Hidden 19S IgM rheumatoid factor in adults with juvenile rheumatoid arthritis onset

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SUMMARY Forty-eight adult patients with juvenile rheumatoid arthritis (JRA) (onset before age 16 years) were evaluated at the age of 17 years or more for the presence of hidden 19S IgM rheumatoid factors (RF), i.e., 19S IgM RF that can be detected by the complement-dependent haemolytic assay in the IgM-containing fraction after separation of the serum by acid gel filtration. The average age of the patients was 25.3 years. The mean duration of disease was 16.5 years. Thirty-two of 48 patients (67%) showed the presence of hidden 19S IgM RF in their serum. Disease activity correlated with hidden RF titres in 62% (55/88) of the evaluations. The results indicate that patients with seronegative JRA onset continue to have significant titres of hidden 19S IgM RF in their sera into early adulthood.

Key-word: seronegative rheumatoid arthritis.

Classical 19S IgM rheumatoid factors (RF) as detected by the latex fixation test (LFT) are usually found in the sera of only 5–10% of cases of children with juvenile rheumatoid arthritis (JRA)¹ as compared with 80% of adults with rheumatoid arthritis (RA).² Hidden 19S IgM RF, i.e., 19S IgM RF detected in the IgM-containing fraction of serum after separation by acid gel filtration, were initially described by Allen and Kunkel³ in adults with severe RA who were seronegative for 19S IgM RF by the LFT. Moore *et al.*⁴ first showed the presence of hidden 19S IgM RF in children with JRA. They found these RF by means of the LFT in 46% of seronegative JRA patients. Moore *et al.*^{5,6} found hidden RF in 59–68% of children with seronegative JRA by the complement-dependent haemolytic assay for detection of RF. Thus hidden RF as determined by the haemolytic assay or by the LFT could be shown to be present in approximately 75% of children with JRA. In a longitudinal study hidden 19S IgM RF correlated strongly with disease activity in JRA patients.⁷

The present study was undertaken to determine

whether hidden 19S IgM RF were still present in the sera of JRA patients who reached young adulthood. The relationship of these hidden RF to disease activity and duration of disease was also evaluated.

Materials and methods

PATIENTS

Sera were obtained from 48 adult (≥ 17 years of age) patients who had a diagnosis of JRA made before they were 16-years old by criteria established by the Committee on the Classification of JRA of the American Rheumatism Association.⁸ Sera were also obtained from 65 children, 35 healthy and 30 patients with connective tissue disease (CTD), and from 50 adults, 25 healthy and 25 with CTD, to act as controls. The CTD controls were mainly patients with systemic lupus erythematosus, mixed connective tissue disease, progressive systemic sclerosis, polymyositis, seronegative spondyloarthropathies and other related CTD. All specimens were drawn in clot tubes and allowed to clot for 30 minutes at room temperature. Sera were obtained by centrifugation at 4°C and stored at –70°C until tested. The 48 patients were attending the Arthritis Clinic at St Louis University Medical Center or the private offices of Division rheumatologists. 79% (38/48) had been under our observation both as children and as

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adults; the remaining 21% had been followed up elsewhere before adulthood. Age range at the time of evaluation was 18–38 years; mean age 25.3 years. The method for determining disease activity was established before the onset of the study and activity of disease was assessed at the time of each evaluation. Active disease was defined on a two point scale: (–) indicated no active joint disease; and (+) indicated one or more swollen painful joints.

