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Pattern of humoral reactivity to type II collagen in rheumatoid arthritis

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SUMMARY

Humoral immunity directed against type II collagen (CII) is a common although not specific feature of rheumatoid arthritis (RA). We have shown that 10 to 15% of the sera either from RA patients ($n=88$) or from healthy controls ($n=149$) reacted with native human CII. Conversely, autoantibodies to the alpha-1 (II) chains were significantly more frequent in the RA group (26·1% *versus* 6·0%, $P<0·001$), suggesting that denatured CII may be an autoantigen in RA. Thus, human CII was cleaved with cyanogen bromide (CB), and immunoblotting techniques were performed on 19 RA and 21 normal sera. Among the four major CB peptides, CB10 and CB11 were recognized by most of the sera tested without distinction between normal or RA sera. Inhibition experiments using an ELISA have shown that: (i) antibodies to the native CII molecule did not cross-react with those recognizing the CB peptides, and vice-versa; (ii) the binding of the sera to native CII was partially inhibited by pre-incubation with alpha-1 (II) chains, and vice-versa; (iii) pre-incubation of the sera with CB peptides partially blocked the binding to alpha-1 (II) chains, whereas pre-incubation of the sera with alpha-1 (II) chains totally inhibited the reactivity against CB peptides; and (iv) a substantial proportion of the epitopes recognized by anti-CII autoantibodies was neither species specific nor type specific. Taken together, these findings reveal the existence of several populations of anti-CII autoantibodies: some antibodies react exclusively with conformational determinants of the CII molecule, and others are directed towards linear structures of alpha-1 (II) chains.

Keywords type II collagen autoantibodies rheumatoid arthritis

INTRODUCTION

For many years, a role for type II collagen (CII) as an autoantigen in rheumatoid arthritis (RA) has been suspected, and many investigations have concentrated on humoral immunity to CII in chronic inflammatory rheumatic diseases (Trentham *et al.*, 1981; Stuart *et al.*, 1983; Clague *et al.*, 1983; Wooley *et al.*, 1984; Klimiuk *et al.*, 1985; Rowley, Gershwin & Mackay, 1988). The reported incidences of antibodies directed against CII in RA sera or synovial fluids varied largely according to the investigators but it was consistently assumed that the antibodies binding to the heat-denatured molecule occurred with higher incidence than those reacting with native CII. Although autoimmunity against CII is not specific for RA, the highest titres of anti-CII antibodies were found in this disease. However, most of the authors failed to correlate the presence or the levels of anti-CII antibodies and clinical features or biological parameters (Verbruggen *et al.*, 1986). Indication that collagen reactivity in humans is under the genetic control of HLA-DR4 has been reported by some investigators (Solinger, Bhatnagar & Stobo, 1981; Rowley *et al.*, 1986), but was not supported by the findings of others (Wooley *et al.*, 1984; Kammer & Trentham, 1984; Ström, Al-Balaghi & Möller, 1984). These apparent contradictions may relate to the existence of epitope(s), the recognition of which is crucial for the pathogenesis of RA whereas reactivity against the other determinants has no relation to the disease. Evidence supporting this hypothesis comes from animal studies; the injection of native CII of either xenogeneic or homologous origin into rats (Trentham, Townes & Kang, 1977) or mice (Courtenay *et al.*, 1980; Boissier *et al.*, 1987) results in the development of a polyarthritis and production of circulating anti-CII antibodies, some of which recognize arthritogenic epitopes (Wooley *et al.*, 1985; Englert *et al.*, 1987).

The native soluble CII molecule is assembled from three identical polypeptide chains coiled together in a triple helical fashion and designated alpha-1 $(II)_3$. This structure can be destroyed by heat denaturation, a process known to yield random-coiled alpha-1 (II) chains, each of which is characterized by the presence of the typical Gly-X-Y repeating sequence throughout 90% or more of its entire length. These biochemical properties of CII indicate that, according to its degree of degradation, the CII molecule may elicit specific antibody responses against antigenic determinants which are either

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conformational or directed at linear structures. Accordingly, the present study was designed to analyse the specificity of anti-CII auto-antibodies in the sera of patients with RA.

PATIENTS AND METHODS

Patients

A group of 88 unselected patients with definite or classical RA (Rose *et al.*, 1958) were screened for anti-CII antibodies. All of them were outpatients or inpatients of the Department of Rheumatology at Hôpital Cochin. Control subjects ($n=149$) were healthy blood donors.

