Fine specificity of serum anticollagen molecules in experimental immune synovitis

THOMAS F KRESINA, ITZHAK A ROSNER, VICTOR M GOLDBERG, AND ROLAND W MOSKOWITZ

From the Departments of Medicine, Orthopaedics, and Pathology, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106

SUMMARY Serum anticollagen antibodies to the native and denatured interstitial collagens were measured by solid phase radioimmunoassay (RIA) in a rabbit model of IgG-induced immune synovitis. Serum antibodies binding the native interstitial collagens and denatured type II collagen were observed in 100% of the animals tested (n=6). Titerable antibodies to the $\alpha 1(\text{III})$ collagen polypeptide chain were observed in 83% of the animals, whereas serum antibodies to denatured type I collagen were observed in 33%. Inhibition studies showed that the observed serum anticollagen antibodies were conformationally dependent and collagen type specific. In addition these antibody populations varied in their affinities by as much as a factor of 2.81 for the specific substrates. Mean value of the average binding constants (K_a) for synovitis anticollagen antibodies binding native type II collagen was $5.47 \times 10^6/\text{mol}$; while the K_a determined for synovitis antibodies binding denatured type III collagen was $1.94 \times 10^6/\text{mol}$. The data indicate that unique anticollagen antibody populations are expressed in the serum of animals with experimental IgG-induced chronic immune synovitis.

Key words: arthritis, immunoglobulins, collagen.

The immune response to collagen has been implicated as playing a major part in the pathogenesis of human rheumatic diseases.^{1,2} Immunoserological studies have been performed to show the existence of antibodies directed against the genetically distinct collagens in the sera of patients with relapsing polychondritis,³ psoriatic arthritis,⁴ rheumatoid arthritis,⁴⁻⁷ and degenerative joint disease.⁸ Other studies have observed antibodies to various collagen types in rheumatoid synovial tissues,⁹ cells,¹⁰ and fluid.¹¹⁻¹³

With regard to patients with traumatic synovitis some controversy exists about the presence of antibodies to the native and denatured interstitial collagens: Cracchiolo *et al.*¹² observed antibodies to native and denatured collagens in a majority of the traumatic synovial fluids assayed. However, only rarely were these antibodies observed in the sera of the patients. In addition other studies^{11,13} found

post-traumatic synovial effusions negative for anti-collagen antibodies.

Experimental antigen-induced immune synovitis has been widely used as a model for the study of the pathophysiology of human rheumatoid arthritis. Similarities between the experimental models of synovitis and the human disease include deposition of immune complexes and complement in joint cartilage,^{14,15} hyperplasia of the synovium,^{16,17} antibody synthesis by the inflamed synovium,^{18,19} cell-mediated immune responses to the genetically distinct interstitial collagens,²⁰ pannus formation, cartilage erosion, and ligament destruction.^{16,17,20,21} Experimental antigen-induced arthritis has been described in rabbits immunised to homologous or heterologous fibrin,¹⁶ and in immune rabbits receiving intra-articular injection of egg albumin,¹⁶ bovine serum albumin,¹ cartilage proteoglycan subunits,²² and immunoglobulin.²¹ Studies characterising antibody synthesis in these models have noted a localised deposition in the synovium of antibody specific for the arthritis initiating immunogens.^{14,15} However, these studies have not considered the

Accepted for publication 22 November 1984.

Correspondence to Dr Thomas F Kresina, Division of Rheumatology, Department of Medicine, Case Western Reserve University, Cleveland, Ohio 44106.

prevalence of anticollagen antibodies in these animal models of arthritis. The present study describes the presence and specificity of anticollagen antibodies in an animal model of synovitis, with homologous rabbit IgG used for the induction of immune synovitis.

Materials and methods

RABBITS

Outbred New Zealand White female virgin rabbits 2–2.5 kg in weight were obtained from H and E Rabbitry (Cleveland, Ohio).

INTERSTITIAL COLLAGENS

Native interstitial type I, type II, and type III collagens were isolated from pepsin digests of tissues rich in the particular isotype of collagen²³ as described previously.²⁰ In brief, preparations of type I and type III collagens were isolated from rabbit dermis, and preparations of type II collagen were isolated from rabbit xyphoid process. Solubilised collagen was purified by differential salt precipitation from neutral salt or acid solutions.²⁴ Collagen preparations were further purified by native state carboxymethylcellulose chromatography (for native type I and type III collagen) and diethylaminoethane cellulose chromatography (for native type II collagen) by methods previously described.^{25, 26} Under these conditions native interstitial collagen can be totally resolved from basement membranes or other related collagenous components, and from proteoglycan moieties. Denaturation of native collagen preparation was achieved by incubation at 60°C for 45 minutes just before use.

