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Isotypes of rheumatoid factors in rheumatoid arthritis and chronic liver diseases

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Abstract We studied isotype-specific rheumatoid factors (RFs) to clarify their significance in rheumatoid arthritis (RA) and to verify the difference in RF isotypes between RA and chronic liver diseases (CLD). Isotype-specific RFs in RA and in CLD were measured by enzyme-linked immunosorbent assay (ELISA). Most sera (n = 51, 94.1%) from RA patients contained some kind of RF isotypes (92.1% for IgM RF, 76.4% for IgG RF, and 43.1% for IgA RF), and seronegative RA by ELISA was seen in only 11.8% (n = 6). The most characteristic combination of RF isotypes in active RA was IgG, IgA, and IgM. This combination of RF isotypes changed to IgG plus IgM, according to the diminution of RA activity; then, we found only IgM RF in inactive RA. The titers of each RF isotype also decreased in parallel with the activity of RA. IgA RF seemed to be the most sensitive factor for evaluating the activity of RA. In CLD, almost the same high frequency (n = 49, 89.8% for IgM RF, 59.2% for IgG RF), with the same titer levels seen in RA, was observed. On the other hand, IgA RF was significantly lower in frequency (n = 9, 18.4%) and in titer, compared with the finding in RA. Surprisingly, even in CLD, true seronegativity by ELISA was also found in very few patients (n = 4, 8.1%). In CLD, positive RFs detected by agglutination assay were seen more often in chronic hepatitis than in liver cirrhosis. In RA patients, significant associations of IgA RF and the serum concentration of IgA, and IgG RF and the serum concentration of IgG, were observed. On the other hand, in CLD patients, significant associations of IgG RF and the serum IgG concentration, and of IgM RF and the serum IgM concentration, were observed. These results indicated that IgA RF in active RA is the most characteristic RF isotype distinguishing it from other nonrheumatic diseases, as well as from inactive RA. RF isotypes reflected the background polyclonal B-cell activation in different manners in both diseases. In CLD, RF isotypes seemed to be disease-related immunological disorders reflecting disease progression.

Key words Chronic liver diseases (CLD) · Isotypes · Rheumatoid arthritis (RA) · Rheumatoid factors (RFs)

Introduction

Rheumatoid factors (RFs), which are autoantibodies against IgG, are the most characteristic marker in rheumatoid arthritis (RA), a chronic joint inflammation with unknown pathogenesis. Lymphocytes in the synovium or synovial fluid of RA patients produce large amounts of RFs.[1,2] The precise contribution of RFs to the pathogenesis of RA remains unclarified, because RFs are also found in a variety of infections, neoplastic diseases, chronic inflammatory diseases, and rheumatic diseases other than RA.[3] Even in healthy individuals, low titers of RFs are sometimes found.[4] Among nonrheumatic diseases, chronic liver diseases (CLD), as well as chronic pulmon ary diseases, often manifest RFs in patient sera.

The pathogenic role of RFs in RA has been discussed based on the results of agglutination tests, such as the Waaler–Rose test for detecting IgM RF. Recently, an enzyme-linked immunosorbent assay (ELISA) has been developed to detect immunoglobulin (Ig) G, A, M, and E classes (isotypes) of RFs. Several reports showed the relevance of specific RF isotypes to progression for overt RA,[5,6] the development of bone damage,[7] clinical severity,[8,9] activity,[9,10] activity and bone damage,[11] the presence of extraarticular symptoms,[12] and the presence of vasculitis.[13] However, the significance of RF isotypes in diseases other than RA is not yet known conclusively.

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In this article, isotype-specific RFs in patients with RA and with CLD were measured, and the results are compared with the results of agglutination tests and with serum Ig levels in relation to polyclonal B-cell activation. We then discuss the pathogenic differences of the RF isotypes that appeared in both diseases.

Materials and methods

Patients and sera

Fifty-one patients with RA and 49 with CLD, who were being followed by Nihon University Hospital, were enrolled in this study. The patients with RA fulfilled the classification criteria of ACR. Collected sera were stored at −20°C until assays were performed. RA patients consisted of 11 men and 40 women ranging from 27 to 84 years of age. Six sero-negative RA patients, by particle agglutination tests (RAPA), were included in this study.

