoassays employing gold. Nonetheless, even with less specific agglutination techniques, many¹⁷⁻¹⁹ but not all²⁰ studies have observed decreased rheumatoid factor in patients treated with gold.¹⁸⁻²⁰ In one study¹⁸ 33% of patients actually became seronegative. Another study¹⁹ observed reduced rheumatoid factor titres, despite the therapeutic result, though the titres decreased further in the patients who experienced excellent clinical responses. In contrast with those experiencing clinical improvement, we did not observe a significant reduction of either IgM or IgG RF in those who failed to improve. Therefore, gold did not cause a reduction of rheumatoid factor in all patients since significant reductions occurred only in the subgroup that experienced clinical improvement. The fact that the IgM and IgG RFs of the NSAID group alone and the NSAIDs plus D-penicillamine therapeutic improvement subgroup did not fall significantly in the face of clinical improvement comparable with the improvement of those treated with gold suggests that changes of rheumatoid factor were not simply non-specific secondary to clinical improvement. If rheumatoid factors were generated in response to synovial inflammation they should have been reduced comparably in all the clinical improvement subgroups since they all experienced similar degrees of disease remission.

Our observations suggest that mechanisms responsible for clinical improvement may have been different in the three therapeutic groups. In those treated with gold perhaps a true immunosuppressive effect, at least for the production of rheumatoid factor, occurred. In the other two therapeutic subgroups that improved no significant reductions of rheumatoid factors occurred, suggesting that different mechanisms may have been responsible.

We³ and others²¹ have observed that circulating IgG RF concentration more accurately reflects articular activity than other classes of rheumatoid factor or immune complexes. However, the associations noted in these studies were not strong enough to be clinically useful. Although, as mentioned, the correlations observed in this study must be viewed with reservation, the clinical utility of routinely measuring IgM and IgG RFs would appear to be limited. Only in those treated with gold were the associations of the articular and laboratory changes reliable. This lack of association has previously been noted in those treated with D-penicillamine and those treated with total nodal irradiation.^{22 23} Our study did not consider the usefulness of IgG or IgM RF for following extra-articular disease such as vasculitis, where they may be of more value.^{24 25} It will be of interest to observe the long term prognostic significance of the reduction of IgM or IgG RF noted in some of our patients.

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