Collagen preparations

Human CII was kindly supplied by D. Herbage (CNRS UA 244, Lyon, France); bovine CII and type I collagen (CI) were purchased from Bioetica, Lyon, France. Purity of all collagens was assessed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE). All native molecules were dissolved at 1 mg/ml in 0·1 M acetic acid. Denatured CII (alpha-1 (II) chains) or CI were prepared by placing the solutions of native collagens in a water bath at 60°C for 1 h and either used for antibody testing or further cleaved with cyanogen bromide (CB) in 70% (v/v) formic acid, according to the method described by Miller (1971). Briefly, CB equal in weight to a 150-fold molar excess relative to the number of methionine residues of the dissolved CII was added. The reaction mixture flushed with N_2 was incubated at 30°C overnight, diluted 10-fold with water, and freeze-dried. The CB peptides were then re-dissolved in distilled water.

Anti-CII antibody determination

The sera were collected and stored at −20°C until they were tested (diluted 1:20) for antibodies directed against human or bovine CII, using an ELISA, as previously described (Boissier *et al.*, 1987). For the determination of antibodies to native CII (or CB peptides), plates were coated with antigen and incubated with the sera, both performed at 4°C. The antibodies to alpha-1 (II) chains were measured by incubating for 1 h at 60°C the plates filled with native CII solution, and performing all the subsequent steps at room temperature. The presence of anti-CII antibodies was determined using rabbit anti-human IgG coupled to alkaline phosphatase (Behring, Marburg, FRG). The results were expressed as optical density (OD) $\times 10^3$ in experimental wells minus the baseline OD obtained in the absence of antigen coating. Sera with levels above the upper limit (mean value for the blood donors plus 2 s.d.) were considered positive for anti-CII antibodies.

Western blot analysis of autoantibodies to human CB peptides

Antibody reactivity with the major CB peptides was evaluated using immunoblotting methods. The CB peptides were separated by SDS–PAGE (8 to 25% gradient) using the Phast system (Pharmacia). In preliminary experiments, the four major CB fragments of alpha-1 (II) chains were identified after electrophoretic migration by silver staining, namely, in order of decreasing mol. wt, CB10, CB11, CB8 and CB9,7. The CB peptides were then transferred onto nitrocellulose (BA 85 Schleicher and Schüll, Dassel, FRG) in 0·02 M Tris, 0·15 M glycine containing 20% (v/v) methanol at 150 mA for 18 to 20 h.

Table 1. Incidence of circulating anti-type II collagen (anti-CII) autoantibodies in patients with rheumatoid arthritis (RA) and in control subjects, evaluated by ELISA

Subjects	Autoantibodies to Native CII (%)	Autoantibodies to Alpha-1 (II) (%)
RA ($n=88$)	14·8	26·1
Controls ($n=149$)	11·4	6·0
P*	NS	<0·001

*Statistical analysis using χ^2-test; NS, not significant.

Proteins were identified by staining the nitrocellulose with 0·2% ponceau S (w/v) (Serva, Heidelberg, FRG) in 3% TCA and individual lanes were separated by cutting. Non specific protein binding was blocked by treating the blot with phosphate-buffered saline (PBS) containing 10% fetal calf serum and 0·2% triton X-100 (PTB). Antigen specificity was then determined by incubating each parallel lane of the blot with the appropriate serum. The sera to be tested were diluted 1:20 in PTB, layered onto nitrocellulose blots, incubated overnight at 4°C, then for 2 h at room temperature on a rotating platform, and washed for 1 h with six changes of PTB. The blots were then further incubated for 2 h at room temperature with a 1:250 dilution of peroxidase-conjugated goat anti-human IgG (Nordic, Tilburg, The Netherlands) and were washed for 10 min six times with PTB and twice with PBS. Peroxidase activity was revealed using a solution of diaminobenzidine (50 mg/100 ml Tris–HCl buffer) and 0·01% hydrogen peroxide. After a 45-sec incubation with the substrate, the reaction was stopped by washing with water.

Competitive inhibition assay

The specificity of serum binding to CII was determined using competitive inhibition in ELISA or immunoblotting methods. The sera to be tested were incubated for 2 h at room temperature with varying concentrations (ranging from 1 to 125 μg/ml) of the appropriate collagen preparation, prior to being added to pre-coated plates or layered onto nitrocellulose blots. The standard technical procedures were then performed as described above.