Classification of RA activity

The activity of RA was classified into three groups according to the Lansbury articular index: "highly active," with more than 70%; "moderately active,"with 40–69%; and "inactive,"with less than 39%. Age, gender, diagnosis, complications, and etiology of the patients with CLD are listed in Table 1. Patients with CLD who had been treated with interferon -Éø or cytotoxic agents were excluded from this study. Patients with autoimmune hepatitis were also excluded. The diagnosis of chronic hepatitis or liver cirrhosis was based on liver biopsy or clinical symptoms and signs, including findings of computed tomography and ultrasonography of the abdomen. Sera from healthy volunteers, age-matched to the RA patients, were also obtained.

RAPA tests

The agglutination test to detect RF was done by a modification of the Waaler–Rose test, using heat-denatured rabbit IgG-sensitized artificial gelatin particles (Serodia-ra; Fuji Rebio, Tokyo, Japan). Endpoint titers were determined by serially diluting serum samples. Titers >1:40 were considered positive.

Table 1. Profile of patients with chronic liver disease (CLD): 49 cases

Chronic hepatitis: 27 cases (13 male, 14 female)
Age: 17–50 years (mean, 45 years)
Complication: gastric cancer (1 case)
Etiology
HB virus infection (5 cases)
HCV infection (18 cases)
Unknown etiology (4 cases)
Liver cirrhosis: 22 cases (14 male, 8 female)
Age: 23–70 years (mean, 57.2 years)
Complication: hepatic cell carcinoma (1 case)
Etiology
HB virus infection (1 case)
HCV infection (18 cases)
Unknown etiology (3 cases)

Determination of serum immunoglobulin levels

Serum levels of IgG, IgA, and IgM were determined by laser nephrometry with specific antibodies against each Ig class (Date Behring, Tokyo, Japan).

ELISA for detection of RF isotypes

A semiquantitative assay for the detection of RF isotypes was established. Briefly, microtiter plates (no. 3861-096; Iwaki, Tokyo, Japan) were coated with 100 µl of rabbit IgG (20 µg/ml) in coating buffer containing 1.59 g/l of sodium carbonate and 2.93 g/l of sodium bicarbonate at pH 7.4. The plates were left overnight at 4°C. After extensive washing with phosphate-buffered saline (PBS) Nissui Co., (no. 05913; Iwaki) at pH 7.4, nonspecific binding was blocked by 1% bovine serum albumin (BSA) in coating buffer. After two washings with phosphate-buffered saline (PBS), 100 µl test serum serially diluted by PBS at pH 7.4 was added to the wells, in duplicate, and the samples were left for 2 h at room temperature. The initial dilution of each sample was set at 1:30; then it was serially diluted to 1:65 610 with PBS. After three washings with PBS, 100 µl alkaline phospha-tase-conjugated mouse monoclonal antibody to human IgG, IgA, or IgM (Calbiocheam Novabiochem, CA, USA) was added to the wells. Optimal dilution of enzyme-conjugated antibodies to each immunoglobulin class had been determined previously; 1:4000 for IgG, 1:400 for IgA, and 1:2000 for IgM were employed. The plates were left for 2 h at room temperature. The plates were washed five times with PBS; then, 100 µl freshly prepared substrate solution (1 mg/ml Sigma 104E in diethanolamine buffer at pH 9.6) was added. All procedures were done in a moist chamber. The reaction was stopped at 30 min with 3N NaOH.

The absorbance was read using a spectrophotometer at 405 nm. In the assay, appropriate reagent blanks, positive assay-control serum from an identical RA patient, and negative assay-control serum from a normal individual were included on every plate with the same dilution. Previously, 30 frozen stock sera from healthy individuals, confirmed as having no clinical symptoms or elevation of serum CRP levels, were assayed for RF isotypes with the same dilution as samples. Mean ± 3 SD of optical density (OD) values were determined to be "negative range" in each dilution. If the OD values of test samples reached the "negative range" by serial dilution, the former dilution was determined to be the endpoint titer. A representative titration curve for detecting IgM RF of an active RA patient, and its endpoint titer, are shown in Fig. 1A. The slope of the titration curves differed in each sample. Such titration curves for detecting IgM RF in active patients are shown in Fig. 1B. Furthermore, the prozone phenomenon was frequently observed in the measurement of IgG RF. These

