The functional affinity of IgM rheumatoid factor is related to the disease duration in patients with rheumatoid arthritis

A Saraux, B Bendaoud, M Dueymes, P Le Goff, P Youinou

Abstract

***Objective*—To determine the relevance of the functional affinity of IgM rheumatoid factor (RF) to the clinical and serological characteristics of patients with rheumatoid arthritis.**

***Methods*—The functional affinity of IgM RF of 57 seropositive rheumatoid arthritis patients was evaluated by an enzyme linked immunosorbent assay based on the use of a chaotropic agent. The inhibition index was taken as an estimate of functional affinity. The patient group was divided into high functional affinity subgroup 1 (functional affinity < 0.5, n = 37) and low functional affinity subgroup 2 (functional affinity > 0.5, n = 20). The medical records of all patients were reviewed with a particular note of the disease activity and the articular damage score.**

***Results*—The disease duration was shorter (P < 0.01) in subgroup 1 patients [7.9 (SD 6.4) years] than in subgroup 2 patients [13.4 (11.29) years], so that Ritchie's, Lee's, and Steinbrocker's indices were lower in the former than in the latter (P < 0.01, 0.001, and 0.01, respectively). In contrast, erythrocyte sedimentation rates, C reactive protein concentrations, antinuclear antibody, and HLA DR4 prevalences were similar in the two subgroups.**

***Conclusions*—Different forms of RF are present during progression of the disease.**

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Rheumatoid factors (RF) are polyclonal antibodies that are directed against the Fc fragment of IgG. Rheumatoid arthritis is characterised by raised concentrations of RF. However, although RF are traditionally claimed to be found in rheumatoid arthritis, they are now widely acknowledged to be products of any ongoing normal immune response. It is not clear thus far whether there are distinct forms of RF, one pathological and another physiological. In vitro studies of affinity suggest that RF of rheumatoid arthritis patients are different of those of normal individuals.[1–4] In vivo, the polyclonality of RF in rheumatoid arthritis makes it difficult to characterise qualitative properties, such as specificity or avidity, which are likely to be important in determining their pathogenicity. However, earlier studies have developed a reproducible method for measuring the avidity index of RF. Therefore they estimated the avidity of RF in subjects with rheumatoid arthritis and controls. This has been termed "functional affinity". A higher functional affinity of RF in the rheumatoid arthritis patients' sera than in healthy controls has been described, suggesting the absence of a downregulating mechanism to limit the functional affinity of RF in rheumatoid arthritis[5–7] or an antigen driven response which would lead to antibody selection. Given the dearth of information regarding the functional affinity of RF, the purpose of this study was to compare IgM RF from rheumatoid arthritis patients with high functional affinity to those having low functional affinity, and to address the issue as to whether extra-articular complications occur more frequently in patients with IgM RF of high functional affinity compared with the remainder.

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Methods

PATIENTS

Fifty seven patients, all fulfilling the American College of Rheumatology revised criteria for the classification of rheumatoid arthritis,[8] were evaluated in a cross sectional prospective study. There were 43 women and 14 men. Serum samples were stored at −70°C until analysed.

Each patient had a standard evaluation including a physical examination, laboratory tests for erythrocyte sedimentation rate (ESR), C reactive protein, RF, antinuclear antibodies (AAN), and HLA type (A, B, DR).

PROCEDURES

Raynaud's phenomenon was defined as episodic well demarcated pallor and/or cyanosis in response to cold or emotional stress that was relieved by rewarming.

Disease activity was evaluated clinically by Ritchie's[9] and Lee's[10] indices. Articular damage to the hands was scored according to the method of Steinbrocker, on a scale of 1-4.[11]

RF were measured by the latex test (Biolyon, Paris, France). Positive values for this assay were considered to be titres equal to or greater than 1/40.

Antinuclear antibodies were detected by indirect immunofluorescence (IIF) using established laryngeal carcinoma cell lines (HEp-2) as substrate, and titres of at least 1/100 were considered positive.

HLA typing was performed by a standard microcytotoxicity test on B lymphocytes using a panel of antisera from France-Transplant.