HAEMOLYTIC ASSAY

Sera were subjected to acid gel filtration on a Sephadex G-200 column (2.5×85 cm) essentially by the method of Allen and Kunkel, as previously described.³ The IgM and IgG fractions were concentrated to the original serum volume by vacuum dialysis. Purity of fractions was determined by immunodiffusion against rabbit antihuman IgG, IgA, and IgM (Behring Diagnostics, Somerville, NJ). The sera and IgM and IgG fractions were then heat inactivated at 56°C for 30 minutes and absorbed by washed sheep red blood cells (SRBC) (Alban Co., St Louis, MO) for 90 minutes at 37°C. A haemolytic assay was then carried out on the serum, IgM, and IgG fractions, as previously described in detail⁶: 0.1 ml of serum, IgM, or IgG fractions was serially diluted in 0.1 ml of 0.15 M veronal-buffered saline containing 0.1% gelatin, 0.005 M MgCl₂, and 0.00015 M CaCl₂ at pH 7.4 (GVB). 0.1 ml of 1% sensitised SRBC was added to each dilution. The target cell SRBC were sensitised with a 1:100 dilution of rabbit anti-SRBC IgG haemolysin (Colorado Serum, Denver, CO), as previously described.^{5,6} After incubation at 37°C for 60 minutes and at 4°C for 60 minutes or overnight the cells were washed with 1 ml of cold GVB and centrifuged at 4°C at 2000 rpm; the supernatant was discarded. 1 ml of GVB and 0.4 ml of 1:40 diluted guinea pig serum (Pel-Freez Co., Helena, AR) were added to the target cells. Guinea pig serum previously absorbed by SRBC was a source of complement. After incubation for 60 minutes at 37°C with vortexing every 15 minutes the reaction was stopped by addition of 1.5 ml of cold 0.15 M phosphate-buffered saline (PBS), pH 7.3. The tubes were centrifuged for 5 minutes at 4°C at 2000 rpm. Haemoglobin released into the supernatant was assayed spectrophotometrically at 412 nm. Buffer and serum background controls and 100% water haemolysis controls were included in all assays. Titres for RF > 1:16 on the serum, IgM-, or IgG-containing fractions were considered positive, since that titre differs significantly from the titre for age-matched healthy controls, children with systemic lupus erythematosus, polymyositis, etc., and from the titre for children with other illnesses.⁵

LATEX FIXATION TEST (LFT)

The LFT was performed on the whole serum after heat inactivation for 30 minutes at 56°C by the method of Singer and Plotz⁷ with a commercial test kit (Difco Laboratories, Detroit, MI). Titres greater than 1:20 were considered positive.

ANTINUCLEAR ANTIBODIES (ANA)

ANA were determined by indirect immunofluorescence on sections using mouse kidney or human epithelial (HEp-2) cells as substrate and fluorescein-labelled antihuman IgG or IgM antibody. Titres > 1:20 were considered positive.

Results

Data on the patients' clinical findings are summarised in Table 1. Results of testing for 19S IgM RF, hidden 19S IgM RF, and ANA are also tabulated.

Six men and 42 women with JRA diagnosed in childhood were studied. Thirty-seven patients had polyarticular onset, nine pauciarticular onset, and two systemic onset disease. The age of onset ranged from two to 15 years, with a median age of 9.4 years. The onset of disease was between ages two and five years for 13 patients, between six and 10 years for nine patients, and between 11 and 15 years for 26 patients. The average age of the patients when last studied was 25.3 years. The duration of the disease when tested after the age of 17 years ranged from five to 31 years, with a mean duration of 16.5 years.

Forty-eight patients were evaluated over a 10-year period. Seven patients (15%) were positive for RF detected by the LFT, with titres ranging from 1:40 to 1:5120. The serum was positive (titre > 1:16) for RF detected by haemolytic assay in 12 cases (25%). Five patients were positive for 19S IgM RF detected by both tests; the titres determined by the LFT were higher in all five patients. Hidden 19S IgM RF (> 1:16) were found on at least one assessment in 32 patients (67%) and correlated well with fluctuations in disease activity. Overall, in 88 serum samples evaluated in the 48 patients disease activity correlated with IgM hidden RF titres in 55/88 (62%). Twenty-one patients were evaluated before and after their 17th birthday. Hidden RF persisted in seven and remained negative in two. Five patients were positive before the age of 17 years and negative when tested after 17. Four patients were negative before the age of 17 years and positive after 17.

ANA were present in 22 of 48 patients (46%) with titres ranging from 1:40 to 1:1600. Twelve (54%) had a speckled pattern and 10 (46%) a diffuse pattern.