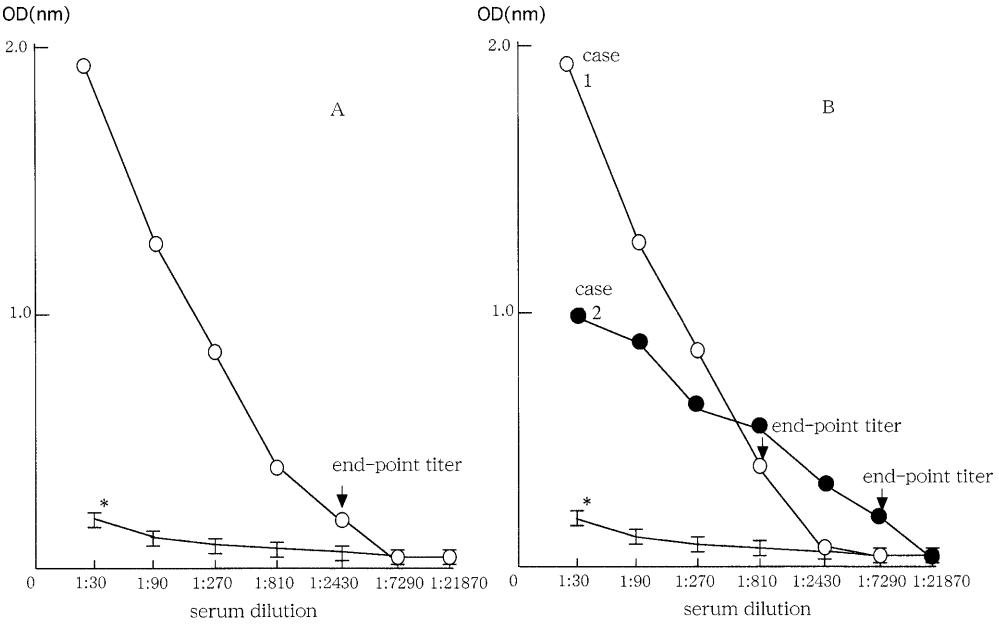

Fig. 1. **A** Determination of the endpoint titer. **B** Different slope of titration curve in IgM rheumatoid factor (RF). * Optical density (OD) values from 30 normal sera (mean ± 3SD)

Table 2. Frequency of positive RAPA tests in patients with RA or CLD and in normal individuals

Sera	Total no. of cases	No. of RAPA positive (%)
RA	51	45 (88.2)
CLD	49	34 (69.3)
Chronic hepatitis	27	23 (85.1)]*
Liver cirrhosis	22	11 (50.0)]*
Normal individuals	37	1 (2.7)

RAPA, rheumatoid arthritis particle agglutination; RA, rheumatoid arthritis; CLD, chronic liver disease
* $P = 0.0007$

observations indicated that serum having a higher OD value in its initial dilution does not always have a higher endpoint titer. Before ELISA for the detection of IgG RF, serum samples were digested with 1.5% (w/w) pepsin at 37°C for 22 h, and digestion was neutralized with 1 M Tris-base 10% BSA.

Statistical analysis

Statistical analyses were performed using the Mann–Whitney *U* test, Student's *t* test, and the determining Pearson's correlation coefficient. In particular, semiquantitative analyses of the results shown in Fig. 2, Table 3, and Table 4, the Kruskal–Wallis rank test (step 1) and the Mann–Whitney *U* test (step 2), were employed.