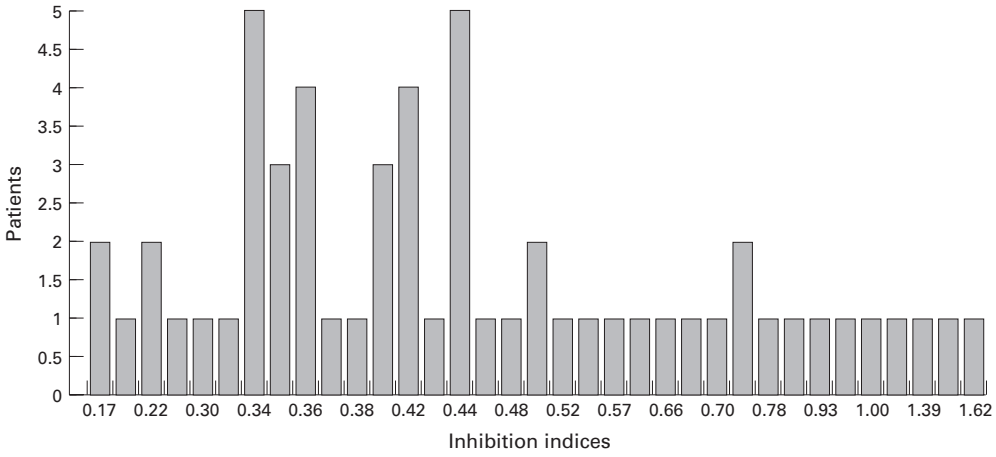

Figure 1 Inhibition indices of RF.

Serum samples were examined for the functional affinity of IgM RF. This procedure has been described previously.[7] Briefly, functional affinity of IgM RF was determined using the chaotropic agent diethylamine in enzyme linked immunosorbent assay (ELISA). Samples were serially diluted on plates (Nunc) coated with Fc fragments of human IgG (Jackson), in the presence or absence of 10 mM diethylamine (Sigma). The plates were incubated at 37°C for 90 minutes, and washed three times with phosphate buffered saline containing 0.05% Tween 20 (PBS-T). HRP conjugated F(ab')2 anti-human IgM (Jackson) was then added at 37°C for 60 minutes. After three washings, colour was developed with H_2O_2 and *O*-phenyl-enediamine and absorption was measured at 492 nm on a Titertek Multiskan (Flow). Dose-reponse curves were plotted and the fall in log titre was taken as an estimate of functional affinity. Thus high functional affinity interactions between RF and IgG were reflected by a low inhibition index, whereas low functional affinity interactions gave high inhibition index values. The arithmetic mean of inhibition indices was 0.53 (SD 0.31). The inhibition indices of RF in the samples studied are given in fig 1.

STATISTICAL ANALYSIS

All the results are expressed as an arithmetic mean and range. Data were analysed using χ^2 test (or Fisher exact test where appropriate), and by the non-parametric Kruskall-Wallis H test. Linear regression analysis were used where indicated. All tests were performed using the Epi-Info and SPSS statistical analysis packages.

Results

We found no correlation between the age of the patients and the inhibition indices of IgM RF ($r = 0.18$, $P > 0.05$, data not shown), whereas the disease duration was correlated with the inhibition indices of IgM RF ($r = 0.41$, $P = 0.002$) (fig 2). As expected, we found a significant correlation between the inhibition indices and Ritchie's index ($r = 0.28$, $P = 0.05$)(fig 3), Lee's index ($r = 0.35$, $P = 0.01$)(fig 4), and radiological indices (interphalangeal, metacarpophalangeal, and carpal scores, $r = 0.51$, 0.27, and 0.37 respectively, $P = 0.01$, 0.05, and 0.01 respectively) (fig5). However, in our population, two subgroups of patients tended to be separated on the basis of these figures. The patients were thus arbitrary divided into two subgroups: subgroup 1 consisted of 37 patients with high functional affinity of IgM RF (< 0.5), and subgroup 2 consisted of 20 patients with low functional affinity of IgM RF (> 0.5). The two groups were statistically treated in similar fashion.

