

## Low molecular weight IgM in juvenile chronic arthritis

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**SUMMARY** Low molecular weight IgM, the monomeric subunit of pentameric IgM, was clearly detected by immunoblotting and filtration chromatographic techniques in six patients with juvenile chronic arthritis and in trace quantities in a further eight of 24 patients studied. This low molecular weight IgM moiety contributed up to 33% of the total circulating IgM and was strongly associated with raised serum concentrations of IgM and the presence of antinuclear antibodies, extractable antinuclear antibodies, and rheumatoid factor. Immunoblot analysis of positive serum samples showed small quantities of other low molecular weight oligomers of IgM in addition to monomeric IgM. It is postulated that the presence of low molecular weight IgM in the serum of patients with juvenile chronic arthritis reflects a disorder of the intracellular assembly of IgM subunits during a stimulated IgM immune response. The pathogenetic role of low molecular weight IgM remains uncertain.

Low molecular weight IgM is the monomeric subunit of pentameric IgM, the latter being the normal circulating form of IgM. It is not found in serum from healthy adults but traces are seen in a few samples from neonates.<sup>1 2</sup> It is also described in certain autoimmune, infective, immunodeficient, and B cell lymphoproliferative disorders, and in these disorders low molecular weight IgM may constitute a considerable proportion of the total serum IgM.<sup>1</sup> The clinical significance of low molecular weight IgM is far from clear. Various acquired and autoantibody binding activities have been ascribed to this molecular moiety of IgM and its frequent occurrence in immune complex associated diseases suggests that it may have an important pathogenic role.<sup>2</sup> Furthermore its common association with chronic infective disorders such as chronic viral hepatitis<sup>3</sup> and syphilis<sup>4</sup> raises the possibility that it may be less efficient at clearing microorganisms from the host than the pentameric molecule; indeed, the agglutinating and precipitating activity of synthetically prepared low molecular weight IgM antibody is either weak or non-existent.<sup>5</sup>

Low molecular weight IgM has not been previously described in either healthy or diseased children. In this study we have observed its presence and that of other IgM oligomers in a number of patients with

juvenile chronic arthritis, and have noted its association with certain autoantibodies and with raised serum IgM concentrations.

### Patients and methods

Twenty four patients with juvenile chronic arthritis as defined by European League Against Rheumatism (EULAR) criteria were studied.<sup>6</sup> Of these, one girl had systemic onset disease, 19 children had pauciarticular onset (15 girls and four boys), and four had polyarticular onset (three girls and one boy). Seventeen of the patients had antinuclear antibodies (16 girls and one boy), and four had HLA B27 (three boys with pauciarticular onset, and one girl who also had antinuclear antibodies). Two girls with antinuclear antibodies and polyarticular onset also had weakly positive latex agglutination tests for rheumatoid factor, and one of these girls had an elder sister with rheumatoid arthritis. All patients had had active disease for at least one year before the study and most were taking non-steroidal anti-inflammatory drugs; several were also having agents to encourage remission (gold or penicillamine).

Control subjects comprised 15 healthy adults, five children with minor atopic disorders, and 21 immediate family members of the propositi (in-

cluding 13 siblings). Blood was obtained by ante-cubital venepuncture in all instances and the serum samples were stored in small aliquots at  $-70^{\circ}\text{C}$ .

Low molecular weight IgM was measured by two methods: firstly, by gel filtration chromatography with IgM enzyme linked immunosorbent assay (ELISA). This was done as described by Koh *et al.*<sup>7</sup> Serum was chromatographed on a Sephacryl S300  $90 \times 1.5$  column and the fractions analysed for IgM by competitive inhibition ELISA. A second IgM peak eluting just before the IgG marker position indicated the presence of low molecular weight IgM and its concentrations were quantified by planimetry.

The second method used was IgM immunoblotting. This was done as described by Harries *et al.*<sup>8</sup> Serum was subjected to sodium dodecyl polyacrylamide (3.6%) gel electrophoresis and the separated proteins transferred to nitrocellulose. IgM bands were identified with a  $\mu$  chain specific antiserum (Dakopatts) and visualised with an avidin biotin, alkaline phosphatase, substrate system.