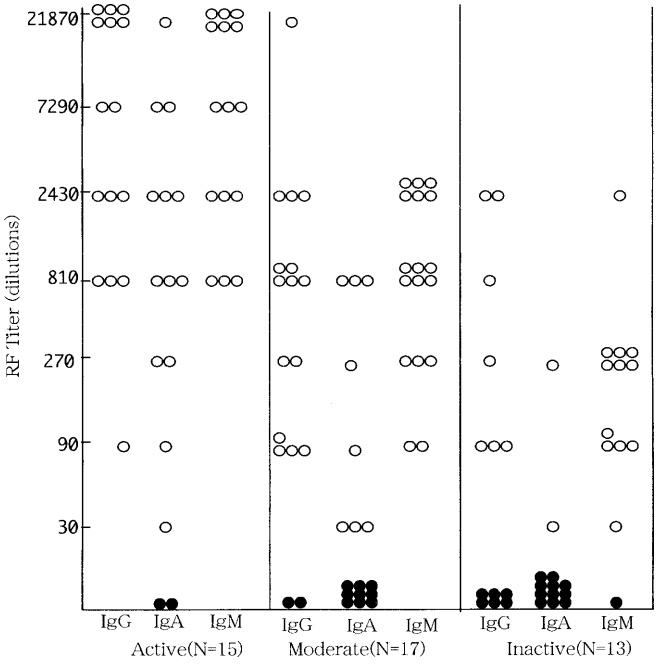

Fig. 2. Activity of RA and RF isotypes. Activity of RA was classified into three groups according to the Lansbury articular index as described in Materials and methods. *Open circles*, positive cases; *solid circles*, negative cases

Results

Frequency of positive RAPA test in patients with RA and CLD and in normal individuals

The titers of the RAPA test of sera from patients with RA or CLD and from normal individuals were determined (see Table 2); 45 of 51 patients (88.2%) with RA showed positive RAPA tests and the remaining 6 (11.8%) RA patients showed seronegativity. Positive RAPA tests were found in 34 sera (69.3%) from patients with CLD of 49 sera tested. RAPA-positive sera were found more often in patients with chronic hepatitis (85.1%) than in these with liver cirrhosis (50.0%); the difference was significant ($p = 0.0007$). In normal individuals, only 1 serum (2.7%) of 37 sera was

Table 3. Rheumatoid factor (RF) isotypes in RAPA-positive and RAPA-negative sera

Sera	Positive IgM RF (%)	Positive IgG RF (%)	Positive IgA RF (%)
RA			
Total no. of cases (n = 51)	47 (92.1%)	39 (76.4%)	22 (43.1%)
RAPA-positive (n = 45)	44 (97.7%)	38 (84.4%)	20 (44.4%)
RAPA-negative (n = 6)	3 (50.0%)	1 (16.6%) *	2 (33.3%) **
CLD			
Total no. of cases (n = 49)	44 (89.8%)	29 (59.2%)	9 (18.4%)
RAPA-positive (n = 34)	34 (100%)	22 (64.7%)	5 (14.7%)
RAPA-negative (n = 15)	10 (66.6%)	7 (46.7%)	4 (26.6%)
Normal individuals			
Total no. of cases (n = 37)	1 (2.7%)	1 (2.7%)	0 (0%)
RAPA-positive (n = 1)	1 (100%)	1 (100%)	0 (0%)
RAPA-negative (n = 36)	0 (0%)	0 (0%)	0 (0%)

* $P < 0.0001$; ** $P = 0.0001$

Table 4. Combination of RF isotypes in RAPA-positive and RAPA-negative sera

Combination of RF isotype	RA				CLD	
	RAPA-positive			RAPA-negative	RAPA-positive	RAPA-negative
	Active (n = 15)	Moderate (n = 17)	Inactive (n = 13)	(n = 6)	(n = 34)	(n = 15)
M + G + A	13 (86.7%)*	8 (47.0%)	1 (7.6%)*	1 (16.6%)	3 (8.8%)	2 (13.3%)
M + G	2 (13.3%)*	9 (52.9%)	7 (53.8%)*	0	18 (52.9%)	6 (33.0%)
M + A	0	0	0	1 (16.6%)	0	2 (13.3%)
G + A	0	0	0	0	0	0
M	0	0	5 (38.5%)	1 (16.6%)	13 (38.2%)	2 (13.3%)
G	0	0	0	0	0	0
A	0	0	0	0	0	0
None	0	0	0	3 (50.0%)	0	4 (26.7%)

* $P < 0.05$: percentages of patients with each combination of RF isotypes were compared between active and inactive patients by determining Pearson's correlation coefficient

positive in the RAPA test. None of the patients with CLD, and none of the normal individuals, had clinical symptoms of RA.