Control studies for hidden 19S IgM RF were performed on 35 healthy children ages one to 16

Table 1 Summary of clinical and laboratory data of adults with juvenile rheumatoid arthritis onset

Patient	Sex	Age of onset (years)	Current age	Type of onset	Serum RF (LFT)*	Serum RF (HA)†	Hidden RF (IgM fraction) (HA)‡	ANA§
1	F	11	23	Pa	Neg.	1:4	1:4	Neg.
2	F	2	21	Pa	Neg.	1:8	1:64	1:320, S
3	F	6	21	Po*	Neg.	1:64	1:64	Neg.
4	M	12	38	Po	Neg.	1:8	1:4	Neg.
5	F	7	30	Po	Neg.	1:4	1:2	1:20, D
6	F	13	19	Po	1:5120	1:512	1:1024	1:640, S
7	F	11	25	Po	Neg.	1:512	1:1024	1:40, S
8	F	9	21	Po	Neg.	1:8	1:32	Neg.
9	F	2	24	Pa	Neg.	1:32	1:128	1:400, D
10	F	6	23	Po	Neg.	1:16	1:256	1:160, S
11	F	10	18	Po	Neg.	1:4	Neg.	1:640, S
12	M	9	30	Po	Neg.	1:4	1:128	Neg.
13	F	2	26	Po	Neg.	1:8	1:8	1:64, S
14	F	11	29	Po	1:5120	1:64	1:512	1:520, S
15	F	13	23	Sy**	Neg.	1:16	1:128	Neg.
16	F	2	19	Po	Neg.	1:64	1:64	1:40, S
17	F	15	37	Po	1:640	1:128	1:32	1:100, D
18	F	13	28	Po	1:40	1:4	1:32	Neg.
19	F	15	25	Po	Neg.	1:16	1:32	Neg.
20	F	4	34	Sy	Neg.	1:512	1:64	Neg.
21	F	3	20	Pa	Neg.	1:16	1:16	Neg.
22	F	12	23	Po	Neg.	1:8	1:64	1:200, D
23	F	5	30	Po	Neg.	1:32	1:64	Neg.
24	F	13	36	Po	Neg.	Neg.	1:2	Neg.
25	M	11	20	Po	Neg.	1:8	1:128	Neg.
26	F	15	31	Po	Neg.	Neg.	1:16	1:20, D
27	F	15	26	Po	Neg.	Neg.	Neg.	Neg.
28	M	12	31	Po	Neg.	1:4	1:64	Neg.
29	F	5	33	Po	Neg.	1:8	Neg.	Neg.
30	F	15	24	Po	Neg.	Neg.	1:64	Neg.
31	F	15	32	Po	1:5120	1:512	1:1024	1:100, D
32	F	12	27	Po	Neg.	1:8	1:32	Neg.
33	F	7	37	Po	Neg.	1:8	1:32	1:200, D
34	F	11	31	Po	Neg.	1:8	1:32	Neg.
35	F	11	32	Po	Neg.	1:16	1:16	Neg.
36	F	13	22	Po	Neg.	1:8	1:32	Neg.
37	F	7	34	Po	1:160	1:16	1:32	1:80, D
38	F	15	21	Po	Neg.	Neg.	Neg.	1:640, S
39	F	14	19	Pa	Neg.	1:8	1:16	1:20, S
40	F	10	18	Pa	Neg.	1:16	1:64	1:80, S
41	F	5	25	Po	Neg.	1:4	1:512	1:80, D
42	F	13	28	Po	Neg.	1:2	1:64	1:80, D
43	F	4	20	Po	Neg.	1:8	1:1024	Neg.
44	F	2	20	Pa	Neg.	1:64	1:512	1:100, D
45	M	11	18	Po	Neg.	1:8	Neg.	1:160, S
46	F	11	21	Pa	Neg.	1:16	1:64	Neg.
47	M	2	19	Pa	Neg.	Neg.	1:16	1:160, S
48	F	4	22	Po	1:640	1:32	1:32	1:1600, D

*Serum 19S IgM rheumatoid factor (RF) as detected by latex fixation test (LFT) (titre > 1:20 considered positive).

†Serum 19S IgM rheumatoid factor (RF) as detected by haemolytic assay (HA) (titre > 1:16 considered positive).

‡Hidden 19S IgM rheumatoid factor (RF) in IgM fraction as detected by haemolytic assay (HA) (titre > 1:16 considered positive).

§Antinuclear antibody (ANA), diffuse (D) or speckled (S) pattern.

||Pauciarticular (Pa) onset type juvenile rheumatoid arthritis.

¶Polyarticular (Po) onset type juvenile rheumatoid arthritis.