RESULTS

Detection of autoantibodies to CII in RA patients and controls

The sera of 88 patients with RA and 149 healthy subjects were screened for the presence of autoantibodies directed against CII. As shown in Table 1, very similar percentages of patient and control sera recognized the native CII molecule. In contrast, the incidence of sera reacting with the alpha-chain of human CII was significantly higher in the patient group than in the control group ($P<0·001$).

In order to further investigate the determinants on human CII which are recognized by the sera, we cleaved the alpha-1 (II) chains with CB and, using a Western blot technique, we analysed the pattern of reactivity of patient and control sera.

Table 2. Incidence of antibody reactivity with cyanogen bromide (CB) peptides from human type II collagen (CII) assayed using immunoblotting methods

Subjects	Incidence (%) of sera reacting with		
	CB10	CB11	CB8 and 9,7
Rheumatoid arthritis ($n=19$)	78·9	68·4	26·3
Controls ($n=21$)	71·4	57·1	14·2

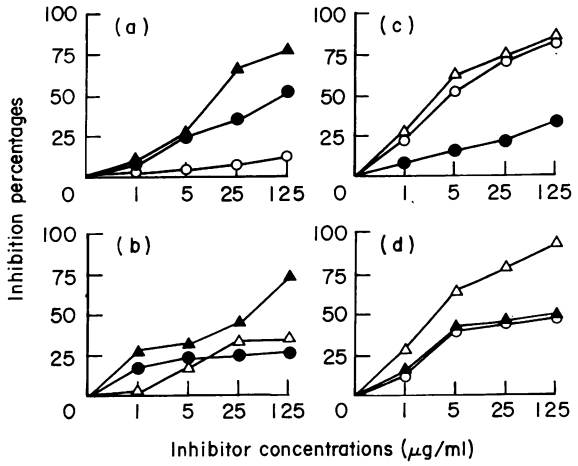

Fig. 1. Species cross-reactivities of anti type II collagen (anti-CII) antibodies in a rheumatoid arthritis (RA) serum using competitive inhibition ELISA. Results are expressed as percent inhibition of binding to human native CII (a), bovine native CII (b), human alpha-1 (II) chains (c) or bovine alpha-1 (II) chains (d), after pre-incubation of serum with varying concentrations of the following inhibitors: human native CII (●) or alpha-1 (II) chains (○); and bovine native CII (▲) or alpha-1 (II) chains (△).

Table 2 shows that most of the sera reacted with CB10, the largest CB cleavage product (29 kD). Fewer sera recognized the CB11 peptide (25 kD) and only a small percentage of them bound to CB8 and CB9,7 (14 and 11 kD, respectively). Although reactivity was always higher in the RA patient group, the incidence of positive sera was not significantly different from that of control subjects.

Cross-reactivity patterns of RA sera towards human and bovine CII

Two sera from RA patients who had high levels of anti-CII autoantibodies were tested for their reactivities against human and bovine CII in either their native or denatured form, using a competitive ELISA. The binding of the sera to human native CII was blocked by pre-incubation with either human or bovine native CII (Fig. 1a). The higher competitive capacity of bovine CII in comparison to that of human CII may reflect a stronger affinity of antibodies for the conformational determinants on the xenogeneic than on the homologous protein. Moreover, the antibodies recognizing determinants of bovine native CII were, as expected, inhibited by bovine native CII but only partially affected by prior addition of human native CII (Fig. 1b). These findings suggest that the sera tested recognize multiple epitopes on the native CII molecule some of which are present on both human and bovine CII, while others are restricted to bovine CII. This observation may account for the higher levels of antibodies detected against the xenogeneic protein. As far as the reactivity to determinants present on the primary or secondary structures of the CII molecule is concerned, similar conclusions can be reached. Indeed, pre-incubation with alpha-1 chains from bovine CII was as effective as that with alpha-1 chains of human origin in inhibiting the binding of the sera to the human alpha-1 chains (Fig. 1c), whereas in the reverse situation a partial cross-reactivity was observed (Fig. 1d). Thus, circulating anti-CII antibodies were shown to recognize antigenic epitopes shared by bovine and human CII and others specific for the xenogeneic molecule.