Isotypes of RFs in RA and CLD patients and in normal individuals

Isotypes of RFs in RA, CLD, and normal individuals were determined by ELISA. In RA patients, a high frequency of each RF isotype was found (92.1% for IgM RF, 76.4% for IgG RF, and 43.1% for IgA RF) (see Table 3). In CLD, a high frequency of IgM RF was also found in 44 sera (89.8%) of 49 examined. On the other hand, lower frequencies of IgG RF (59.2%) and IgA RF (18.4%) in CLD, compared with those in patients with RA (76.4% of IgG RF and 43.1% of IgA RF, respectively), were observed. These differences were significant in their frequencies ($P < 0.0001$ in IgG RF; $P = 0.0001$ in IgA RF) and in their titers by semiquantitative analysis ($P < 0.0001$ in IgG RF; $P = 0.0001$ in IgA RF). Surprisingly, even in the sera with negative RAPA tests, both in RA and in CLD, positive IgM, IgG, and IgA RFs were found (50.0%, 16.6%, and 33.3% for RA; 66.6%, 46.7%, and 26.6% for CLD), although IgM RF was found less frequently and in lower titers than in RAPA-positive sera, in both diseases. In normal individuals, only 1 RAPA-positive serum had low titers of both IgM and IgG RF.

Activity of RA and RF isotypes

Relationships of RA activity and the titers of RF isotypes were studied using RAPA-positive sera (n = 45) (see Fig. 2). The activity of RA was classified by the Lansbury articular index into three groups, as described in Materials and methods. High frequencies of IgG, IgA, and IgM RF isotypes (100%, 86.6%, and 93%, respectively) in the highly active RA group were observed, and they decreased along with the diminution of disease activity (88%, 47%, and 64% in the moderately active group; 69%, 15.3%, and 38.5% in the inactive group). The titers of each RF isotype also declined in parallel with disease activity. Semiquantitative analyses demonstrated that the differences in titers between active and moderately active RA patients, moderately active and inactive RA patients, and active and inactive RA patients, in any RF isotypes, were statistically significant ($p < 0.05$). IgM RF seemed to be poorly sensitive to disease activity in terms of its frequency, although its titer was significantly lower in the inactive group than in the highly active group ($p < 0.01$). The difference in the frequencies of positive IgG RF (15 of 15 cases; 100%) and positive IgA RF

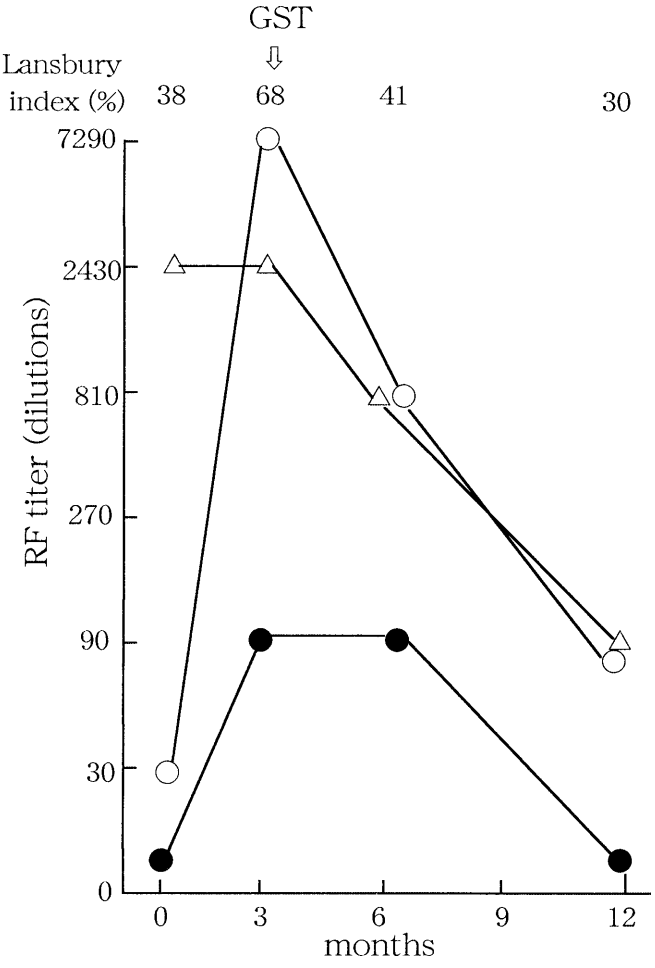

Fig. 3. Clinical course and periodic change of RF isotypes of an RA patient. *Open circles*, IgG RF; *closed circles*, IgA RF; *triangles*, IgM RF; *GST*, gold sodium thiomalate