Serum IgM was measured by rate nephelometry (Beckmans ICS), which is not influenced by the size of the protein being quantified.<sup>2</sup> Antinuclear antibodies was measured by indirect immunofluorescence on HEp-2 cell line substrates using standard techniques. All serum samples were initially screened at a dilution of 1:40 and a positive reaction recorded if nuclear fluorescence was detected at this dilution. Extractable antinuclear antibodies were measured by counterimmunoelectrophoresis in 1% agarose using rabbit thymus extract and K562 cell culture extract as the extractable antigens. Rheumatoid factor was assessed by latex agglutination (Behring). All these immunological techniques have been standardised and are in regular use in our department.

## Results

Monomeric IgM was clearly evident in six patients and trace quantities were observed in a further eight

Table Association of low molecular weight IgM, autoantibodies and serum IgM in juvenile chronic arthritis

	Low molecular weight IgM		
	Positive (n=6)	Trace* (n=8)	Negative (n=10)
Mean IgM concentration (g/l)	3.18	1.56	1.33
Antinuclear antibodies present	6	7	4
Extractable antinuclear antibodies present	4	1	2
Rheumatoid factor present	2	0	0

\*Low molecular weight IgM band just visible on immunoblot.

patients with the immunoblot technique (table). Furthermore, small quantities (faint bands) of dimeric IgM and species migrating above and below this moiety were also evident in these six patients (fig 1) indicating the presence of other low molecular weight IgM oligomers. In each of these six patients the presence of low molecular weight IgM was verified by the filtration chromatography technique, which showed that it constituted between 10 and 33% of the total IgM as assessed by planimetry

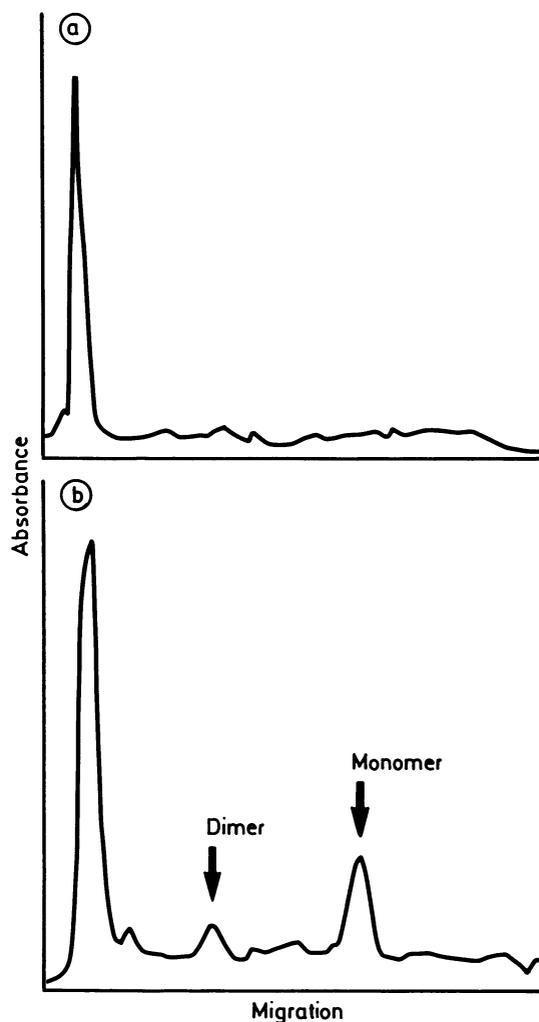


Fig 1 Densitometric scan of immunoblot of serum from healthy control showing only the presence of pentameric IgM (panel a), and of serum from patient with chronic juvenile arthritis containing monomeric IgM and other low molecular weight IgM oligomers in addition to pentameric IgM (panel b).

(fig 2). Of the six patients clearly positive for low molecular weight IgM, four had pauciarticular and two had polyarticular onset juvenile chronic arthritis, and all cases had detectable antinuclear antibodies with titres of 1:40 (four patients), 1:80, and >1:320. In addition, the mean serum IgM concentration for these patients was significantly higher than for the patients without low molecular weight IgM ( $p < 0.01$ ,  $\chi^2$  test), four of these patients had undefined extractable antinuclear antibodies, and two had low titres of rheumatoid factor (latex agglutination 1:20) (table). In contrast, none of the 15 healthy adults or five atopic children had low molecular weight IgM as assessed by the filtration chromatography technique. Trace quantities of low molecular weight IgM were visible, however, using the immunoblot technique in two of the samples from the healthy

adults and eight of the samples from the family members, and faint but clearly discernible low molecular weight IgM bands were observed in a further seven family members. Of these latter subjects, six were clinically asymptomatic, one had rheumatoid arthritis, and all but one had antinuclear antibodies or extractable antinuclear antibodies, or both, and had a mean serum IgM concentration of 1.64 g/l. None of the three boys with B27 associated juvenile chronic arthritis had either low molecular weight IgM or autoantibodies, but low molecular weight IgM was seen in one B27 positive girl who had pauciarticular onset juvenile chronic arthritis and antinuclear antibodies.