**Systemic (Sy) onset type juvenile rheumatoid arthritis.

years, 25 healthy adults ages 17 to 40 years, 30 CTD patients ages one to 16 years and 25 CTD controls ages 17 to 40 years. The 35 healthy children showed a median hidden RF titre of 1:7 by the haemolytic assay, the 30 CTD controls in this age group 1:5, the

25 healthy adults 1:6, and the 25 adults with CTD 1:5. The combined controls of these 115 patients showed an overall median titre of 1:6. We, therefore, considered that two standard deviations above the median titre (titres > 1:16) were significant.⁵

Discussion

Allen and Kunkel³ had shown that hidden IgM RF with agglutinating ability were present in the serum of a small number of adults with seronegative RA, but Robbins and Moore,¹⁰ on the contrary, were not able to detect complement-fixing hidden IgM RF in adult seronegative RA patients. Complement-fixing hidden IgM RF had previously been detected in the majority of children with seronegative JRA.⁴⁻⁷

In this study complement-fixing hidden 19S IgM RF were found in adult patients with JRA onset in 67% (32/48) of cases, results similar to those (59-68%) previously noted in children with JRA.^{5,6} Complement-fixing hidden RF titres correlated with disease activity in 62% (55/88) of instances, a lower incidence than that found in children with JRA where there was a 92% (93/101) correlation.⁷ In these patients the percentage of hidden RF (67%) was greater than that of ANA (46%), positive serum RF determined by the haemolytic assay (25%), or by the LFT (12%).⁷

Thirty patients (62%) had active disease when examined as adults, but only 12 (25%) had severe, erosive disease. Nineteen of 30 patients with active disease were either currently being treated with or had previously received 'remission inducing' or immunosuppressive agents, such as, gold salts, D-penicillamine, methotrexate, or azathioprine. Of 18 patients with inactive disease seven were previously treated with gold salts and five were continuing on maintenance gold therapy; 13 patients had remained on non-steroidal anti-inflammatory drugs. Of 31 patients who received 'remission inducing' agents 27 (87%) initially had positive hidden RF; four of these subsequently became hidden RF negative, while 23 continued to show positive hidden RF. There was no reduction in titres of hidden RF in these patients.

The presence of RF determined by LFT correlated with the age of onset of JRA. Hanson *et al.*¹¹ showed a rise in the incidence of RF determined by the LFT from 3.8% up to the age of four years, to 25% between four and 12 years, and to 77% at ages 12 to 16 years. In our patients only one of 13 children with onset of disease up to five years of age was positive for RF determined by the LFT, but eight were positive for IgM hidden RF. In the six- to 10-year-old onset group one of nine had a positive LFT for RF, but seven had a positive test for IgM hidden RF. For those with onset at 11 to 15 five of 26 were seropositive by LFT and 17 of 26 had a positive test for IgM hidden RF. The presence of hidden RF in the child with JRA therefore did not change significantly with age. Jeremy *et al.*¹² looked at the incidence of RF in patients with JRA whose

disease had persisted into adulthood. They found no correlation between the presence of RF as detected by LFT and duration or severity of the disease. In our study in the age group 17 to 25 years hidden RF were found in 20 of 27 patients, at 26- to 30-years old in six of nine patients, and above 31 years in six of 12 cases. There was no significant reduction in hidden RF positivity with age. Overall, 19S IgM RF seemed independent of age of onset in the JRA onset group and did not change significantly in young adulthood. There was also no change related to duration of disease.

Hidden RF have been demonstrated in all three onset types of JRA but more frequently in the polyarticular onset group.^{5,6} This group most frequently has long-term disease activity.^{1,12,13} Thirty-seven of 48 patients in this study had a polyarticular onset, 26 of whom had raised titres of hidden RF at one time during the course of their disease.

Although the pathogenic role for IgM RF and hidden IgM RF in JRA has not been completely understood, the correlation of hidden RF with disease activity and with polyarticular onset disease and its resultant higher incidence of a chronic and more severe illness strongly suggests a pathogenic role for hidden 19S IgM RF in JRA. This potential has also been suggested by studies that showed the presence of 19S IgM RF and hidden 19S IgM RF in immune complexes in these patients.^{14,15}

In conclusion complement-fixing hidden 19S IgM RF were shown to be present in the majority of seronegative adult arthritis patients with childhood onset JRA. The presence of hidden RF correlated with disease activity. In contrast, previous studies on seronegative adult onset RA patients showed no evidence of complement-fixing hidden 19S IgM RF in these patients.¹⁰ These studies suggest that patients with seronegative JRA onset may have different pathophysiological mechanisms of disease than patients with adult onset seronegative RA.

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