(13 of 15 cases; 86.6%) in the active RA group was not significant ($P = 0.16$), whereas the frequency of positive IgG RF (7 of 13 cases; 69%) was significantly higher than that of positive IgA RF (2 of 13 cases; 15.3%) in the inactive RA group ($P = 0.018$). These results indicated that IgA RF was the most sensitive RF isotype for disease activity.

The kinetic changes of RF isotypes by treatment with antirheumatic agents of an RA patient are shown in Fig. 3. In this case, IgA RFs disappeared, but IgG and IgM RFs were still present at lower titers at 12 months after treatment. The same association of RF isotypes and disease activity in individual patients was found in four of five other cases studied.

Titration of RF isotypes in CLD

RF titers for each isotype were determined. IgM RF was higher in titers in RAPA-positive sera than in RAPA-negative sera ($P < 0.01$). There were no differences in titers of IgG RF ($P > 0.05$) and IgA RF ($P > 0.05$) between RAPA-positive and RAPA-negative sera. The IgM RF titer from RAPA-positive sera in patients with CLD was almost comparable to that from sera in patients with "moderately active" RA. On the other hand, the IgG and IgA RF titers in patients with CLD were significantly lower than those from sera in patients with "moderately active" RA ($P < 0.05$).

Combination of RF isotypes

The combination of IgG, IgA, and IgM RF was the most typical pattern in the active RA group (86.7%) (see Table 4). This combination of RF isotypes changed to IgG plus IgM (53.8%), and to only IgM RF (38.5%), in the inactive RA group, with decreased titers of each RF isotype. low titers of RF isotypes, with various combinations, were observed even in RAPA-negative sera from patients with RA, and true seronegative RA was seen only in 50% of the RAPA-negative patients. In CLD, serum containing all three classes of RFs was found in only three (8.8%) RAPA-positive and in only 2 (13.3%) RAPA-negative patients. The combination of IgM plus IgG RF was the most frequent in both RAPA-positive (52.9%) and RAPA-negative patients (33.0%). Single-positive IgM RF was found more frequently in RAPA-positive patients (38.2%) than in RAPA-negative patients (13.3%). True seronegativity in CLD by ELISA was found in 26.7% of RAPA-negative sera. Among CLD patients, a difference of the combination of RF isotypes between chronic hepatitis and liver cirrhosis was not observed. The IgM RF titer in the sera from chronic hepatitis was higher than that from liver cirrhosis ($P < 0.05$) (data not shown).

Relationship between RF isotypes and serum immunoglobulin levels

To understand the serum background in relation to RF isotype expression, serum levels of each immunoglobulin class were measured and compared with the titers of RF isotypes. There were no differences in the respective serum levels of immunoglobulin classes between RAPA-positive and RAPA-negative sera from RA patients (1501 versus 1440 mg/dl for IgG, 225 versus 268 mg/dl for IgA, and 168 versus 198 mg/dl for IgM) and from CLD patients (2104 mg/dl versus 2271 mg/dl for IgG, 320 mg/dl versus 372 mg/dl for IgA, and 297 mg/dl versus 202 mg/dl for IgM). In RA patients, there were significant associations between serum IgG levels and the titer of IgG RF ($p < 0.001$, $r = 0.47$), and between serum IgA levels and the titer of IgA RF ($p < 0.05$, $r = 0.42$), but no significant association was found between serum IgM levels and IgM RF.