### Discussion

This study has clearly shown monomeric IgM and other low molecular weight IgM oligomers in six of 24 patients with juvenile chronic arthritis. Low molecular weight IgM seemed to occur most often in those patients with high serum concentrations of IgM and with the autoantibodies antinuclear antibodies, extractable antinuclear antibodies, and—to a lesser extent—rheumatoid factor. Small quantities of low molecular weight IgM and autoantibodies were also seen in other asymptomatic family members of these six patients, but were not seen in healthy adults or in five children with minor atopic disorders. No attempt was made in this study to correlate the quantity of low molecular weight IgM with clinical indices that reflected disease activity or severity because of the limited clinical data made available to the investigators.

The role of low molecular weight IgM in human disease is unknown.<sup>2</sup> It has been previously associated with a number of autoimmune conditions such as rheumatoid arthritis, systemic lupus erythematosus, and primary biliary cirrhosis, and is usually found in those patients who have more florid disease, often associated with high serum concentrations of IgM, autoantibodies, and immune complexes.<sup>1</sup> It has also been described in a number of chronic infective disorders including infective endocarditis,<sup>7</sup> chronic viral hepatitis,<sup>3</sup> and tertiary syphilis.<sup>4</sup> In the last two conditions, low molecular weight IgM occurs in a high proportion of affected subjects, in contrast to the acute initial infection where specific IgM is found only in the normal pentameric state. It is therefore possible that the presence of low molecular weight IgM leads to ineffective clearance of the micro-organism from the host, thereby facilitating chronic infection. Unfortunately in the current study we were unable to comment on the presence or absence of any possible infective illness either preceding the onset of the

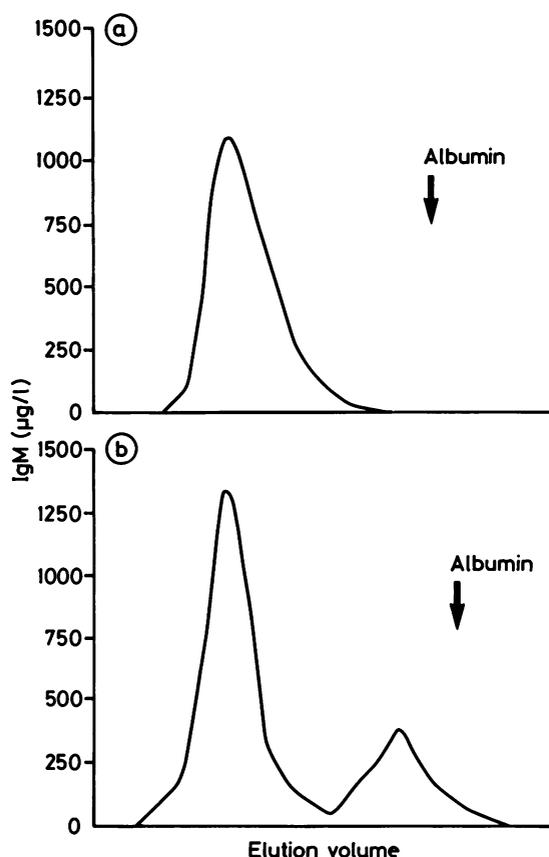


Fig 2 Sephacryl S300 filtration chromatography IgM elution profiles. Eluting position of marker proteins are indicated. Panel a: serum from healthy control and panel b: serum from patient with chronic juvenile arthritis illustrating presence of low molecular weight IgM.

arthritic illness or immediately before the collection of blood samples for low molecular weight IgM analysis.