Conformational specificity of anti-CII antibodies

Next, we examined whether the anti-CII antibodies were reactive with the same determinants on the native and on the heat-denatured antigen. As seen in Fig. 1, the binding of the sera to the triple helix of human CII was poorly inhibited by the human alpha-1 chains (Fig. 1a) and vice-versa (Fig. 1c), suggesting that most of the antibodies do not react with the same determinants on the native and the heat-denatured molecule of human CII. However, the degree of cross-reactivity of the anti-bovine CII antibodies was higher, since the binding of sera to both bovine CII structures was reciprocally inhibited by 35–50% (Fig. 1b and d). In order to further investigate the conformational specificities of anti-CII antibodies, these competitive inhibition experiments were extended to the reactivity of the sera with CB peptides derived from bovine CII. Sera from four RA patients and two healthy controls that reacted with both native and heat-denatured bovine CII were evaluated for antibodies directed against antigenic determinants that are either conformational or sequential in nature. Both RA and control sera exhibited identical inhibition curves. Figure 2 shows such a representative experiment with serum from a patient with RA, using the competitive ELISA. The binding of sera to native CII-coated plates was partially inhibited by pre-incubation with heat-denatured CII but was not affected by the presence of a solution of unseparated CB peptides derived from bovine CII (Fig. 2a). Reciprocally, addition of native bovine CII to the sera failed to inhibit their binding to CB peptide-coated plates, whereas alpha-1 chains exhibited a blocking effect similar to the control curve obtained with CB peptides (Fig. 2c). Finally, antibodies to denatured CII were partially cross-reactive with those to the native molecule but they were only moderately inhibited by prior addition of the CB peptides (Fig. 2b). These observations were confirmed using immunoblotting methods. Figure 3 exemplifies the pattern of reactivity of the same RA serum as that used for the ELISA represented in Fig. 2. Each lane includes dots for native and denatured CII and Western blots of the four major CB peptides of CII. The RA patient's serum (lane a) but not the control negative serum (lane e) reacted with all the CII structures. Prior incubation with native CII (lane d) inhibited the binding of the serum to both native and denatured CII dots but did not affect the intensity of labelling of the CB peptides, and vice-versa, when the serum was

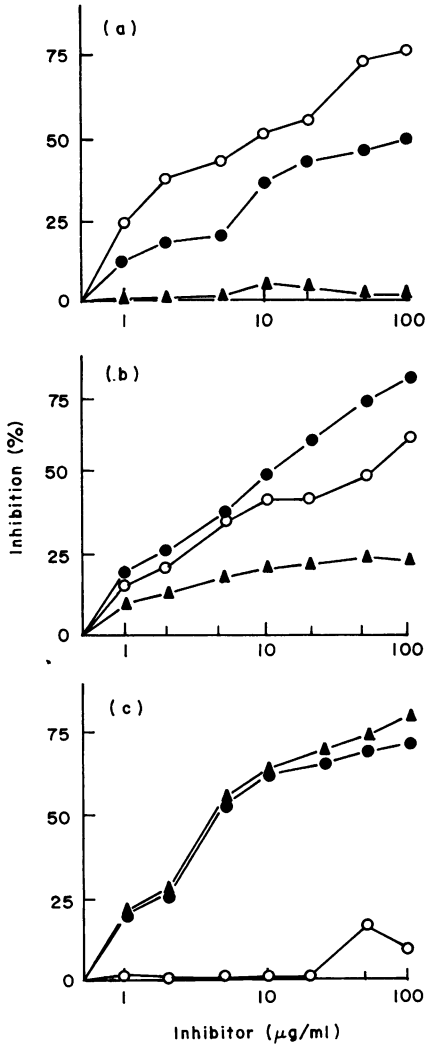

Fig. 2. Conformational specificity of anti type II collagen (anti-CII) antibodies: a representative experiment with serum from a patient with rheumatoid arthritis (RA), using competitive inhibition ELISA. The serum was pre-incubated without or with increasing concentrations of the appropriate inhibitor and tested for its binding to native CII (a), heat-denatured CII (b) or cyanogen bromide (CB) peptides (c). Results are expressed as percentages of inhibition in the presence of the competing antigen. Inhibitors: ○, native CII; ●, heat-denatured CII; and ▲, CB–CII.

