

1 α 2 α 3 α Collagen is arthritogenic

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SUMMARY Native 1 α 2 α 3 α collagen (500 μ g per rat) was both immunogenic and arthritogenic in Alderley Park rats (46% developed arthritis) but only immunogenic in Sprague-Dawley rats. Conversely, native type II collagen (500 μ g per rat) was immunogenic and arthritogenic in both strains (64% arthritic in Alderley Park strain, 57% arthritic in Sprague-Dawley strain). The inflammatory polyarthritis induced by 1 α 2 α 3 α collagen was similar to that produced by native type II collagen in clinical appearance, time of onset, and histology. Antibodies raised to native bovine type II collagen cross-reacted with native 1 α 2 α 3 α collagen and vice versa. Thus the minor collagen component of cartilage, the 1 α 2 α 3 α collagen, as well as the major collagen component, type II collagen, are immunogenic and arthritogenic in the rat, with strain differences.

Native type II collagen, the major collagen component of cartilage, is immunogenic and arthritogenic in rats^{1–3} and mice.⁴ The major noncollagenous component, proteoglycan, although immunogenic is not arthritogenic¹ (personal observations). A minor collagen component (1 α 2 α 3 α collagen) has recently been described in human articular cartilage⁵ and bovine nasal cartilage.⁶ We have therefore investigated whether this collagen is arthritogenic in a similar manner to native type II collagen, or nonarthritogenic like proteoglycans and noncartilage collagens such as type I and type III collagen.^{1,3} This is of particular interest, as some earlier preparations of type II collagen used may have contained very small amounts of 1 α 2 α 3 α collagen.

Materials and methods

Preparation of native bovine type II collagen. Native bovine type II collagen was prepared from nasal septa after treatment of the cleaned cartilage with 2 M magnesium chloride in 0.05 M Tris-HCl, pH 7.4.⁷ The cartilage was pepsin solubilised in 0.5 M acetic acid (HAc) and the type II collagen precipitated by dialysis into 0.5 M HAc containing 4% sodium chloride (NaCl). The precipitate was removed by centrifugation. The supernatant, which was depleted

of type II collagen, was used for the isolation of 1 α 2 α 3 α collagen (see below) and the precipitate redissolved in 0.45 M NaCl/0.05 M Tris-HCl, pH 7.4.

Further purification of native type II collagen. Native type II collagen was precipitated by dialysis into 0.02 M disodium hydrogen orthophosphate and dissolved in 0.1 M HAc. The type II collagen was then precipitated by dialysis into 0.1 M HAc containing 4% NaCl, by 16% alcohol, by dialysis into 3.42 M NaCl/0.02 M Tris-HCl, pH 7.4, and by dialysis into 0.02 M disodium hydrogen orthophosphate, the precipitate being dissolved in 0.45 M NaCl/0.02 M Tris-HCl (pH 7.4) between each step. The now pure type II collagen was dissolved in 0.1 M HAc, dialysed exhaustively against 0.1 M HAc, and centrifuged at 21 000 g for 90 minutes at 4°C.

The native type II collagen was pure by polyacrylamide gel electrophoresis and by amino acid analysis. No uronic acid could be detected by the method of Bitter and Muir,⁷ indicating that there was no proteoglycan contamination.

Preparation of 1 α 2 α 3 α collagen. 1 α 2 α 3 α collagen was prepared from the 4% NaCl supernatant of the pepsin digest (see above).⁸ Additional dialysis against phosphate-buffered saline (PBS), pH 7.2, selectively precipitated the 1 α 2 α 3 α collagen, leaving type II collagen in solution (Ayad and Weiss, in preparation). The 1 α 2 α 3 α collagen was pure as judged by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis. However, the PBS precipitation step was repeated twice to ensure the

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complete removal of type II and cartilage-phosphate soluble collagens. No traces of these collagens were found in the final PBS supernatant.

