Incidence and correlation between serum IgG and IgM antibodies to native type II collagen in patients with chronic inflammatory arthritis

ROY B. CLAGUE, MARY J. SHAW, AND P. J. L. HOLT

From the Department of Rheumatology, University of Manchester Medical School, Stopford Building, Oxford Road, Manchester M13 9PT

SUMMARY Raised serum IgG and IgM antibody levels to native type II collagen were found in patients with rheumatoid arthritis and in patients with juvenile chronic arthritis. There was a good correlation between the serum IgG and the IgM antibody levels in rheumatoid arthritis and a weaker correlation in juvenile chronic arthritis. Raised serum IgM antibody levels to native type II collagen were found in only 1 patient each with ankylosing spondylitis and psoriatic arthritis, and in these groups there was no correlation between the serum IgG and the IgM antibody levels. The highest serum IgG and IgM antibody levels to native type II collagen were found in rheumatoid arthritis. These results, together with the results on serum antiglobulin levels, indicate that patients with rheumatoid arthritis produce antibodies of both IgG and IgM immunoglobulin class which may have pathogenetic significance in the more severe arthritis found in this condition.

We have previously found raised serum IgG antibody levels to native type II collagen in 49% of patients with rheumatoid arthritis (RA) 46% of patients with ankylosing spondylitis (AS) with peripheral joint involvement, but no patients with psoriatic arthritis or AS with spinal involvement alone.¹ Patients with RA and juvenile chronic arthritis (JCA) may have raised serum antiglobulins of both IgG and IgM antibody class.² We have therefore investigated the incidence and correlation between the serum IgG and IgM antibody levels to native type II collagen in patients suffering from the chronic inflammatory arthritides.

Patients and methods

PATIENTS

Thirty-three patients with definite or classical RA,³ 18 with AS,⁴ 14 with seronegative polyarthritis associated with psoriasis,⁵ 25 (20 females, 5 males; aged 2-27 yr) with JCA⁶ were studied. Many of the patients were those seen in previous study.¹ Five of the patients with JCA had antiglobulins in their serum, and 2 (1 male and 1 female) had the HLA B27 antigen. Three (12%) female children had chronic

iridocyclitis, all of which were longstanding (5-16 years) and associated with pauciarticular disease. Three of the adult patients with AS had a previous history of acute iridocyclitis.

COLLAGEN

Native bovine type II collagen was extracted from articular cartilage by pepsin solubilisation after previous treatment with 2M MgCl₂⁷ and appeared pure on polyacrylamide gel electrophoresis. The collagen was lyophilised and stored at -20°C.

IMMUNOCHEMICALS

Rabbit antihuman IgG and IgM were obtained from Miles Laboratories. Human IgG was isolated from pooled normal human sera by DEAE-cellulose chromatography.⁸ Human IgM was isolated from pooled human sera by fractionation on a Sephadex G 200 (Pharmacia) column.⁸ The purity of the isolated IgG and IgM was checked by immunoelectrophoresis. The human IgG or IgM was linked by cyanogen-bromide activated Sepharose 4B (Pharmacia), and this immunoabsorbent was used to separate specific antihuman IgG or IgM antibody from their respective rabbit antisera. The antihuman IgG or IgM was eluted with 2.5 M potassium thiocyanate, extensively dialysed against

Incidence and correlation between serum IgG and IgM antibodies to native type II collagen 7

phosphate buffered saline (PBS), concentrated to 1 mg/ml in an Amicon Ultrafiltration unit, and stored at -20°C in 1 ml aliquots.

RADIOLABELLING

The antihuman IgG or IgM was labelled with ^{125}I to a specific activity of $0.5 \mu\text{Ci}/\mu\text{g}$ by a modification of Hunter and Greenwood's method⁹ and free ^{125}I removed by application to a Sephadex G25 (Pharmacia) column. The ^{125}I -antihuman IgG or IgM was stored in 1 ml aliquots in 0.1% bovine serum albumen (BSA) in PBS at -20°C . The ^{125}I -antihuman IgG or IgM was centrifuged at 20 000 *g* for 40 minutes just before use to remove aggregates.

RADIOIMMUNOASSAY

The levels of serum IgG or IgM antibodies to native type II collagen were determined using the previously described radioimmunoassay¹ except for the use of either ^{125}I -antihuman IgG or IgM in the final stage depending on which immunoglobulin was being determined.