CNBr PEPTIDES

Purified preparations of type I, type II, and type III collagens were reduced before CNBr cleavage by the method of Adelstein and Kuehl²⁷ as described previously.²⁵ This procedure facilitates the complete cleavage of collagen molecules with CNBr by converting oxidised methionyl residues to methionine. Cleavage of methionyl residues was achieved by incubation of the reduced collagen preparations with CNBr for 4 h at 37°C in formic acid.²⁸ Collagen CNBr preparations were subsequently characterised by carboxymethylcellulose chromatography with a sodium acetate buffer as described previously.²⁸

INDUCTION OF SYNOVITIS

Antigen-induced immune synovitis was induced by a modified method of Goldberg *et al.*²¹ Female virgin rabbits were primed subcutaneously with 4.0 mg homologous rabbit IgG emulsified in complete

Freund's adjuvant. One week later the animals were boosted with 2.0 mg of IgG subcutaneously and two days later skin tested for reactivity to IgG and purified protein derivative (PPD). Only responding rabbits were utilised for induction of immune synovitis. During the subsequent four weeks each rabbit received intra-articularly in the left knee 1 mg of rabbit IgG solubilised in saline, twice weekly. During weeks five to eight the administration schedule was reduced to a single injection per week. Normal rabbits received no injections. Adjuvant control rabbits without synovitis were given complete Freund's adjuvant and intra-articular injections of saline. IgG immune controls without synovitis were primed with IgG and boosted, and saline was administered intra-articularly to rabbits with a positive skin test. Reference antibody binding curves and binding constants were obtained from antibodies derived from hyperimmune rabbits. These rabbits were inoculated (five times) biweekly with 100 µg collagen emulsified in complete Freund's adjuvant.

PATHOLOGY SCORING

A pathology score is derived by grossly assigning equal weight to each of three parameters—cartilage, synovium, and synovial fluid. *Cartilage* (patellar, femoral, tibial): 0=normal, 1=fine diffuse surface pitting, 2=softening, undulation of the surface, marginal erosions, 3=focal ulceration/denudation of the articular surface, 4=diffuse denudation of the articular surface and joint disorganisation. *Synovial fluid*: 0=normal, 1=minimal excessive clear fluid, 2=turbid fluid, 0.5–2.0 ml collected, 3=thick yellow pus extending into synovial cysts, 4=haemorrhagic pus. *Synovitis*: 0=normal synovium, 1=hyperaemia and minimal proliferation, 2=pannus at joint margins, 3=pannus covering intra-articular structures, 4=replacement of joint structures with pannus.

RADIOIMMUNOASSAY FOR ANTICOLLAGEN ANTIBODIES

Serum anticollagen antibodies were measured by solid phase²⁹ radioimmunoassay. Polyvinyl microtitre plates were incubated with 20 µl of a neutral pH sodium bicarbonate solution containing the particular isotype of collagen (1 g/l) for 24 h at 4°C. After the adherence of antigen to the wells the plates were washed with a bicarbonate buffer pH 7.5 and filled with a solution of 2% BSA (bovine serum albumin) in bicarbonate for 24 h at 4°C. This procedure reduced the non-specific background binding by blocking remaining sites of adherence in the wells. In parallel wells nanogram quantities of radiolabelled collagens (2200–3000 cpm/ng) were added to these collagen solutions, and it was

determined that approximately 0.1% of the quantity of the collagen added to the wells irreversibly adhered to the plates under these conditions. After this incubation 20 µl of bicarbonate buffer and 20 µl of serum dilution (1:10, 1:50, 1:100, 1:1000, 1:10 000) were incubated in the wells for 18 h at 4°C. The wells were washed thoroughly with bicarbonate buffer and filled with 50 µl (125 ng) of ¹²⁵I-labelled goat antirabbit IgG (Cappel Laboratories, PA). This reagent was incubated in the wells of the plates for 12 h at 4°C. The wells were washed 12 times with bicarbonate buffer, dried by air, and counted in a Beckman 5500 gammacounter. For inhibition studies 1 µg of unlabelled collagen was preincubated with 20 µl of a 1:20 serum dilution for 30 minutes at room temperature just before incubation in the antigen-coated wells.

