

# Quantitation and evaluation of low molecular weight IgM in rheumatoid arthritis

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**SUMMARY** Laser nephelometric estimation of IgM in the eluate fractions following Sepharose 6B chromatography has enabled the calculation of the proportion of low molecular weight IgM (7S IgM) in normal and pathological sera. This figure has then been used to determine the absolute amount of 7S IgM. Twenty-seven of 36 (75%) patients with rheumatoid arthritis had 7S IgM with a mean value of 17 mg/100 ml (170 mg/l) (range 2.5–59 mg/100 ml). No sera from 10 healthy controls were found to contain 7S IgM. Patients with active rheumatoid arthritis had significantly more 7S IgM than those with inactive disease, but there was no significant difference between those patients with and without rheumatoid vasculitis. Significant correlations occurred between 7S IgM and the absolute IgM level ( $P < 0.01$ ), the Rose-Waaler titre ( $P < 0.01$ ), and the erythrocyte sedimentation rate ( $P = 0.01$ ). However, there was no significant correlation with the age of the patient, the duration of the disease, or the level of circulating immune complexes as measured by the C1q binding assay. It is concluded that 7S IgM commonly occurs in rheumatoid arthritis, and it is postulated that a common immunological stimulus is responsible for the production of 7S IgM and rheumatoid factors, serological abnormalities that characterise this disease.

Low molecular weight or 7S IgM is the monomeric subunit of classical 19S IgM.<sup>1,2</sup> It has been described in serum and synovial fluid of patients with rheumatoid arthritis<sup>3,4</sup> and in other immunological disorders, including systemic lupus erythematosus, lymphoid neoplasia, IgA deficiency, and in certain chronic infectious diseases.<sup>5,6</sup> The significance of this form of IgM is uncertain. It does not occur in healthy individuals nor does it represent an *in vivo* or *in vitro* catabolism of 19S IgM.<sup>6,7</sup> Solomon and McLaughlin<sup>8</sup> have suggested that its biosynthesis might represent a phylogenetically and ontogenetically primitive immune response resulting from prolonged antigenic stimulation.

The 7S form of IgM appears to function as a univalent antibody which forms small soluble immune complexes,<sup>9</sup> and because of this unusual property its relationship to vasculitic complications of rheumatoid arthritis (RA) has been studied by Stage and Mannik<sup>10</sup> and Theofilopoulos *et al.*<sup>11</sup> Both these groups of investigators have noted its

frequent occurrence in rheumatoid vasculitis and have suggested that it may be involved in the pathogenesis of this complication.

In this study we have determined the absolute amount of 7S IgM in RA sera using filtration chromatography combined with laser nephelometry. The 7S IgM has been compared with other variables of the rheumatoid process, including the presence or absence of vasculitis and the level of circulating immune complexes as measured by the C1q binding assay.

## Patients and methods

Thirty-six Caucasian patients with definite or probable RA (ARA criteria) were chosen from the rheumatological ward and outpatient department. There were 20 females and 16 males and the mean age was 59 years (range 23–82 years). The majority were taking nonsteroidal anti-inflammatory drugs. Some were also taking prednisolone (11), penicillamine (9), gold (6), or azathioprine (2). The patients were divided into 2 groups. One group consisted of patients with active arthritis as defined by the presence of joint pain, swelling, and tenderness (27

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patients), while the other group were inactive or suppressed arthritis had no evidence of joint inflammation (9). There were 8 patients with rheumatoid vasculitis, defined by the presence of mononeuritis multiplex (6 patients), necrotising arteritis/venulitis (3), or skin ulcers not due to other causes (3). Healthy laboratory personnel were chosen as controls.

Venous blood was allowed to clot at room temperature and serum obtained within 4 hours and stored either at  $-80^{\circ}\text{C}$  (for C1q binding assay) or  $-20^{\circ}\text{C}$  for other tests.