Both collagens were lyophilised and stored under vacuum at -20°C until used. They were dissolved in 0.45 M NaCl/0.02 M Tris buffer (pH 7.5) at a concentration of either 20 or 1000 μ g/ml before emulsification at a ration of 1:1 with Freund's incomplete adjuvant (ICFA, Miles Laboratories Ltd).

Experimental procedure. Outbred female Sprague-Dawley rats (150-250 g) from the Manchester Medical School colony and outbred male Alderley Park rats (Wistar derived; 250-310 g) were used. Rats were injected intradermally on the back with 1 ml of emulsion as in Tables 1 and 2. Rats were examined throughout the experiments for evidence of arthritis and nonarticular lesions, and serum samples were collected.

Serum IgG antibodies to native bovine type II collagen and to native bovine 1 α 2 α 3 α collagen were measured using an adaptation of the solid-phase double-antibody radioimmunoassay described by Clague *et al.*⁹ Results were expressed as the amount of radioactively labelled (I¹²⁵) antirat IgG bound by the collagen-specific antibodies in the rat sera.

Results

INCIDENCE OF ARTHRITIS

(a) *Alderley Park rats (Wistar derived).* The incidence of arthritis in Alderley Park rats is shown in Table 1. Only rats immunised with 500 μ g of native type II collagen or with 500 μ g of native 1 α 2 α 3 α collagen developed arthritis. The time of onset was similar in both groups.

Arthritis induced by either collagen was clinically indistinguishable. With both the onset was sudden and involved gross swelling and erythema of one or more hind limbs, particularly the ankle and tarsal joints. No forelimb involvement or nonarticular lesions were seen, although these have been seen previously in this strain of rat when immunised with native type II collagen (personal observation).

Table 1 Incidence and onset of arthritis in Alderley Park rats. Rats examined up to 50 days after immunisation

Collagen injected: II	1 α 2 α 3 α	Total no. of rats	No. of arthritic rats	% Arthritic	Time of onset (days)
500 μ g	—	33	21	64	11-23
10 μ g	—	10	0	0	—
1 μ g	—	10	0	0	—
—	500 μ g	24	11	46	10-28
—	10 μ g	10	0	0	—

Table 2 Incidence and onset of arthritis in Sprague-Dawley rats. Rats examined up to 50 days after immunisation

Collagen injected: II	1 α 2 α 3 α	Total no. of rats	No. of arthritic rats	% Arthritic	Time of onset (days)
500 μ g	—	14	8	57	12-17
500 μ g	500 μ g	7	3	43	12-15
500 μ g	10 μ g	6	3	50	14-20
—	500 μ g	32	0	0	—
—	10 μ g	8	0	0	—

Histologically, arthritic joints from rats immunised with 1 α 2 α 3 α collagen showed extensive synovial hypertrophy, periosteal new bone formation, cellular infiltration and oedema containing neutrophils and mononuclear cells, and erosion of cartilage and bone by pannus at the cartilage—bone junction. This picture was also seen in rats immunised with native type