AFFINITY OF ANTICOLLAGEN ANTIBODIES

Antibody affinities for hyperimmune anticollagen molecules and serum-derived (from rabbits with synovitis) anticollagen molecules were determined by the method of Desbuquois and Aurbach.³⁰ In brief, microgram quantities of collagen molecules were radiolabelled with ¹²⁵I as described below. The fraction of antigen bound to antibody was measured in triplicate by 10% polyethylene glycol precipitation at various antigen and antibody concentrations. The precipitates were harvested by centrifugation, washed, and counted in a Beckman 5500 gammacounter. Total ¹²⁵I-labelled collagen was determined by precipitation with 10% trichloroacetic acid (TCA). Non-specific binding and precipitation was determined with non-immune rabbit sera. The effect of proportionately decreasing antigen and antibody concentrations on the fractional amount of antigen bound to antibody was noted, and the affinity calculated with a Heath kit H89 disk drive computer.

IODINATION OF COLLAGEN

Purified collagen preparations were dialysed against 0.05 M trometamol hydrochloride (TRIS-HCl), 1.0 M NaCl, pH 7.5. 100 µl of a 1 g/l solution of each collagen type was radiolabelled by the chloramine-T method.³¹ The specific activity of ¹²⁵I-collagens used as substrates was 2200–3000 cpm/ng. Radioactivity in the collagen samples was greater than 90% TCA precipitable.

Results

GROSS PATHOLOGY

The results of gross pathological scoring are presented in Table 1. Normal rabbits, adjuvant control rabbits, and IgG immune control rabbits had no evidence of synovitis, excess synovial fluid, or

Table 1 Gross pathology

Group	Synovitis*	Synovial	Cartilage
Normal rabbits† nos. 1, 2	0	0	0
Adjuvant control‡ nos. 3, 4, 5,	0	0	0
IgG immune control§ nos. 6, 7, 8	0	0	0
Immune synovitis nos. 10	3.0	2	2
11	1.5	1	1
12	3.0	3	3
13	3.5	4	ND¶
14	3.0	3	ND
15	4.0	3	ND

*For scoring basis 0–4 synovitis, synovial fluid, cartilage, pathology scoring section in 'Methods'.

†Rabbits randomly chosen from stock, receiving no injection.

‡Rabbits immunised with complete Freund's adjuvant (two times) and receiving intra-articular injections of saline.

§IgG primed and boosted rabbits receiving intra-articular injections of saline.

||IgG primed and boosted rabbits receiving intra-articular injections of homologous IgG in saline.

¶ND=Not determined.

Animal 9 did not survive to the end of the experiment and was not included in the study.

cartilage breakdown when killed. Rabbits which were immunised and given IgG intra-articularly for eight weeks were observed to have classical arthritic pathological lesions. Five of six animals had pannus formation covering intra-articular structures. Only animal no. 11 showed minimal synovitis with hyperaemia and minimal proliferation. All rabbits were observed to have excessive synovial fluid, with five of six rabbits presenting with thick turbid to yellow or haemorrhagic pus extending into synovial cysts. No bacterial growth, however, was cultured from the purulent-like joint fluid. Cartilage integrity ranged from fine diffuse surface pitting to denudation of the articular surface.

ANTICOLLAGEN ANTIBODY BINDING ACTIVITY

Sera from normal rabbits, adjuvant control rabbits, and IgG immune control rabbits were found not to contain titerable antibodies to the native and denatured interstitial collagens (data not shown). Sera from six immune rabbits with synovitis were examined for native and denatured collagen binding activity. As shown in Fig. 1a all six sera samples were observed to contain titerable binding activity for native homologous type I collagen. Four sera (animals nos. 10, 11, 14, and 15) were observed to have binding curves approximating to or below that of the anti-type I collagen hyperimmune serum. On the other hand, sera from synovitic animals (nos. 12 and 13) were observed to have substantially greater

Fig. 1 Binding curves (—) for serum antibodies from six immune synovitic rabbits (nos. 10–15) with native and denatured interstitial collagens as antigens. Data are presented as serum assayed (μ l) versus 125 I-labelled ligand (Δ cpm) specifically bound. Δ cpm is defined as the cpm of bound labelled ligand derived from immune synovitis samples (eight weeks postimmunisation) minus the cpm of bound preimmune synovitis sera. The curves are based on triplicate determinations of all serum dilutions. Serum samples from normal rabbits, adjuvant control rabbits, and IgG immune control rabbits were not found to have binding activity for the native or denatured interstitial collagens. Reference binding curves (---) for antibodies from hyperimmune rabbits binding native type I, II, and III collagens are also presented.

binding activity compared with the hyperimmune serum. Samples of serum from synovitic rabbits 13 and 14 showed minimal binding activity to denatured type I collagen, and serum samples 10, 11, 12, and 15 displayed no measurable activity to it (Fig. 1b).