Statistical analysis was carried out by the 2-tailed Mann-Whitney U test and the Kendall rank correlation coefficient (τ).¹⁰

Results

The levels of serum IgM antibody against native bovine type II collagen in the various patient groups are shown in Fig. 1. The mean (± 1 SD) of the 15

Fig. 1 Serum IgM antibody levels to native bovine type II collagen in patients with rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriatic arthritis (Psor A), juvenile chronic arthritis (JCA), and normal controls. In AS group: ● patients with peripheral arthritis, ▲ patients with spinal involvement alone.

normal controls was $0.46 (\pm 0.22)$ mg/l, giving an upper limit of normal of 0.90 mg/l (mean ± 2 SD).

The median serum IgM antibody level to native type II collagen in the 33 patients with RA was 1.07 (range 0.135 – 3.68) mg/l. There was a difference in this group from the normal controls, and this difference was statistically significant ($U = 438$, $z = 4.24$, $P < 0.001$). The 18 patients with AS had a median serum IgM antibody level to native type II collagen of 0.56 (range 0.03 – 1.22) mg/l. This group was not significantly different from the normal controls ($U = 100$, $P > 0.1$, NS). The median serum IgM antibody level to native type II collagen of the 14 patients with psoriatic arthritis was 0.586 (range 0.218 – 1.09) mg/l, and there was no significant difference in this group from the normal controls ($U = 72$, $P > 0.1$, NS). The 25 patients with JCA had a median serum IgM antibody level to native type II collagen of 0.822 (range 0.156 – 1.56) mg/l. This group was different from the normal controls, and this difference was statistically significant ($U = 80$, $P = 0.001$).

Eighteen (54%) patients with RA, 1 patient with AS, 1 with psoriatic arthritis, and 9 (36%) with JCA had raised serum IgM antibody levels to native type II collagen. The highest levels were found in RA.

CORRELATION BETWEEN SERUM IGG AND IGM ANTIBODY LEVELS TO NATIVE TYPE II COLLAGEN

The correlation between the serum IgG and IgM antibody levels to native type II collagen in the 33 patients with RA is shown in Fig. 2, and this correlation was highly significant ($\tau = 0.49$, $P = 0.001$). Thirteen (40%) patients had raised serum IgG antibody levels to native type II collagen and 19 (57%) had raised serum IgM antibody levels to the same antigen. In this group of rheumatoid patients 13 had raised levels of both serum IgG and IgM antibodies, 6 had only a raised serum IgM antibody level, and 14 had normal levels of both serum IgG and IgM antibodies to native type II collagen. No patient had only a raised serum IgG antibody level.

In the 18 patients with AS there was a lack of correlation between the serum IgG and IgM antibody levels to native type II collagen (Fig. 3; $\tau = 0.15$, $P > 0.1$, NS). Five patients had a raised serum IgG antibody level, but only 1 of these had a raised serum IgM antibody level. This patient had severe ankylosing spondylitis with synovial thickening and synovitis in both knees. His serum was negative for antiglobulins, but he had a persistently raised erythrocyte sedimentation rate. None of the 3 patients with a previous history of acute iridocyclitis had raised serum IgG or IgM antibody levels to

Fig. 2 Correlation between the serum IgG and IgM antibody levels to native bovine type II collagen in 33 patients with rheumatoid arthritis.

native type II collagen. There was also a lack of correlation between the serum IgG and IgM antibody levels to native type II collagen in the 14 patients with psoriatic arthritis (Fig. 4; $\tau = -0.19$, $P > 0.1$, NS). No patient had a raised serum IgG antibody level, but 1 patient with marked synovial thickening in both knees had a slightly raised serum IgM antibody level to native type II collagen.

There was a significant correlation between the serum IgG and IgM antibody levels to native type

II collagen in the 25 patients with JCA (Fig. 5; $\tau = 0.24$, $P = 0.03$). Sixteen (64%) patients with JCA had raised serum IgG and/or IgM antibody levels. Seven patients had only a raised serum IgG antibody level, 2 had only a raised serum IgM antibody level, and 7 had raised levels of both serum IgG and IgM antibodies to native type II collagen.

ANTIBODIES TO NATIVE TYPE II COLLAGEN IN A S

Fig. 3 The lack of correlation between the serum IgG and IgM antibody levels to native bovine type II collagen in 18 patients with ankylosing spondylitis.

ANTIBODIES TO NATIVE TYPE II COLLAGEN IN PSORIATIC ARTHRITIS

Fig. 4 The lack of correlation between the serum IgG and IgM antibody levels to native bovine type II collagen in 14 patients with psoriatic arthritis.