Disease duration was longer in subgroup 2 patients than in the subgroup 1 patients ($P = 0.01$). Subgroup 2 patients had a lower Ritchie's index ($P = 0.01$) and Lee's index ($P = 0.001$) than subgroup 1 patients, but disease duration was significantly longer in subgroup 2 than in subgroup 1. Furthermore, the radiological scores appeared to be significantly worse ($P < 0.01$) and disease duration significantly longer ($P = 0.01$) in subgroup 2 than in subgroup 1 (table 2). In contrast, extra-articular manifestations, ESR, C reactive protein, AAN and HLA DR4 prevalences were similar in the two subgroups (tables 1 and 2).

The most striking observation was thus the association between disease duration and the functional affinity of RF. These data provide evidence for the existence of distinct functional affinity forms of IgM RF in rheumatoid arthritis patients early and late in disease.

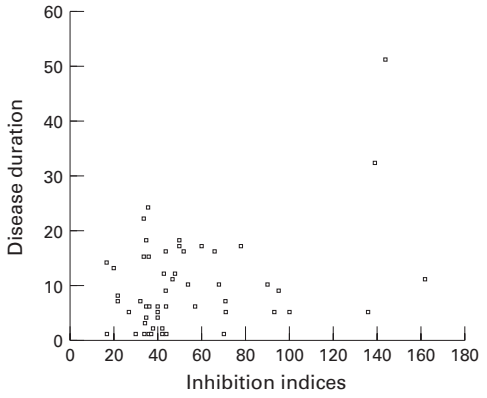

Figure 2 Linear correlation of inhibition indices of IgMRF and disease duration.

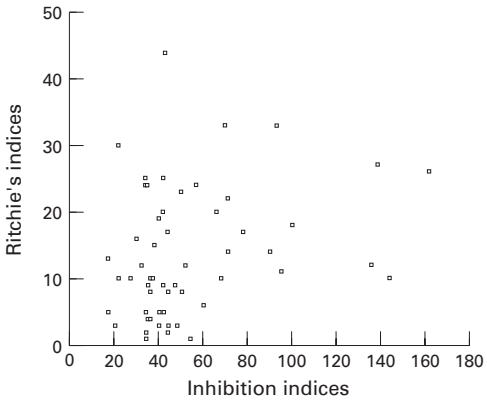

Figure 3 Linear correlation of inhibition indices of IgMRF and Ritchie's indices.

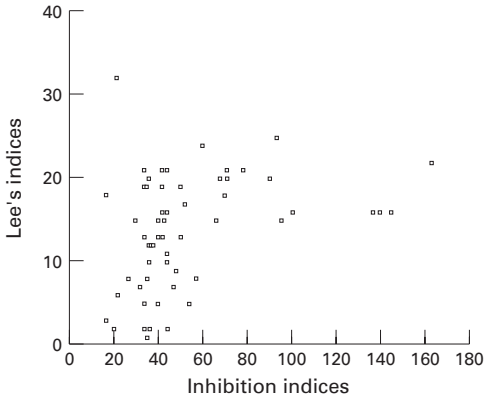

Figure 4 Linear correlation of inhibition indices of IgMRF and Lee's indices.

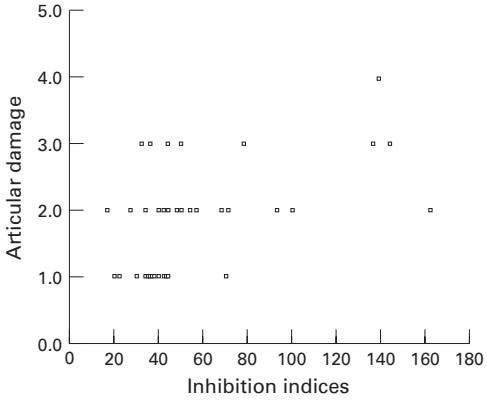

Figure 5 Linear correlation of inhibition indices of IgMRF and interphalangeal articular damage (Steinbrocker's indices).