The binding curves for synovitis serum samples versus native type II collagen (Fig. 1c) were comparable with those observed for native type I collagen. Thus all six sera were observed to have binding activity to native type II collagen molecules, and sera 10, 11, 14, and 15 were proximal to the binding activity of the native type II hyperimmune serum tested. In addition samples 12 and 13 were again 'high' binding samples compared with the hyperimmune serum. As shown in Fig. 1d all serum samples

were observed to have titerable binding activity to denatured type II collagen. In addition samples of sera from synovitic animals 12 and 13 can be classified as 'high' binding antisera, while samples 10, 11, 14, and 15 were observed to have relatively low binding activity to $\alpha 1(\text{II})$ polypeptide chains.

Fig. 1e shows the binding curves for the synovitis sera versus native type III collagen. It can be noted that all samples of sera were observed to have binding activity to native type III collagen. Serum samples 12 and 13 show higher binding activity than the type III hyperimmune serum sample, while serum samples 10, 11, 14, and 15 are comparable with the hyperimmune serum reference. Five of the six synovitis samples tested against denatured type III collagen (Fig. 1f) were observed to have

titerable activity towards $\alpha 1(\text{III})$ polypeptide chains. Serum from the synovitic rabbit no. 10 did not show any measurable binding activity. With regard to the samples binding denatured type III collagen sera 12 and 13 were 'high' binding sera, while sera 11, 14, and 15 were relatively low responders.

The binding studies can be summarised as follows. All synovitis rabbit sera tested were observed to have measurable binding activity to native type I, II, and III collagens and denatured type II collagens. Five of six synovitic rabbits were observed to have binding activity to denatured type III collagen, while two of six serum samples were positive when tested against denatured type I collagen.

SYNOVITIS ANTICOLLAGEN ANTIBODY SPECIFICITY

Inhibition studies with preparations of native and denatured interstitial collagens and the CNBr peptides of type I, II, and III collagens were performed to determine the binding specificities of the anticollagen antibodies observed in the serum of chronic immune synovitic rabbits. As shown in Table 2 anticollagen antibodies binding native type I collagen were effectively inhibited by preincubation with native type I collagen but not with identical quantities of native type II or type III collagens. In addition denatured types II and III collagens and the CNBr peptides of types I, II, and III collagen were ineffective inhibitors of the binding of synovitis anti-type I collagen antibodies and native type I collagen. In this assay denatured type I collagen was slightly inhibitory. This minimal inhibition could reflect the blocking of anti-type I collagen antibodies directed to the terminal truncated non-helical regions of the native collagen molecule.

Synovitis anticollagen antibodies binding native type II collagen were effectively inhibited by preincubation with native type II collagen but not by preincubation with native types I and III collagens and denatured types I and III collagens or their CNBr peptides. The marginal inhibition observed with denatured type II collagen and its CNBr peptide preparation could represent the binding of antibodies directed to the non-helical terminal regions of the collagen polypeptide. In the same manner synovitis anti-type III collagen antibodies were inhibited by native type III collagen but not by the other native interstitial collagens and denatured interstitial collagens or their CNBr peptides.

The inhibition data indicated that three populations of antibodies directed to collagenous epitopes were present in the sera of immune synovitic rabbits. They were (1) antibodies directed to the helical epitopes of native collagen molecules. However, a small percentage of these antibodies may bind the non-helical terminal regions of the molecule. (2) Antibodies directed to only denatured collagen polypeptide chains; and (3) antibody molecules recognising denatured collagen polypeptides and their CNBr peptide preparations.