Fig. 5 The correlation between the serum IgG and IgM antibody levels to native bovine type II collagen in 25 patients with juvenile chronic arthritis.

The 2 highest serum IgG and IgM antibody levels occurred in children with seropositive rheumatoid arthritis. Only 1 of the 3 children with chronic iridocyclitis had a raised serum IgG antibody level to native type II collagen.

Discussion

Antibodies to collagen have been demonstrated in the sera of patients with RA by various techniques which are relatively insensitive and lack measurement of class-specific immunoglobulins.¹¹⁻¹⁴ The use of a class-specific double-antibody solid phase radioimmunoassay has allowed us to determine the incidence of raised serum IgG and IgM antibody levels to native type II collagen in a group of patients suffering from the chronic inflammatory arthritides.

These studies have demonstrated raised serum IgG antibody levels to native type II collagen in 39% of patients with RA, 42% of patients with AS with peripheral joint involvement, but in no patients with psoriatic arthritis or ankylosing spondylitis with spinal involvement alone. Serum IgM antibody levels to native type II collagen were raised in 57% of patients with RA, in only 1 patient each with ankylosing spondylitis and psoriatic arthritis, and in 36% of patients with JCA.

There was a very good correlation between the serum IgG and IgM antibody levels in patients with RA, though in 5 patients only the serum IgM antibody levels were raised. The correlation between the serum IgG and IgM antibody levels in JCA was far weaker, though significant. This was due to the fact that this group is composed of all the different

arthritides. The highest serum IgG and IgM antibody levels to native type II collagen were found in RA. There was a lack of correlation between serum IgG and IgM antibody levels to native type II collagen in the patients with ankylosing spondylitis and psoriatic arthritis.

Native type II collagen is found mainly in articular cartilage but also in the eye¹⁵ and the intervertebral disc.¹⁶ As ocular involvement is a feature in many of the arthritides, the development of immunity to native type II collagen could play a role in the pathogenesis of the eye lesions. However, none of the 3 patients with ankylosing spondylitis and a previous history of acute iridocyclitis (uveitis) had a raised serum IgG or IgM antibody level to native type II collagen, and only 1 of the 3 patients with juvenile chronic arthritis and long-standing chronic iridocyclitis had raised serum IgG and IgM antibody levels to native type II collagen. Although only 1 patient had his serum tested within 1 month of the onset of the eye lesion, the fact that all the patients with very high serum IgG and IgM antibody levels to native type II collagen had no overt eye disease suggests that humoral immunity to native type II collagen does not play a role in the pathogenesis of the ocular lesions associated with the arthritides.

The patients with rheumatoid arthritis appear capable of producing high serum levels of both IgG and IgM antibodies to native type II collagen. Patients suffering from the other forms of chronic inflammatory arthritides are capable of producing only moderately raised serum antibody levels, which are almost exclusively IgG antibody to native type II collagen. These findings are in keeping with the results on serum antiglobulin levels, which are highest in patients with RA and in these patients are composed of both IgG and IgM antibodies.² Low levels of raised serum IgG antiglobulin may be found in patients suffering from the 'seronegative' chronic inflammatory arthritides.^{2 17-19}

The reasons for the highest levels of serum antibodies to native type II collagen occurring in RA are unknown. Rheumatoid patients with high titres of antiglobulins (< 1/1280) and nodules, frequently possess the major histocompatibility antigen HLA DWR3, though RA is associated with HLA DWR4.²⁰ In inbred mice the level of humoral immune response to native type II collagen has been linked with the major histocompatibility complex H2.²¹ Thus a possible association between high serum antibody levels to native type II collagen and the HLA system requires investigation. However, patients with RA usually suffer more severe joint destruction than the majority of patients with seronegative arthritis, so that the greater release of

native type II collagen from articular cartilage could lead to greater stimulation of the immune response. Further studies on these factors are in progress.

Normally IgM antibody production is short-lived after an antigenic stimulus and falls back to normal within 6 weeks. In contrast IgG antibody production can persist at high levels for prolonged periods, especially after antigenic boosting. In rheumatoid arthritis, however, there appears to be an immunological abnormality in that IgM, as well as IgG, antibody production may persist at high levels. The cause of this persistent production of IgM antibody is unknown, though one reason may be the persistence of antigenic stimulation. This immunological abnormality may have important pathogenetic significance, in both the intra- and extra-articular manifestations of RA, as IgM antibody binds complement more efficiently than IgG. This increased complement activation by IgM antibody could then lead to a more severe inflammatory response and result in the more severe articular and extra-articular lesions associated with RA. The pathogenetic significance of IgG and IgM antibodies to native type II collagen is now being investigated.