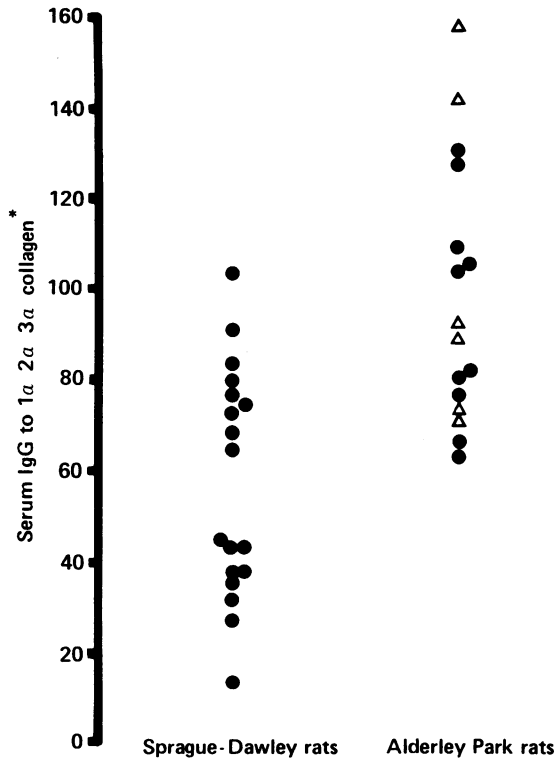


Fig. 1 Serum IgG levels of 1 α 2 α 3 α collagen in rats 21 days after immunisation with 500 μ g of 1 α 2 α 3 α collagen in ICFA. ● = Nonarthritic rats. Δ = Arthritic rats. All figures: * = measured as μ g of I¹²⁵I-labelled antirat IgG bound by anticollagen antibodies per 1 ml of serum.

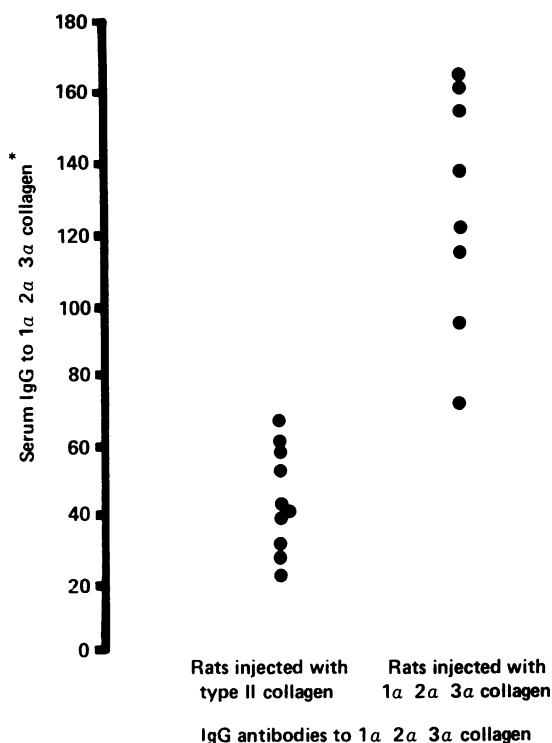


Fig. 2 Serum IgG levels of 1 α 2 α 3 α collagen in rats. Alderley Park rats immunised with either 500 μ g of 1 α 2 α 3 α collagen or 500 μ g type II collagen. See also legend to Fig. 1.

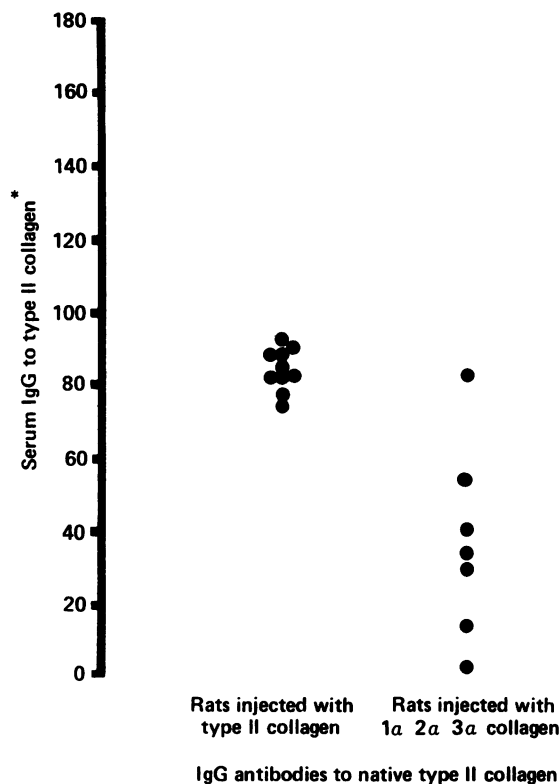


Fig. 3 Serum IgG levels of native type II collagen in the same rats as in Figure 2. See also legend to Fig. 1.