ANTICOLLAGEN ANTIBODY AFFINITY

The affinity of synovitis anticollagen antibodies for native and denatured interstitial collagen preparations was measured. Table 3 lists the average binding affinities, K_a , for the antibodies derived from sera from synovitic animals nos. 10 to 15 and the K_a for rabbit anti-type I and anti-type II collagen hyperimmune serum. For the sera tested there was little interanimal variation for K_a determined. This is, the average binding constants for the synovitis

Table 2 Percentage inhibition of binding by preincubation of 1 μg collagenous proteins*

Synovitis antisera	Inhibitors								
	Native collagens			Denatured collagens			CNBr peptides		
	I	II	III	I	II	III	I	II	III
Native type I collagen†	73±15‡	1±1	5±2	10±4	5±3	3±3	3±4	5±3	4±2
Native type II collagen†	1±1	87±13	4±3	3±2	9±11	2±1	4±2	12±6	4±2
Native type III collagen†	3±2	0±0	70±11	3±2	2±1	5±3	2±2	4±2	6±1
Denatured type I collagen†	9±9	4±5	2±3	49±11	0±0	0±0	20±11	8±8	4±4
Denatured type II collagen†	1±1	3±2	4±2	5±4	66±10	4±3	4±2	73±12	2±1
Denatured type III collagen†	6±4	4±2	4±3	1±1	2±2	45±17	3±2	5±1	20±9

*Inhibition assay: 1 μg of inhibitors (native and denatured interstitial collagens and their CNBr peptides) was incubated with 20 μl of 1:20 dilution of synovitis rabbit antisera for 30 minutes at room temperature. Samples were subsequently incubated in collagen-coated microtitre plates for four hours at room temperature. Microtitre wells were subsequently washed and developed with ^{125}I -labelled antirabbit IgG (see 'Methods'). Data represent values determined in triplicate for six individual synovitis rabbit serum samples. Data presented for synovitis antibodies directed to denatured type III collagen and denatured type I collagen are for serum samples previously shown by R1 to have titerable anticollagen antibodies.

†Purified collagen preparation which coated microtitre wells.

‡Values are given as means±SEM.

Table 3 Average binding constant ($K_a \times 10^{-6}/\text{mol}$) of serum anticollagen antibodies derived from immune synovitic rabbits*

Animal number†	Native collagens‡			Denatured collagens‡		
	I	II	III	I	II	III
10	4.32	5.71	2.30	—	4.69	—
11	4.19	5.74	2.34	—	4.95	1.99
12	4.33	5.24	2.36	—	4.85	2.11
13	4.06	5.41	2.38	2.85	4.80	1.90
14	4.13	5.39	2.28	2.13	4.76	1.76
15	4.00	5.37	2.38	—	4.29	1.96
Mean±SEM	4.17±0.06	5.47±0.08	2.34±0.02	2.49±0.4	4.72±0.09	1.94±0.06
Hyperimmune rabbits§	4.03	3.88	ND	ND	ND	ND

*Average binding constants determined by 10% polythelene glycol precipitation as described in 'Methods'. Values presented are means of three individual determinations.

†Synovitic rabbits – IgG immune rabbits administered IgG intra-articularly.

‡Purified collagen preparations isolated and purified as described in the text.

§Hyperimmune rabbits – rabbits repeatedly (five times) immunised intradermally with purified preparations of collagen, emulsified in complete Freund's adjuvant.

||ND=Not determined.

antibodies from the individual rabbits showed little variation for each genetically distinct collagen type assayed. However, comparisons of the average binding constants obtained for the synovitis antibodies directed to native and denatured collagens (i.e. native type I v native type III; denatured type II v denatured type III) did show variation.

Determination of the average binding constants allowed the grouping of antibody populations with regard to observed association constants. An initial group comprised synovitis antibodies directed to native and denatured type II collagen and native type I collagen. The average binding constants obtained for both native and denatured type II collagen as ligand were 5.47 and $4.72 \times 10^6/\text{mol}$ respectively. The average binding constant obtained for native type I collagen as ligand was $4.17 \times 10^6/\text{mol}$. A second grouping comprised molecules binding the native type III and denatured type I and III collagen ligands. The K_a values obtained for these antibody populations are 2.34 , 2.49 , and $1.94 \times 10^6/\text{mol}$ respectively.

Discussion

The present study details the prevalence and specificity of anticollagen molecules observed in the sera of chronic immune synovitic rabbits. Serum anticollagen antibodies as measured by solid phase radioimmunoassay were not observed in animals which did not display synovitis gross pathology. These groups include normal rabbits, rabbits administered complete Freund's adjuvant and saline intra-articularly, and IgG immune rabbits administered saline intra-articularly. IgG immune rabbits

administered IgG intra-articularly were observed to have synovitis lesions including pannus formation, excessive synovial fluid, and cartilage degradation. These synovitic rabbits also displayed titrable serum collagen antibodies in the following prevalence: native types I, II, and III collagen and denatured type II collagen – 100%; denatured type III collagen – 83%; and denatured type I collagen – 33%.