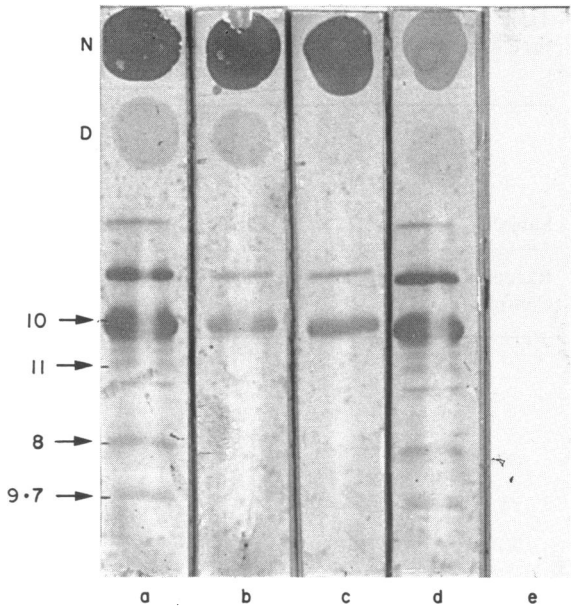

Fig. 3. Antibody reactivity with native type II collagen (CII) (N), denatured CII (D) and the four major cyanogen bromide (CB) peptides of CII (identified by their number). Immunoblots of the same rheumatoid arthritis (RA) serum as in Fig. 2 made in the absence of inhibitor (lane a); or in presence of 100 μg/ml of CB peptides (lane b); alpha-1 (II) chains (lane c); and native CII (lane d). Lane e was run with the negative control serum.

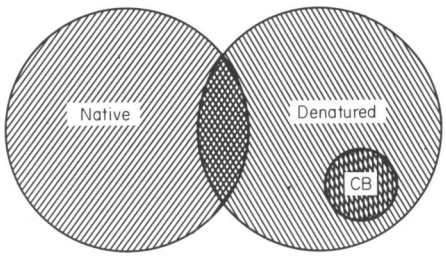

Fig. 4. Schematic representation of the specificity of human anti type II collagen (CII) antibodies according to the data presented in Figs 3 and 4. CB, cyanogen bromide.

pre-incubated with the CB peptides (lane b). The pre-incubation with alpha-1 (II) chains (lane c) abrogated the reactivity of the serum with denatured CII and CB peptides but only partially inhibited the binding to native CII. Taken together, our experiments indicate the existence of cross-reactive and non cross-reactive populations of anti-CII antibodies, as illustrated in Fig. 4.

Type specificity of collagen antibodies

Since RA sera were shown to contain antibodies directed against CI (Stuart *et al.*, 1983; Morgan *et al.*, 1987; Charrière *et al.*, 1988), we investigated whether the anti-CII antibodies circulating in the patients' sera could cross-react with antigenic determinants present on the CI molecule. The sera of two RA patients and one control were tested in competitive-inhibition ELISA, using the native and heat-denatured forms of CI and CII as the competitor. Table 3 reports the results of a representative experiment with one RA serum and shows that, although antibody levels to CI were lower than those to CII, there was an important cross-reactivity between both. Indeed, serum binding to native CII or denatured CII was inhibited by 50% in the presence of, respectively, native CI and denatured CI. However, a large majority of epitopes recognized on the CI molecule did not depend on the triple helix structure, since the binding to native CI was blocked by the alpha-chains of CI, and vice versa (Table 3). These data indicate that most of the non-conformational antigenic determinants expressed on native and denatured CII are also shared by native and denatured CI.

Table 3. Cross-reactivity of antibodies to type I and II collagens (CI and CII) evaluated using competitive inhibition ELISA

Serum binding to	Antibody levels (OD × 10^3)	Inhibition* (%) of binding in the presence of				
		Native CI	Denatured CI	Native CII	Denatured CII	CB peptides of CII
Native CI	85	90	74	41	61	29
Denatured CI	196	79	93	66	78	28
Native CII	266	51	49	78	38	2
Denatured CII	238	54	46	44	81	39
CB peptides CII	192	40	32	14	70	79

*Inhibitor concentrations used were 125 μg/ml.
CB, cyanogen bromide; OD, optical density.

Interestingly, the poor inhibitory effect of CB (II) peptides indicates that these common determinants are destroyed by CB cleavage.