A representative clinical course, and the changes of RF isotypes and serum immunoglobulin levels by treatment with antirheumatic agents in an RA patient, are shown in Fig. 4. Serum IgG and IgG RF, and serum IgA and IgA RF, were decreased in parallel; however, IgM RF was unchanged, despite the decrease of serum IgM levels by treatment with antirheumatic agents. The same association of RF isotypes and counterpart Ig classes in individual levels was observed in three of seven other cases tested. In CLD,

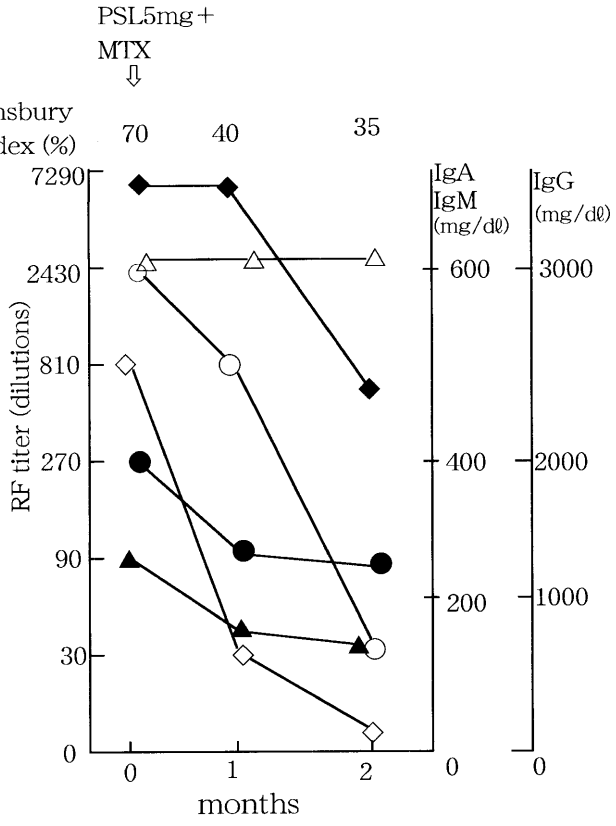

Fig. 4. Clinical course and periodic changes of RF isotypes and serum immunoglobulin levels of a RA patient. *Open triangles*, IgM RF; *open circles*, IgG RF; *open diamonds*, IgA RF; *closed triangles*, serum IgM concentration; *closed circles*, serum IgG concentration; *closed diamonds*, serum IgA concentration; *PSL*, prednisolone; *MTX*, methotrexate

there were significant associations between titers of IgM RF and serum IgM levels ($p < 0.0001$, $r = 0.692$), and between IgA RF and serum IgA levels ($p < 0.0001$, $r = 0.753$). No association was found between the titer of IgG RF and serum IgG levels.

Discussion

Rheumatoid arthritis (RA) is characterized by elevated levels of RFs. Standard assays for the detection of RF, Waaler–Rose tests or RAPA tests, reflect mainly IgM RF. Some 80% of sera from RA patients show positive RAPA tests, and the remaining RA patients have been named seronegative RA, according to agglutination assays. The contribution of RFs to the pathogenesis of RA has been argued on the basis of results of agglutination assays. An immunochemical technique, such as ELISA, enables the measurement of isotype-specific RFs, and several attempts have demonstrated an association between RF isotypes and clinical features of RA.

In our study, the association between IgG and IgA RF isotypes and disease activity was clearly found in a mass study (see Fig. 2). In periodic studies, at a single-patient level, the association between IgA RF and disease activity was also found (see Fig. 3). The association with disease activity was stronger for IgA RF than for IgG RF. Clinical significance of IgA RF for diagnosis,[14] severity,[8,15] severity, activity and duration of disease,[9] and extraarticular manifestation[16] in RA has been reported. The specific meanings of IgA RF in RA are unknown. Hajeer et al. reported[17] the possibility that IL-10 promoter gene polymorphism leads to low IL-10 production and specific IgA RF production in patients with RA.