Serum samples were considered positive for anticollagen antibodies by solid phase radioimmunoassay when binding values exceeded those determined for preimmune sera. Therefore any inherent non-specific binding activity is not recorded in the present data. In addition synovitis serum samples observed to contain anticollagen binding activity did not display binding activity above background levels in bovine serum albumin coated microwells. The data indicate that non-specific anti-IgG binding activity is not part of the tabulated anticollagen binding activity. Such anti-IgG binding activity is a criticism of solid phase radioimmunoassays.⁷

Although no attempts were made to quantify the serum anticollagen antibodies, some comparative statement can be derived from the binding studies. In the sera of chronic immune synovitic rabbits antibodies to native helical type I collagen are prevalent compared with antibodies directed to denatured type I collagen (sequential determinants). However, antibody populations directed to native and denatured type II collagen are roughly equivalent. Synovitis anticollagen antibodies specific for native type III collagen appear in slightly excess of the antibodies directed to the $\alpha 1(\text{III})$ collagen polypeptides. This latter difference may

not be quantitative but may reflect the differences in binding affinities for the two populations of antibody molecules.

Inhibition studies with native, and denatured interstitial collagens and their CNBr peptides indicate that synovitis anticollagen antibodies are collagen type specific and conformationally dependent. The data indicate that anticollagen antibodies which bind native helical collagen molecules are not reactive with other native interstitial collagens, their denatured polypeptide chains, or CNBr peptides. However, a small population of antibodies which bind native collagen preparations may be directed to the terminal non-helical regions of the collagen molecule and thereby recognise these determinants on nascent polypeptide chains. Analogous observations were made for synovitis antibodies binding native type II and type III molecules. The fine antigenic specificity of synovitis anticollagen antibodies directed to the denatured polypeptide chains of the interstitial collagens (sequential epitopes) is more complex. Although these antibody molecules do not bind the native collagen molecules, a substantial percentage of the antibodies recognise epitopes on the collagenous CNBr peptides of the specific collagen type. These binding studies indicate that antisynovitis antibodies comprise three distinct populations with regard to antigenic fine specificity. These are (1) anticollagen antibodies which recognise only the helical epitopes of the collagen molecule; (2) anticollagen antibodies which recognise only sequential epitopes of collagen alpha chains; and (3) antibodies which bind both specific denatured collagen polypeptide chains and their CNBr peptide fragments.

It is of interest to note the differences in conformational dependent anticollagen antibodies elicited in experimental immune synovitis. Normal rabbits on immunisation with collagen and complete Freund's adjuvant do not display synovitis pathologic lesions but generate predominantly serum antibodies to non-helical and terminal region determinants.³²⁻³⁵ This compares with the observation that virtually all native helical binding anti-type I antibodies and equal quantities of antibodies bind native or denatured collagens for types II and III collagen in the sera of synovitic rabbits. These observations indicate that an enhanced immune repertoire is observed in immune synovitis, that is, unique B cell clones not triggered on hyperimmunisation with collagen are stimulated to produce antibody in chronic immune synovitis. Whether such B cell clones produce 'pathologic' anticollagen antibodies or specific antibodies responsible for the observed synovitis lesions is unknown.

The determination of the average binding con-

stants for synovitis anticollagen antibodies allows a comparison of binding affinities for anticollagen antibodies to be made. The mean binding constants range from 5.47×10^6 to $1.94 \times 10^6/\text{mol}$ for synovitis anticollagen antibodies to native type II collagen and denatured type III collagen, respectively. These values represent a difference in binding by a factor of 2.82. The K_a values calculated for synovitis antibodies directed to the remaining native and denatured collagens and the K_a values for antibodies directed to native type I and II collagens derived from hyperimmune rabbits are within these limits. It can also be noted that the K_a values for native types I and II and denatured type II collagen antibodies are relatively higher compared with the K_a values obtained for antibodies directed to native type III collagen and denatured type I and type III collagen. Therefore the antibodies directed to native types I and II collagens and the antibodies to the $\alpha 1(\text{II})$ polypeptide chain require comparatively half to a third of the ligand concentration for 50% binding of soluble antigen.