#### ESTIMATION OF 7S IGM

1 to 2 ml of serum was applied to an  $85 \times 2.5$  cm Sepharose 6B (Pharmacia) column. Gel filtration was performed in phosphate buffered saline (PBS), pH 7.3, at room temperature using upward flow at the rate of 20 ml/hour. The eluate was monitored with an LKB Uvicord 11 Recorder. 5ml fractions were collected with an LKB Ultrarac fraction collector. IgM concentrations were then determined in alternate fractions with a laser nephelometer (Behring) adapted with a single flow cell and a graph recorder (Camag Wand W 1100) to record mV of light scatter. 20  $\mu\text{l}$  of anti-IgM (Dakopatts) were added to 480  $\mu\text{l}$  of eluant taken from each alternate column fraction, allowed to stand at room temperature for 1 hour, and the resultant precipitation measured and compared with a standard curve constructed from normal human serum of known IgM concentration. Blanks (i.e., 500  $\mu\text{l}$  of eluant from column fractions without IgM antisera) for subtraction were included in every assay. The minimum amount of IgM detected by this method is 0.1 mg per 100 ml (1 mg/l). To determine IgM concentrations within the standard curve some column fractions from sera with high IgM levels had to be diluted several-fold. An IgM profile was then obtained and the percentage 7S IgM was calculated by planimetry where the relative amounts of 19S and 7S fractions were derived. The absolute quantity of 7S IgM was then calculated by multiplying the percentage 7S IgM with the total serum IgM level. Since it was not possible accurately to calculate values of 7S IgM less than 2.5% of the total IgM, this value was therefore used as a threshold for the absence or presence of 7S IgM. Duplicate determinations of the percentage 7S IgM for 3 pathological sera did not vary by more than 3.1% (mean difference).

#### C1Q BINDING ASSAY

The method used was that described by Zubler *et al.*<sup>12</sup> The results were expressed as  $\mu\text{g}$  of a standard preparation of heat aggregated human IgG (HAGG-Cohn Fraction 11  $\gamma$ -globulin mg/ml heated at  $65^{\circ}\text{C}$  for 30 min) per ml of serum.

#### OTHER HAEMATOLOGICAL AND IMMUNOLOGICAL MEASUREMENTS

The erythrocyte sedimentation rate (ESR—Westergren) and Rose-Waaler titre were measured by standard laboratory techniques. Serum IgM was measured by an automated immune precipitation nephelometer (Technicon). Unlike radial immunodiffusion this nephelometric technique is independent of the size of the protein.<sup>13</sup> The anti-IgG (rheumatoid factor) titre was measured by observing the degree of precipitation in the laser nephelometer between 100  $\mu\text{g}$  of HAGG and 500  $\mu\text{l}$  of alternate fractions obtained after filtration chromatography.

#### STATISTICAL ANALYSIS

Comparison between variables was calculated by linear regression, while comparison between groups was by the Wilcoxon sum of ranks method.

#### Results

Low molecular weight IgM was not detected in any of the 10 control subjects. In contrast, 25 of the 36 RA patients (75%) had 7S IgM with a mean of 17 mg/100 ml (170 mg/l) (range 2.5–59 mg/100 ml). This represented 7S IgM making up 5–43% of the total IgM level. In Fig. 1 the profile is shown from a

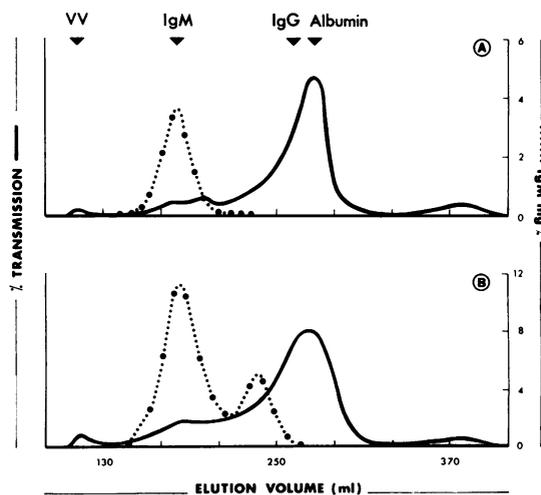


Fig. 1 The percentage transmission and IgM elution profile following Sepharose 6B filtration chromatography of 1.5 ml of serum. The eluting positions of marker proteins detected in the serum are shown. Upper panel—Normal human serum. Lower panel—RA serum containing 7S IgM which accounted for 28% of the total IgM profile and was equivalent to 101 mg/100 ml (1010 mg/l), i.e., 28% of total IgM level of 360 mg/100 ml (3600 mg/l).

patient with 51 mg/100 ml (510 mg/l) 7S IgM, which is equivalent to 28% of the total IgM.