In another rheumatic disease, systemic lupus erythematosus (SLE), patients who have high titers of RFs have been reported to have low disease activity and to be protected against renal involvement,[18,19] rather than to be more pathogenic. On the other hand, the significance of RF isotypes in normal individuals, or in nonrheumatic diseases, is still unknown. In this study, positive RAPA tests were demonstrated in 69.3% of sera from patients with CLD. This frequency of positive RAPA tests is almost as that in RA patients (88.2%). Among CLD, chronic hepatitis showed a higher proportion of positive RAPA tests (85.1%) compared with liver cirrhosis (50%), in contrast to the report by Wang et al.[20] The different frequency of RAPA positivity between chronic hepatitis and liver cirrhosis mainly reflected the different titers of IgM RF determined by ELISA.

Even in RAPA-negative sera, RFs were found with a single isotype or with various combinations of isotypes, in both RA and CLD patients. True seronegativity by ELISA was seen in 50% of RAPA-negative RA and in 26.7% from RAPA-negative CLD. A hidden RF, consisting of complexes with high-affinity IgG RF and autologous IgG, has been considered to be a reason for seronegativity in RA.[21,22] Our results indicated that the presence of either IgG or IgA RFs, alone or in combination, with a low titer of IgM RF, also leads to seronegativity detected by agglutination assay, in both diseases. Less seronegativity in RA sera by ELISA, similar to our results, has been reported.[23,24]

It is now accepted that nonpathogenic autoantibodies encoded in the germline gene may differentiate into pathogenic autoantibodies by antigen stimulation and polyclonal activation. Through such differentiation, isotype switching and affinity maturation may occur. In general, nonpathogenic autoantibodies are polyreactive and of the IgM class, whereas pathogenic autoantibodies usually posses somatic mutations and are monoreactive, high affinity, and of the IgG or IgA class.[25] These explanations seemed to be consistent with RF.[26] In this regard, the results presented here, and those of others,[8–10] that IgG and IgA RF isotypes are associated with clinical activity of RA , seem reasonable. These data indicated that isotype switching is important for the activity of RA. On the other hand, our data also showed that a high frequency of IgG RF isotypes, together with the IgM isotype and less frequently IgA RF, was observed in CLD. This finding indicates that class switching of RFs, especially to the IgG class, occurred not only in RA but also in nonrheumatic diseases without any pathogenic significance. The low frequency and lower titers of IgA RF in CLD seem to be the essential points in distinguishing it from RA.

Because polyclonal hypergammaglobulinemia was present at almost the same levels in RAPA-positive and RAPA-negative sera from patients with both RA and CLD, it is doubtful that polyclonal B-cell activation is mainly responsible for the RF production. On the other hand, IgG and IgA RFs in RA, and IgM and IgA RFs in CLD, reflected each serum level of immunoglobulin classes. These results indicated that polyclonal B-cell activation in some immunoglobulin classes may play a role in production of counterpart RF isotypes. In particular, IgA-class-specific B-cell activation appears to be important for the disease activity of RA. The contribution of immunoglobulin class-restricted polyclonal hypergammaglobulinemia to the pathogenesis of RA is a problem to be solved. Aho et al.[27] demonstrated, by a long-term mass study, that serum IgG and IgA concentrations before the onset of RA have been predictive for RF-positive RA.

It is well known that hepatitis B- and C-related CLD may be associated with various immunological disorders.[28,29] These disorders include RF production, mixed cryoglobulinemia,[30] and other autoantibody production, such as anti-GOR antibodies,[31] antinuclear antibodies, and antimicrosomal antibodies.[32] In the case of antinuclear antibody (ANA) in CLD, it was reported that the production of ANA was correlated with disease progression, and sometimes positive ANA was a good serologic indicator for the presence of hepatocytic carcinoma.[33] This result, together with our different manner of RF production, indicated that the autoantibodies may act as markers at different states of CLD. These results also suggest that the mechanisms of autoantibody production in CLD are heterogeneous.

Exceptional patients with hepatitis C virus (HCV) infection, with positive RF and rheumatic manifestations indistinguishable from RA, have been reported.[34] Although class switching of RF isotypes, including to IgA isotype, and class-specific polyclonal B-cell activation, seemed to be important background for RA, our results allow the speculation that other pathogenic factors in addition to RFs are required to cause rheumatic manifestations in CLD. Looking at the findings in CLD, the production of RFs and RF isotypes seemed to be disease-related disorders reflecting different immunological alteration by the progression of CLD.

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