Discussion

The B cell compartment producing the Fc binding to the Fc fragment of IgG is expanded in rheumatoid arthritis patients and the repertoire of these B cells is shifted toward autoantibodies with monoreactivity and high affinity for the Fcγ autoantigen.[1] In contrast to rheumatoid arthritis patients, RF production in normal subjects is a transient phenomenon which is the consequence of antigenic stimulation and it subsequently decreases in level. These findings suggest that B cells producing RF might be under strict regulatory control both in normal subjects and in patients with rheumatoid arthritis. It is an intriguing question as to how RF are regulated.

Table 1 Extra-articular manifestations, antinuclear antibodies, and HLA DR4 in patients with rheumatoid arthritis

	Group I (high avidity)	*Group II (low avidity*	*P*
Sex ratio F/M	30/7	13/7	0.19
Raynaud's phenomenon	3/37 (8%)	1/20 (5%)	0.56
Vasculitis	2/37 (5%)	0/20 (0%)	0.42
Nodules	9/37 (24%)	7/20 (35%)	0.39
Antinuclear antibodies	14/37 (38%)	6/20 (30%)	0.64
HLA DR4	26/30 (87%)	9/11 (82%)	0.60

Table 2 Disease activity in patients with rheumatoid arthritis. Values are means (SD)

	Group I (high avidity)	*Group II (low avidity)*	*P*
Age (years)	58.1 (14.75)	63.6 (13.72)	0.3
Disease duration (years)	7.9 (6.37)	13.4 (11.29)	0.01
Lee's index	11.89 (7.05)	17.35 (4.88)	0.001
Ritchie's index	11.29 (9.6)	17.05 (8.8)	0.009
Steinbrocker's index	2.12 (0.75)	2.73 (0.46)	0.007
C reactive protein (mg l^{-1})	45.81 (36.29)	42.3 (43.19)	0.48
ESR (mm/h)	57.43 (35.59)	54.35 (33)	0.78
Fibrin (g l^{-1})	48.6 (17.5)	49.58 (18)	0.89
Haemoglobin (g l^{-1})	11.19 (16.31)	10.84 (15.55)	0.31
Xrays:			
Interphalangeal	1.57 (0.74)	2.35 (0.74)	0.003
Metacarpophalangeal	1.78 (0.83)	2.62 (0.96)	0.007
Carpal	2 (0.94)	2.89 (1.02)	0.006

The fact that there is no increase in affinity with the accumulation of mutations in a group of clonally related RF from an immunised normal donor supports the view that there is a controlling mechanism to limit the affinity of RF autoantibodies. Relevant to this interpretation is the recent finding of the rescue of plasma cells from apoptosis by bone marrow and rheumatoid arthritis synovium fibroblasts.[12] As a result, RF might be overproduced within the synovium, leading to mutations and higher affinity of the antibodies. In rheumatoid arthritis patients, IgM RF can undergo affinity maturation[2] without controlling mechanism.[3] The higher affinity of the rheumatoid arthritis derived RF may contribute to the pathogenesis of the disease.

However, locally produced RF may be more important than circulating RF in disease pathogenesis. Interestingly, the functional affinity of IgM RF has been reported to be reduced after a two month treatment with slow acting drugs. The concomitant effect on IgG glycation suggest that there is some connection between RF activity and the carbohydrate load of the targeted molecules.[13 14]

The goal of this study was to compare the features of rheumatoid arthritis patients with IgM RF of high and low functional affinity. The only difference between the two subgroups was the disease duration. In contrast, extra-articular manifestations and biological findings did not differ between the two groups.

Interestingly, Newkirk *et al* [15] did not find significant differences in age, swollen joint

index, extra-articular manifestations (excluded number of nodules), and ESR between rheumatoid arthritis patients with high and low avidity of RF. In contrast, they did not find significant differences in disease duration between the two groups, but disease duration was longer (and with a lower range) in their study than in our own work. The absence of clinical or radiological differences between high and low functional affinity IgM RF groups suggests that functional affinity has no diagnostic values in rheumatoid arthritis.

In conclusion, our findings support the contention that different forms of RF are present throughout the development of the disease. These data suggest that rheumatoid patients downregulate the functional affinity of IgM RF without effect on clinical features.

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