DISCUSSION

Previous studies have documented the presence of autoantibodies to CII in RA (Trentham *et al*, 1981; Stuart *et al.*, 1983; Clague *et al.*, 1983; Wooley *et al.*, 1984; Klimiuk *et al.*, 1985; Rowley *et al.*, 1988). In our study we investigated whether the recognition of some antigenic determinants is indicative feature of RA by comparing the specificities of anti-CII antibodies found in RA sera with those of circulating natural autoantibodies of normal subjects. The levels of anti-CII autoantibodies in positive sera selected by ELISA are similar in both groups. However, the incidence of antibodies directed against the alpha-1 chains of CII (but not to the native molecule) is significantly higher in RA patients than in controls. Moreover, in line with previous reports (Stuart *et al.*, 1983; Clague *et al.*, 1983; Rowley *et al.*, 1988), we have found that RA sera contain higher percentages of antibodies to denatured CII than to native CII. These findings seem to indicate that their presence is a secondary phenomenon following the breakdown of cartilage and yet we failed to find any difference in the pattern of reactivity against the CB peptides of CII, whether the positive sera originated from RA patients or healthy controls. Thus, it seems that each CB peptide expresses several antigenic determinants and that further enzymatic treatment would be necessary to identify an epitope specifically recognized by RA sera. Alternatively, the critical arthritogenic epitope(s) may have been cleaved by CB treatment either because it (they) contain(s) a methionine residue or because it (they) need(s) a conformational structure. In line with this last hypothesis are the experimental studies demonstrating that both rats (Trentham *et al.* 1977) and mice (Courtenay *et al.*, 1980; Stuart, Townes & Kang, 1982) require immunization with native CII in order to develop arthritis. Other types of collagen and denatured CII are not effective in inducing the articular pathology. Moreover, only the IgG that react with conformational determinants of CII are active in inducing clinical arthritis when transferred passively into rats (Englert *et al.*, 1986). More precisely, in rodents the arthritogenic epitopes on CII of either bovine or chick origin were shown to be carried by the alpha-1 (II) CB11 peptide (Englert *et al.*, 1987; Terato *et al.*, 1985). If this observation is extended to human CII, it would imply that the CB11 peptide expresses multiple antigenic epitopes, some of which play a critical role in the induction of arthritis, whereas others only elicit an immune response. The other CB fragments derived from human CII also express antigenic specificities that are more frequently recognized on the CB10 peptide than on the smaller fragments. Whether CB10 (which is the largest CB cleavage product) contains the majority of dominant antigenic domains or includes a higher density of repetitive epitopes shared by the four major CB fragments remains to be elucidated.

In agreement with previous findings in rats (Englert *et al.*, 1986), our data provide evidence that in humans, humoral immunity directed against CII is polyclonal. As illustrated in Fig. 4, at least four populations of anti-CII antibodies with different specificities have been identified. The first group delineates antibodies reacting exclusively with CII in its triple helicoidal form and thus defining conformational epitopes. The second group is composed of antibodies binding to alpha-1 (II) chains but not to the native molecule, indicating that antibodies recognize determinants present on the primary or secondary structure of CII but masked in the native form of CII and modified by the treatment with CB. The third group includes cross-reacting antibodies that bind to both native and heat-denatured CII, most probably recognizing linear sequences. Finally, the fourth group is represented by those antibodies which bind to the CB peptides of CII and also to alpha-1 chains reacting exclusively with primary structure of the molecule. The presence of species-specific and common epitopes has been demonstrated on CII using monoclonal antibodies (Holmdahl *et al.*, 1986; Iribe *et al.*, 1988a). There we have shown that many of the antigenic determinants recognized by RA sera are shared by the human and the bovine CII, regardless of their conformational structure (Iribe *et al.*, 1988b). However, the partial blocking effect of antibody binding to bovine CII by human CII indicates that more antigenic epitopes are recognized in the xenogeneic than in the homologous system. Very similar conclusions have been reached previously in mice immunized with homologous or heterologous CII (Boissier *et al.*, 1987; Holmdahl *et al.*, 1985).

The analysis of the type specificity of circulating anti-CII antibodies revealed a partial blocking effect by either native or denatured CI (Choi *et al.*, 1988). This is not surprising, since CI contains two alpha-1 (I) chains very similar in structure to those of CII (Miller, 1985). Interestingly, the population of antibodies recognizing both CI and CII also cross-react almost completely with native and denatured CII, strongly suggesting that these IgG bind to linear sequences of amino-acid residues expressed on genetically different collagens.