II collagen and was similar to that previously described.¹⁻³

(b) *Sprague-Dawley Rats.* The incidence of arthritis in Sprague-Dawley rats is shown in Table 2. Eight out of 14 rats immunised with native type II collagen developed arthritis between 12 and 17 days after immunisation. No rats immunised with 10 or 500 μ g of 1 α 2 α 3 α collagen developed arthritis.

The incidence of arthritis in rats immunised with mixtures of native type II collagen and native 1 α 2 α 3 α collagen was similar to that in rats immunised with native type II collagen alone. Thus mixing the 2 collagens did not increase the incidence of arthritis as compared with native type II collagen alone.

ANTIBODIES TO THE COLLAGENS

Fig. 1 shows the levels of IgG antibodies to 1 α 2 α 3 α collagen in Sprague-Dawley and Alderley Park rats. The mean level of IgG antibodies to 1 α 2 α 3 α collagen was higher in the Alderley Park rats, though there was an overlap between the groups.

Rats immunised with 10 μ g of 1 α 2 α 3 α collagen had no increase in the background binding of antirat

IgG and therefore had no IgG antibodies to this collagen (<1 μ g of ¹²⁵I antirat IgG bound per ml of serum).

IgG antibodies to native bovine type II collagen were present in the sera of rats immunised with 500 μ g of this collagen. However, rats immunised with 1 or 10 μ g failed to produce detectable levels of antibody to this antigen 21 days after immunisation.

CROSS-REACTIVITY OF ANTIBODIES

The cross-reactivity of antibodies raised in rats injected with native bovine type II collagen or with native 1 α 2 α 3 α collagen is shown in Figs 2 and 3. Rats had higher levels of antibodies to the collagen with which they were injected but also showed considerable cross-reactivity with the other collagen.

Discussion

1 α 2 α 3 α Collagen is a minor collagen component of cartilage, being approximately 10% by weight of the total collagen present. The 1 α and 2 α chains have no similarity to the α 1 (II) chain of type II collagen but

are closely related to though genetically distinct from the $\alpha 1$ and $\alpha 2$ chains of type V collagen. The 3α chain, however, is similar to the $\alpha 1$ (II) chain of type II collagen, although it is more highly glycosylated.

Previous work on native type II collagen-induced arthritis in the rat used native type II collagen which may have been contaminated with 1α 2α 3α collagen at a very low level undetectable by polyacrylamide gel electrophoresis.^{1-3 10} Therefore it is possible that the arthritis could have been induced by the small amounts of 1α 2α 3α collagen, which may also have been present, and not by the type II collagen. The purification of these collagens as described in this paper ensured that they were native collagens free from contamination with denatured collagens and from cross-contamination.

In the Sprague-Dawley rats arthritis was induced only by the native bovine type II collagen and not by the native 1α 2α 3α collagen, and mixing the collagens did not increase the incidence of arthritis above that found with native type II collagen alone, indicating that in Sprague-Dawley rats arthritis was only induced by native type II collagen.

In the Alderley Park rats, however, arthritis could be induced either by native bovine type II collagen or by native 1α 2α 3α collagen, and this arthritis was clinically and histologically similar whichever collagen was used.

1α 2α 3α Collagen was immunogenic in both strains of rat, as was the type II collagen, but the 1α 2α 3α collagen was arthritogenic only in the Alderley Park rats. Antibodies raised to 1α 2α 3α collagen showed cross-reactivity to type II collagen and vice versa. This cross-reactivity was possibly due to the similarity of the 3α chain to that of the $\alpha 1$ (II) chain of type II collagen.

Thus both the major and minor collagen constituents of hyaline cartilage have been shown to be arthritogenic and immunogenic in their own right.

Investigation of the reasons why type II collagen but not 1α 2α 3α collagen was arthritogenic in Sprague-Dawley rats, although both were immunogenic, may lead to a greater understanding of the factors necessary for the induction of arthritis.

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