The observation of serum anticollagen antibodies in chronic experimental immune synovitis, together with previous studies detailing chronic inflammation in antigen-induced arthritis, suggests a role for the immune response to collagen in the maintenance of inflammation in antigen-induced arthritis. Previous studies^{14 18 22} with immune animals and intra-articular injection of numerous molecules to elicit antigen-induced arthritis have noted synthesis in situ of specific antibody. In addition, the intra-articularly injected antigen was selectively retained in the joint.¹⁹ The retained antigen was found to be consistently present in highly collagenous tissues such as the menisci, ligaments, and articular cartilage.¹⁴ Such retention of antigen in collagenous joint structures as free antigen or immune complexes could result in alteration of collagenous components. Interculation of free antigen into collagen fibrils or extracellular matrix could be destabilising or render collagen molecules cross reactive to the host response to antigen. On the other hand immune complex formation and deposition in collagenous tissues could result in destruction of collagenous molecules through complement activation. However, for both of these events collagen molecules could become immunogenic thereby initiating anticollagen antibody synthesis and subsequently heightening destruction of connective tissues. Present studies are considering these postulates and are investigating the deposition of immune complexes and complement components in the collagenous joint structures of synovitic rabbits.

The authors wish to thank Mrs Betty Boja and Ms Candace Finegan for their excellent technical assistance and Mrs Terri

Grenwis-Woodcraft for her secretarial skills. This work was supported by NIH grant AM-30134, the Revco DS Foundation, and the Kroc Foundation.