Those patients with active disease had significantly more 7S IgM than those with inactive or suppressed disease ( $P < 0.05$ ), but there was no significant differences between males and females. All patients with rheumatoid vasculitis had 7S IgM (mean 19 mg/100 ml (190 mg/l), range 5-59 mg/100 ml), but there was no significant difference between those patients with and without rheumatoid vasculitis (Fig. 2).

When 7S IgM was correlated with other clinical and laboratory variables significant correlations were found between 7S IgM and the reciprocal of the Rose-Waaler titre ( $P < 0.01$ ), the ESR ( $P = 0.01$ ), and the absolute IgM level ( $P < 0.01$ ), but other correlations were not significant (Table 1). In particular there was no significant correlation between 7S IgM and the level of circulating immune complexes as measured by the C1q binding assay.

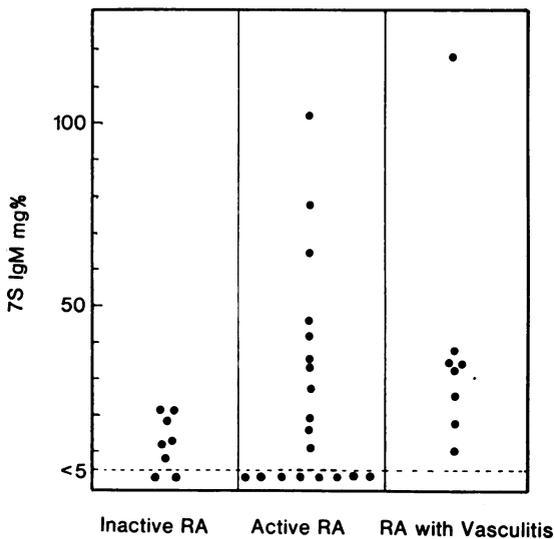


Fig. 2 Absolute level of 7S IgM in active and inactive RA patients and in those with rheumatoid vasculitis

Table 1 Correlation of 7S IgM with other disease variables

No.	Age	Duration	ESR	RW	IgM	C1q
	36	36	34	36	36	23
r	0.18	0.14	0.43	0.77	0.72	0.22
P	NS	NS	0.01	<0.01	<0.01	NS

ESR = erythrocyte sedimentation rate mm/1st hour. RW = Reciprocal of the log<sub>2</sub> Rose-Waaler titre. C1q = C1q binding (µg HAGG equivalents/ml). r = Regression coefficient. P = Probability. NS = not significant.

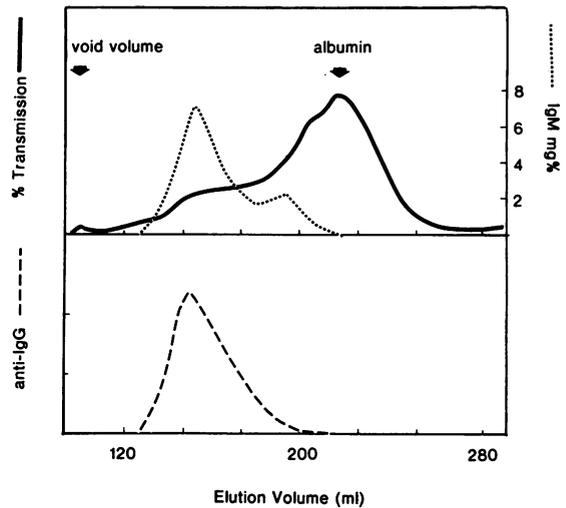


Fig. 3 The percentage transmission, IgM, and anti-IgG profiles following filtration chromatography on Sepharose 6B of 1.0 ml of serum from a seropositive patient. The units for anti-IgG are in millivolts of light scatter as recorded in the laser nephelometer.

The 7S IgM level was followed serially in 5 patients. In 1 patient an increase of 10 mg/100 ml (100 mg/l) was observed over an 18-month period, but in 4 other patients only minor variations were seen over shorter periods up to 6 months despite the development of florid vasculitis in 1 patient.