The finding of low molecular weight IgM in patients with juvenile chronic arthritis and in some of the family members in association with specific autoantibodies and high concentrations of serum IgM is of some interest. Traces of low molecular weight IgM are found only in serum samples from a few neonates, and never in serum from healthy adults.<sup>9</sup> Formal studies have not yet been performed in children but it seems highly unlikely that it will be found in healthy children. What then is the explanation for the occurrence of low molecular weight IgM in these children with juvenile chronic arthritis? A number of theories have been proposed to account for the presence of low molecular weight IgM in human disease.<sup>7</sup> It is not caused by a degradative process either *in vivo* or *in vitro*, and there is good evidence in certain diseases that it is actively secreted in the monomeric form *in vitro*.<sup>10</sup> The close association with high serum IgM concentrations (as are also found in a number of diseases in adults<sup>1,2</sup>) and the presence of other oligomers of IgM, suggest that its presence is a consequence of rapid rates of IgM synthesis and secretion with a resultant disturbance of assembly of the low molecular weight IgM subunit during polymerisation. Possible explanations for this disordered assembly could be alterations in the quantity or function of the J chain polypeptide that seems to play a vital part in polymerisation, structural alterations in the IgM monomeric subunit (for example, glycosylation defects), or alterations in the quantity or activity of the enzymes concerned in polymerisation (for example, sulphhydryl oxidase<sup>11</sup>). *In vitro* studies using an IgM secreting human cell line are currently being performed in an attempt to examine some of these possibilities.

Does the presence of circulating low molecular weight IgM have any pathogenetic consequences in juvenile chronic arthritis? We cannot answer this question. In adults, however, there is good circumstantial evidence linking the presence of low molecular weight IgM to active or florid disease and—in the case of systemic lupus erythematosus—to increased mortality.<sup>12</sup> Furthermore, several autoantibody activities, including antinuclear antibodies, have been ascribed to low molecular weight IgM. Hence we feel that a similar association may be found in juvenile chronic arthritis. If so, it will then be necessary to determine whether the presence of low molecular weight IgM is detrimental to the

nature and course of patients with juvenile chronic arthritis, and see if there are any means of reversing the abnormal IgM humoral response so that it produces fully polymerised IgM (and thereby hopefully arrests the course of the disease).

In conclusion, we have observed the presence of low molecular weight IgM in patients with juvenile chronic arthritis for the first time and have suggested that its occurrence is associated with a disorder of assembly of the IgM subunits during IgM polymerisation.

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#### References

- 1 Roberts-Thomson PJ, Wernick RM, Ziff M. Low molecular weight IgM in rheumatoid arthritis and other rheumatic diseases. *Arthritis Rheum* 1981;**24**:795–802.
- 2 Roberts-Thomson PJ. Low molecular weight IgM in health and disease, Adelaide: University of Adelaide, 1988. (Thesis.)
- 3 Sjogren MJ, Lemon SM. Low molecular weight IgM antibody to hepatitis B core antigen in chronic infections with hepatitis B virus. *J Infect Dis* 1983;**148**:445–51.
- 4 Tanaka S, Suzuki T, Shimada K, Nishioka K. Low molecular weight IgM antibody in syphilis detected by Treponema pallidum immune adherence (TPIA) test. *Med Microbiol Immunol* 1984;**173**:155–65.
- 5 Chavin SI, Franklin EC. Studies on antigen binding activity of macroglobulin antibody subunits and their enzymic fragments. *J Biol Chem* 1969;**244**:1345–52.
- 6 Wood PH. Nomenclature and classification of arthritis in children, In: Munthe E, ed. *The care of rheumatic children*. Basle: European League Against Rheumatism, 1978:47–54.
- 7 Koh LY, Jones ND, Roberts-Thomson PJ. Appearance of low molecular weight IgM during the course of infective endocarditis. *Clin Exp Immunol* 1986;**64**:471–5.
- 8 Harries R, Beckman I, Roberts-Thomson PJ. Low molecular weight IgM. Detection using immunoblotting. *J Immunol Methods* 1986;**88**:97–100.
- 9 Roberts-Thomson PJ, Kennedy A, Koh LY. Frequency of low molecular weight IgM in human cord sera. *J Reprod Immunol* 1987;**11**:321–4.
- 10 Jones DN, Koh LY, Bertouch JV, Roberts-Thomson PJ. *In vitro* synthesis of low molecular weight IgM in rheumatoid arthritis. *Arthritis Rheum* 1985;**28**:112–7.
- 11 Roth RA, Koshland ME. Identification of a lymphocyte enzyme that catalyses pentamer immunoglobulin M assembly. *J Biol Chem* 1981;**256**:4633–9.
- 12 Harrissdangkul V, Barnes TY, Songcharven S, Pennebaker JB. Clinical significance of low molecular weight IgM in patients with systemic lupus erythematosus. *J Rheumatol* 1984;**11**: 638–43.

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