Finally, there is accumulating evidence for a role of humoral autoimmunity to CII in the perpetuation of RA as a consequence of cartilage breakdown (Rowley, Williamson & Mackay, 1987). The tissue destruction may result in the release of CII molecules in their native conformation or after the action of the locally synthesized enzymes in a more or less degraded form. These autoantigens exposing multiple antigenic determinants may in turn elicit polyclonal autoantibody production (Klareskog *et al.*, 1986). Crucial for the arthritogenic process to develop would be the recognition under genetic control of a limited number of epitopes, carried by the triple helix of CII derived from different species, and type-specific. Antibodies to these arthritogenic epitopes may be valuable markers of the extent of existing cartilage damage.

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REFERENCES

Boissier, M.C., Feng, X.Z., Carlioz, A., Roudier, R. & Fournier, C. (1987) Experimental autoimmune arthritis in mice. I. Homologous type II collagen is responsible for self-perpetuating chronic polyarthritis. *Ann. rheum. Dis.* **46,** 15.

Charriere, G., Hartmann, D.J., Vignon, E., Ronziere, M.C., Herbage, D. & Ville, G. (1988) Antibodies to types I, II, IX, and XI collagen in the serum of patients with rheumatic diseases. *Arthritis Rheum.* **31,** 325.

Choi, E.K.K., Gatenby, P.A., McGill, N.W., Bateman, J.F., Cole, W.G. & York, J.R. (1988) Autoantibodies to type II collagen: occurrence in rheumatoid arthritis, other arthritides, autoimmune connective tissue diseases, and chronic inflammatory syndromes. *Ann. rheum. Dis.* **47,** 313.

Clague, R.B., Firth, S.A., Holt, P.J.L., Skingle, J., Greenbury, C.L. & Webley M. (1983) Serum antibodies to type II collagen in rheumatoid arthritis: comparison of 6 immunological methods and clinical features. *Ann. rheum. Dis.* **42,** 537.

Courtenay, J.S., Dallman, M.J., Dayan, A.D., Martin, A. & Mosedale, B. (1980) Immunisation against heterologous type II collagen induces arthritis in mice. *Nature*, **283,** 666.

Englert, M.E., Ferguson, K.M., Raventos Suarez, C., Oronsky, A.L. & Kerwar, S.S. (1986) Passive transfer of collagen arthritis: heterogeneity of anti-collagen IgG. *Cell. Immunol.* **101,** 373.

Englert, M.E., Ferguson, K.M., Suarez, C.R., Sapp, T.M., Oronsky, A.L. & Kerwar, S.S. (1987) Type II collagen arthritis: identification of arthritogenic epitopes using fractionated anticollagen IgG. *Cell. Immunol.* **105,** 447.

Holmdahl, R., Jansson, L., Gullberg, D., Rubin, K., Forsberg, P.O. & Klareskog L. (1985) Incidence of arthritis and autoreactivity of anti-collagen antibodies after immunization of DBA/1 mice with heterologous and autologous collagen II. *Clin. exp. Immunol.* **62,** 639.

Holmdahl, R., Rubin, K., Klareskog, L., Larsson, E. & Wigzell, H. (1986) Characterization of the antibody response in mice with type II collagen-induced arthritis, using monoclonal anti-type II collagen antibodies. *Arthritis Rheum.* **29,** 400.

Iribe, H., Kabashima, H., Kakimoto, K. & Koga, T. (1988a) Induction of an anti-human type II collagen response by monoclonal anti-idiotypic antibody. *J. Immunol.* **140,** 4151.

Iribe, H., Kabashima, H., Ishii, Y. & Koga, T. (1988b) Epitope specificity of antibody response against human type II collagen in the mouse susceptible to collagen-induced arthritis and patients with rheumatoid arthritis. *Clin. exp. Immunol.* **73,** 443.

Kammer, G.M. & Trentham, D.E. (1984) HLA-DR4 is not a requisite for autoimmunity to collagen in rheumatoid arthritis. *Arthritis Rheum.* **27,** 489.

Klareskog, L., Johnell, O., Hulth, A., Holmdahl, R. & Rubin, K. (1986) Reactivity of monoclonal anti-type II collagen antibodies with cartilage and synovial tissue in rheumatoid arthritis and osteoarthritis. *Arthritis Rheum.* **29,** 730.