References

- Glynn L E. Aetiology of rheumatoid arthritis with regard to its chronicity. *Ann Rheum Dis* 1969; **28** (suppl): 3-4.
- Steffen C. Consideration of pathogenesis of rheumatoid arthritis as collagen autoimmunity. *Immunobiology* 1970; **39**: 219-26.
- Foidart J M, Abe S, Martin GR, *et al*. Antibodies to type II collagen in relapsing polychondritis. *N Engl J Med* 1978; **299**: 1203-7.
- Trentham D E, Kammer G M, McCune W J, David J R. Autoimmunity to collagen. A shared feature of psoriatic and rheumatoid arthritis. *Arthritis Rheum* 1981; **24**: 1363-9.
- Andriopoulos N A, Mestecky J, Wright G P, Miller E J. Characterization of antibodies to the native human collagens and to their component α chains in the sera and the joint fluids of patients with rheumatoid arthritis. *Immunochemistry* 1976; **13**: 709-12.
- Michaeli D, Fundenberg H H. The incidence and antigenic specificity of antibodies against denatured human collagen in rheumatoid arthritis. *Clin Immunol Immunopathol* 1974; **2**: 153-9.
- Stuart J M, Huffstutter E H, Townes A S, Kang A H. Incidence and specificity of antibodies to types I, II, III, IV and V collagen in rheumatoid arthritis and other rheumatic diseases as measured by ^{125}I radioimmunoassay. *Arthritis Rheum* 1983; **26**: 832-40.
- Lazzari A A, Cathcart E S, Oliveric R M, *et al*. A high incidence of antibodies to bovine type II collagen (C II) in degenerative joint disease (DJD). *Arthritis Rheum* 1983; **24**: S19.
- Mestecky J, Miller E J. Presence of antibodies specific to cartilage-type collagen in rheumatoid synovial tissue. *Clin Exp Immunol* 1975; **22**: 453-6.
- Steffen C, Ludwig H, Knapp W. Collagen-anticollagen immune complexes in rheumatoid arthritis synovial fluid cells. *Immunobiology* 1974; **147**: 229-35.
- Menzel J, Steffen C, Kolarz G, Kojer M, Smolen J. Demonstration of anticollagen antibodies in rheumatoid arthritis synovial fluids by ^{14}C -radioimmunoassay. *Arthritis Rheum* 1978; **21**: 243-8.
- Cracchiolo A, Michaeli D, Goldberg L S, Fundenberg H H. The occurrence of antibodies to collagen in synovial fluids. *Clin Immunol Immunopathol* 1975; **3**: 567-74.
- Andriopoulos N A, Mestecky J, Miller E J, Bennett J C. Antibodies to native and denatured collagens in synovial fluids of patients with rheumatoid arthritis. *Clin Immunol Immunopathol* 1976; **6**: 209-12.
- Cooke T D, Hurd E R, Jasin H E, Bienenstock J, Ziff M. Identification of immunoglobulins and complement in rheumatoid articular collagenous tissues. *Arthritis Rheum* 1975; **18**: 541-51.
- Dumonde D C, Glynn L E. The production of arthritis in rabbits by an immunological reaction to fibrin. *Br J Exp Pathol* 1962; **43**: 373-83.
- Zvaifler N J. The immunopathology of joint inflammation in rheumatoid arthritis. In: Dixon F J, Kunkel H G eds. *Advances in immunology*. New York: Academic Press, 1973; **16**: 265-336.
- Jasin H E, Ziff M. Immunoglobulin and specific antibody synthesis in a chronic inflammatory focus: antigen-induced synovitis. *J Immunol* 1969; **102**: 355-69.
- Cooke T D, Jasin H E. The pathogenesis of chronic inflammation in experimental antigen induced arthritis. 1. The role of antigen on the local immune response. *Arthritis Rheum* 1972; **15**: 327-37.
- Kresina T F, Rosner I A, Golberg V M, Moskowitz R W. IgG-induced experimental chronic immune synovitis: cell-mediated immunity to native interstitial collagen molecules and their constituent polypeptide chains. *Cell Immunol* 1984; **87**: 504-16.
- Goldberg V M, Lance E M, Davis P. Experimental immune synovitis in the rabbit. Relative roles of cell mediated and humoral immunity. *Arthritis Rheum* 1974; **17**: 993-1005.
- Friedlaender G E, Ladenbauer-Bellis I M, Chrisman O D. Immunogenicity of xenogeneic cartilage matrix components in a rabbit model. *Yale J Biol Med* 1983; **56**: 211-7.
- Gay S, Miller E J. *Collagen in the physiology and pathology of connective tissue*. Stuttgart: Gustav Fischer, 1978.
- Trelstad R L, Catanese V M, Rubin D F. Collagen fractionation: separation of native types I, II and III by differential precipitation. *Anal Biochem* 1976; **71**: 114-8.
- Kresina T F, Miller E J. Isolation and characterization of basement membrane collagen from human placental tissue. Evidence for the presence of two genetically distinct collagen chains. *Biochemistry* 1979; **18**: 3089-97.
- Miller E J. Isolation and characterization of a collagen from chick cartilage containing three identical α chains. *Biochemistry* 1971; **10**: 1652-9.
- Adelstein R S, Kuehl W M. Structural studies on rabbit skeletal actin. I. Isolation and characterization of the peptides produced by cyanogen bromide cleavage. *Biochemistry* 1970; **9**: 1355-64.
- Miller E J, Epstein E H Jr, Piez K A. Identification of three genetically distinct collagens by cyanogen bromide cleavage of insoluble human skin and cartilage collagen. *Biochem Biophys Res Commun* 1971; **42**: 1024-9.
- Gossiau B, Barrach H J. Enzyme-linked immunosorbent microassay for quantification of specific antibodies to collagen type I, II, III. *J Immunol Methods* 1979; **29**: 71-7.
- Desbuquois B, Aurbach G D. Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *J Clin Endocrinol Metab* 1971; **33**: 732-8.
- Kresina T F, Malesud C J. Susceptibility of interstitial rabbit collagens to rabbit articular chondrocyte collagenase. *Collagen Relat Res* in press.
- Michaeli D, Benjamine E, Leung D Y K, Martin G R. Immunochemical studies on collagen II antigenic differences between guinea pig skin collagen and gelatin. *Immunochemistry* 1971; **8**: 1-7.
- Forthmayr H, Beil W, Timpl R. Different antigenic determinants in the polypeptide chain of human collagen. *FEBS Lett* 1971; **12**: 341-4.
- Timpl R, Forthmayr H, Beil W. Maturation of the immune response to soluble rat collagen: Late appearance of antibodies to N-terminal sites of the α -2 chain. *Immunology* 1972; **108**: 119-26.
- Webb F W S, Ford P M, Glynn L E. Persistence of antigen in rabbit synovial membrane. *Br J Exp Pathol* 1971; **52**: 31-7.
- Hollister J R, Mannik M. Antigen retention in joint tissues in antigen-induced synovitis. *Clin Exp Immunol* 1974; **16**: 615-21.
- Van Beosekom H J, Van Den Broek W J M, Van De Putte L B A, Buijs W C A M, Van Den Berg W B. ^{99m}Tc -pertechnetate uptake measurements in the rabbit knee joints. I. Standardization of the method and measurements in experimental arthritis. *J Rheumatol* 1981; **8**: 31-9.