We thank Mrs D. Ward for typing the manuscript, Mrs S. Roe (Department of Medical Illustrations) for preparing the figures, and Miss Linda Hunt (Faculty of Medicine Computational Group) for statistical analysis.

Dr R. B. Clague was in receipt of a Medical Research Council training fellowship, and the work was also supported by the Arthritis and Rheumatism Council and the North-West Regional Area Health Authority.

References

- 1 Clague R B, Shaw M J, Holt P J L. Incidence of serum antibodies to native type I and type II collagens in patients with inflammatory arthritis. *Ann Rheum Dis* 1980; **39**: 000-000.
- 2 Hay F C, Nineham L J, Roitt I M. Routine assay for detection of IgG and IgM antiglobulins in seronegative and seropositive rheumatoid arthritis. *Br Med J* 1975; **iii**: 203-4.
- 3 Ropes M W, Bennett G A, Cobb S, Jacax R, Jessar R A. Diagnostic criteria for rheumatoid arthritis. 1958 Revision. *Ann Rheum Dis* 1959; **18**: 49-53.
- 4 Kellgren J H, Jeffrey M R, Ball J. *The Epidemiology of Chronic Rheumatism*. Oxford: Blackwell, 1963; **1**: 326.
- 5 Wright V. Psoriasis and arthritis. *Ann Rheum Dis* 1956; **15**: 348-56.
- 6 Ansell B M, Bywaters E G L. Prognosis in Still's disease. *Bull Rheum Dis* 1959; **9**: 189-92.
- 7 Trelstad R L, Rubin D, Gross J. Osteogenesis imperfecta congenita—evidence for a generalised molecular disorder of collagen. *Lab Invest* 1977; **36**: 501-8.
- 8 Hudson L, Hay F C. *Practical Immunology*. Oxford: Blackwell, 1976.
- 9 Hunter W M, Greenwood F C. Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature* 1962; **194**: 495-6.
- 10 Siegal S. *Non-parametric Statistics for the Behavioural Sciences*. International Student ed. London: McGraw-Hill Kogakusha, 1956.
- 11 Steffen C, Timpl R. Antigenicity of collagen and its application in the serological investigation of rheumatoid arthritis sera. *Int Arch Allergy Appl Immunol* 1963; **22**: 333-49.
- 12 Michaeli D, Fudenberg H H. The incidence and antigenic specificity of antibodies against denatured human collagen in rheumatoid arthritis. *Clin Immunol Immunopathol* 1974; **2**: 153-9.
- 13 Andriopoulos N A, Mestecky J, Miller E J, Bradley E L. Antibodies to native and denatured collagens in sera of patients with rheumatoid arthritis. *Arthritis Rheum* 1976; **19**: 613-7.
- 14 Trentham D E, Dynesius R A, Rocklin R E, David J R. Cellular sensitivity to collagen in rheumatoid arthritis. *N Engl J Med* 1978; **299**: 327-32.
- 15 Swann D A, Constable I J, Harper E. Vitreous structure. III. Composition of bovine vitreous collagen. *Invest Ophthalmol Visual Sci* 1972; **11**: 735-8.
- 16 Eyre D R, Muir H. Types I and II collagens in intervertebral disc. Interchanging radial distributions in annulus fibrosus. *Biochem J* 1976; **157**: 267-70.
- 17 Howell F A, Chamberlain M A, Perry R A, Torrigiani G, Roitt I M. IgG antiglobulin levels in patients with psoriatic arthropathy, ankylosing spondylitis and gout. *Ann Rheum Dis* 1972; **31**: 129-31.
- 18 Tapaner F J, Rawson A J, Hollander J K. Serum anti-immunoglobulins in psoriatic arthritis. *Arthritis Rheum* 1972; **15**: 153-6.
- 19 Arana R M, de la Vega M T, Porrini A, Morteo O S. Antiglobulins in ankylosing spondylitis. *J Rheumatol* 1975; **2**: 303-7.
- 20 Wooley P H, Panayi G S, Batchelor R J. HLA-DR antigens in rheumatoid arthritis. *Ann Rheum Dis* 1979; **38**: 188.
- 21 Nowack H, Hahn E, Timpl R. Specificity of the antibody response in inbred mice to bovine type I and type II collagen. *Immunology* 1975; **29**: 621-8.