Klimiuk, P.S., Clague, R.B., Grennan, D.M., Dyer, P.A., Smeaton, I. & Harris, R. (1985) Autoimmunity to native type II collagen. A distinct genetic subset of rheumatoid arthritis. *J. Rheumatol.* **12,** 865.

Miller, E.J. (1971) Isolation and characterization of the cyanogen bromide peptides from the alpha-1 (II) chain of chick cartilage collagen. *Biochemistry*, **10,** 3030.

Miller E.J. (1985) The structure of fibril-forming collagens. Biology, chemistry and pathology of collagen. *Ann. NY Acad. Sci.* **460,** 1.

Morgan, K., Clague, R.B., Collins, I., Ayad, S., Phinn, S.D. & Holt, P.J.L. (1987) Incidence of antibodies to native and denatured cartilage collagens (types II, IX and XI) and to type I collagen in rheumatoid arthritis. *Ann. rheum. Dis.* **46,** 902.

Rose, M.W., Bennett, G.A., Cobb, S., Jacox, R. & Jessar, R.A. (1958) 1958 revision of diagnostic criteria for rheumatoid arthritis. *Bull. rheum. Dis.* **9,** 175.

Rowley, M.J., Gershwin, M.E. & Mackay, I.R. (1988) Collagen antibodies in juvenile arthritis and adult rheumatoid arthritis: differences in levels and type-specificity. *J. Rheumatol.* **15,** 289.

Rowley, M.J., Williamson, D.J. & Mackay, I.R. (1987) Evidence for local synthesis of antibodies to denatured collagen in the synovium in rheumatoid arthritis. *Arthritis Rheum.* **30,** 1420.

Rowley, M., Tait, B., Mackay, I.R., Cunningham, T. & Phillips, B. (1986) Collagen antibodies in rheumatoid arthritis. Significance of antibodies to denatured collagen and their association with HLA-DR4. *Arthritis Rheum.* **29,** 174.

Solinger, A.M., Bhatnagar, R. & Stobo, J.D. (1981) Cellular, molecular, and genetic characteristics of T cell reactivity to collagen in man. *Proc. natl. Acad. Sci. USA*, **78,** 3877.

Strom, H., Al-Balaghi, S. & Moller, E. (1984) No demonstrable association between HLA-DR4 and *in vitro* collagen reactivity as determined by the production of leukocyte inhibition factor. *Tissue Antigens*, **24,** 174.

Stuart, J.M., Townes, A.S. & Kang, A.H. (1982) Nature and specificity of the immune response to collagen in type II collagen-induced arthritis in mice. *J. clin. Invest.* **69,** 673.

Stuart, J.M., Huffstutter, E.H., Townes, A.S. & Kang, A.H. (1983) 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.* **26,** 832.

Terato, K., Hasty, K.A., Cremer, M.A., Stuart, J.M., Townes, A.S. & Kang, A.H. (1985) Collagen-induced arthritis in mice. Localization of an arthritogenic determinant to a fragment of the type II collagen molecule. *J. exp. Med.* **162,** 637.

TRENTHAM, D.E., TOWNES, A.S. & KANG, A.H. (1977) Autoimmunity to type II collagen: an experimental model of arthritis. *J. exp. Med.* **146,** 857.

TRENTHAM, D.E., KAMMER, G.M., MCCUNE, W.J. & DAVID, J.R. (1981) Autoimmunity to collagen. A shared feature of psoriatic and rheumatoid arthritis. *Arthritis Rheum.* **24,** 1363.

VERBRUGGEN, L.A., GEERTS, A., WISSE, E. & LAPIERE, C. (1986) Serum and synovial fluid antibodies to collagen in rheumatic diseases: a review. *Clin. Rheumatol.* **5,** 440.

WOOLEY, P.H., LUTHRA, H.S., O'DUFFY, J.D., BUNCH, T.W., MOORE, S.B. & STUART, J.M. (1984) Anti-type II collagen antibodies in rheumatoid arthritis. The influence of HLA phenotype. *Tissue Antigens,* **23,** 263.

WOOLEY, P.H., LUTHRA, H.S., GRIFFITHS, M.M., STUART, J.M., HUS, A. & DAVID, C.S. (1985) Type II collagen-induced arthritis in mice. IV. Variations in immunogenetic regulation provide evidence for multiple arthritogenic epitopes on the collagen molecule. *J. Immunol.* **135,** 2443.