To determine if 7S IgM had anti-IgG activity one IgM and anti-IgG profiles were plotted for 6 patients after infiltration chromatography. There was no relative increase in the anti-IgG profile in the 7S IgM region despite considerable amounts of 7S IgM (Fig. 3). This suggested that 7S IgM had no detectable anti-IgG activity as measured by this nephelometric method.

### Discussion

The nephelometric estimation of IgM in the eluate fractions after filtration chromatography has enabled us to calculate the proportion of 7S IgM in pathological sera. From this the absolute amount of 7S IgM could be derived. The majority of our rheumatoid patients had considerable amounts of 7S IgM, which occurred particularly in those patients with active disease and correlated with the absolute IgM level, the Rose-Waaler titre, and the ESR.

There have been 5 previous studies reporting the presence of 7S IgM in RA, but in none of these has the absolute amount of 7S IgM been measured

(Table 2). The incidence of 7S IgM in RA in the present study (75%) is considerably greater than those reported in these previous studies (Table 2). This may possibly relate to patient selection. However, a more likely explanation may be attributed to the increased sensitivity of the laser nephelometric technique, where minute quantities of IgM can be detected in the eluate following filtration chromatography. This is in comparison to the polyacrylamide method used in 3 of the above studies where 7S IgM was detected by double diffusion in 4–5% polyacrylamide, a relatively insensitive technique, which permits diffusion of 7S but not 19S IgM as originally described by Stobo and Tomasi.<sup>6,7</sup>

Theofilopoulos and colleagues<sup>11</sup> note 7S IgM in 12 of 15 (80%) of patients with rheumatoid vasculitis and suggested that immune complexes containing low molecular weight IgM may be involved in the pathogenesis of rheumatoid vasculitis. Similarly, Stage and Mannik<sup>10</sup> noted a significant association between 7S IgM and rheumatoid vasculitis, with 12 of 13 patients with vasculitis having this particular form of IgM.

The present study has confirmed the observation that patients with rheumatoid vasculitis have 7S IgM, but there was no significant difference between those patients with vasculitis and those without this complication. Furthermore, there was no significant correlation between 7S IgM and the level of circulating immune complexes as measured by the C1q binding assay, an assay which we have found to be of particular value in monitoring patients with rheumatoid arthritis (unpublished). It is possible, however, that complexes containing 7S IgM do not fix C1q. Finally, in 1 patient serial measurements of 7S IgM showed little variation before, during, and after the development of florid vasculitis. From our observations we conclude that 7S IgM is unlikely to be of major importance in the immunopathogenesis of rheumatoid vasculitis.

Table 2 7S IgM in rheumatoid arthritis

Author	Method	No. of patients	Positive for 7S IgM
LoSpalluto <sup>3</sup>	Gel filtration and RID	36	10 (28%)
Dammacco <i>et al.</i> <sup>5</sup>	5% polyacrylamide gel diffusion	43	12 (28%)
Stage and Mannik <sup>10</sup>	4% polyacrylamide gel diffusion	264	25 (9.5%)
Theofilopoulos <i>et al.</i> <sup>11</sup>	4% polyacrylamide gel diffusion	48	18 (37%)
Clark <i>et al.</i> <sup>15</sup>	Ultracentrifugation and RID	14	7 (50%)
Present study	Gel filtration and nephelometry	36	27 (75%)

RID = radial immunodiffusion.

A significant correlation was observed between 7S IgM and the absolute IgM level, the reciprocal of the Rose-Waaler titre, and the activity of the disease and its severity as measured by the ESR. Similarly, Stage and Mannik<sup>10</sup> found a significant association between the presence of 7S IgM and high IgM levels, severe articular disease, high ESR, and frequent subcutaneous nodules. From these observations we would speculate that in active rheumatoid disease a common immunological stimulus is responsible for the production of 19S IgM which is elevated in RA,<sup>14</sup> 7S IgM, and for rheumatoid factors. However, the nature of this common stimulus is unknown. In addition the significant association noted between 7S IgM and the activity of the rheumatoid process would suggest that the quantitation and serial measurements of 7S IgM may be of value in monitoring the progress and treatment response in this disease.

We conclude that 7S IgM frequently occurs in active RA and speculate that a common stimulus is responsible for the production of 7S IgM and rheumatoid factors. However, the precise significance of 7S IgM and its relationship to the immunopathogenesis of RA is still obscure. Further studies of its associations and properties are required.

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