Dependence of proteoglycan induced arthritis in BALB/c mice on the development of autoantibodies to high density proteoglycans

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Abstract

BALB/c mice were immunised with high or low density native human cartilage proteoglycans, or the respective core proteins obtained from chondroitin ABC lyase digestion. Mice injected with high density native proteoglycans developed arthritis whereas mice injected with low density proteoglycans or with core proteins did not. Analysis of the immune response by enzyme linked immunosorbent assay (ELISA) and western blot showed a stronger and more polyspecific response in animals injected with low density proteoglycans compared with mice with arthritis which had been injected with high density proteoglycans. Autoantibodies to mouse high density proteoglycans were only present in mice injected with native human high density proteoglycans, however. The data suggest that an arthritogenic epitope lies within the glycosaminoglycan rich region of the native proteoglycan molecule, which of antibodies to proteoglycans which cross react with native mouse proteoglycan. In the work reported here we have elaborated on this observation by exploring the arthritogenic potential of native low density proteoglycans and high density proteoglycans, and their respective core proteins. We found that although low density proteoglycans induced a higher immune response than high density proteoglycans, only the mice injected with native high density proteoglycans developed polyarthritis. The induction of arthritis in BALB/c mice appears to be related to the specificity of the autoimmune response to high density proteoglycans induced by native human cartilage proteoglycans.

Methods and materials

MICE

Inbred female BALB/c mice (five to seven weeks old) were obtained from Jackson Laboratory (Bar Harbor, MA, USA) and were
under associative conditions at 105 000 g for 70 hours at 10°C (Beckman Ultracentrifuge Model L5–75, 50Ti rotor). The density gradient was then fractionated into five equal volumes and the lower three fifths (A1A2A3) containing high density proteoglycans and the upper two fifths (A4A5) containing the low density proteoglycans were collected and separated from the gel at the top of the gradient. Additional buffer (0·4 M guanidine-HCl, 50 mM sodium acetate, pH 5·8) and solid caesium chloride to a density of 1·63 g/ml for the fraction containing high density proteoglycans and 1·46 g/ml for the fraction containing low density proteoglycans were added and the two fractions were centrifuged again under the same conditions to obtain the lower three fifths (A1A2A3–A1A2A3) of the gradient containing the high density proteoglycans and the middle two fifths (A4A5–A2A3) containing the low density proteoglycans. The proteoglycan fractions were extensively dialysed against water at 4°C, lyophilised, and stored at −70°C. Neither proteoglycan fraction contained any hydroxyproline when analysed spectrophotometrically.13 Proteoglycan fractions were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis as described by Laemmlí,14 stained with silver nitrate,15 and then with toluidine blue O (Allied Chemical) in 0·1 M acetic acid.

PROTEOGLYCAN CORE PROTEINS
Core protein fractions of low and high density proteoglycans from fetal cartilage were prepared by enzyme digestion.16 Proteoglycan (0·17 mg) was digested with one unit of purified chondroitin ABC lyase (ICN ImmunoBiologicals, Lisle, IL, USA) at 37°C for 25 hours in 100 mM Tris-HCl, 30 mM sodium acetate buffer (pH 8·0) containing 0·6 mg/ml bovine serum albumin as a stabiliser, 10 mM EDTA, 10 mM N-ethylmaleimide, 5 mM phenylmethylsulphonyl fluoride, and 0·36 mM peptatin. After digestion, the fractions containing low and high density proteoglycans and a control (with lyase but without proteoglycans) were dialysed against 1·1 of water at 4°C for 48 hours with two buffer changes. After lyophilisation these core protein fractions containing proteoglycans were used for a western blot immunoassay.

IMMUNISATION METHOD
Groups of ten mice were injected intraperitoneally either with 0·1 mg native high density proteoglycans or native low density proteoglycans solubilised in phosphate buffered saline (0·14 M sodium chloride in 0·01 M sodium phosphate buffer, pH 7·2) and emulsified with an equal volume of Freund’s complete adjuvant. A third group of ten control mice was injected with a similar volume (100 μl) of Freund’s complete adjuvant emulsified with an equal amount of PBS alone. Each group of mice was reinjected intraperitoneally with the relevant antigen in PBS and Freund’s complete adjuvant two weeks after the initial immunisation.

In a separate experiment, groups of six mice were injected with 0·1 mg core proteins of high or low density proteoglycans, or with the respective native proteoglycan molecules.

EVALUATION OF ARTHRITIS
The mice were assessed every third day after the second injection. A clinical score from 0 to 3 was assigned to each limb as described previously,17 where 0 = normal, 1 = redness and swelling, 2 = joint deformities, and 3 = loss of flexion and extension. The histopathological features of the joint disease were assessed in affected and unaffected limbs removed from the mice at the end of the trial using histological techniques described previously.17

SEROLOGICAL ANALYSIS
Serum samples were obtained by retro-orbital puncture before immunisation (prebled), and at 4, 6, 8, 10, 12, and 14 week intervals after immunisation. Serum samples were stored at −80°C and assayed for antibodies to proteoglycans and other connective tissue components, and for IgM rheumatoid factor.

ELISA FOR CONNECTIVE TISSUE COMPONENTS
Proteoglycan antibodies
Flat bottomed microtitre plates were coated with 2·5 μg/well human or murine low or high density proteoglycans (native or core molecule) in 100 μl phosphate buffered saline (PBS) overnight at 4°C. Plates were washed with PBS containing 0·05% Tween 80 (PBS/Tween), blocked with PBS containing 5% dried milk proteins (PBS/milk), and rewashed after incubation for 24 hours at 4°C. Serum samples from mice injected with low and high density proteoglycans were diluted appropriately in PBS/milk and 100 μl added to each well. Plates were incubated at room temperature overnight, washed with PBS/Tween, and total bound antibody was detected by the addition of 100 μl goat antimouse polyimmunoglobulin conjugated with alkaline phosphatase (FisherBiotek, Orangeburg, NY, USA), and incubated overnight at room temperature. Plates to determine immunoglobulin isotypes were probed with 100 μl of alkaline phosphatase labelled goat antimouse IgG, IgM, and IgA (FisherBiotek), whereas the binding of IgG subclasses was determined using enzyme labelled goat antimouse IgG1, IgG2a, and IgG2b (FisherBiotek). After washing plates were developed with 1 M p-nitrophenyl phosphate (Sigma), and the absorbance at 405 nm was determined with a microplate photometer (Molecular Devices, Menlo Park, CA, USA). Antibody binding was expressed as absorbance at 405 nm.

Antibodies to type II collagen
Flat bottomed microtitre plates (Dynatech Laboratories, Alexandria, VA, USA) were coated with buffer containing 3·0 μg/well type II collagen or without antigen (control). The type II collagen was isolated from bovine cartilage as previously described.18 Plates were...
washed with PBS/Tween, blocked with PBS/milk and rewashed after incubation for 24 hours at 4°C. Serum samples from mice injected with low and high density proteoglycans were mixed with the appropriate dilutions in PBS/5% milk, and 0·1 ml added to the wells. The plates were incubated overnight at room temperature. After the plates had been washed, the bound antibody was detected as described earlier. This assay is a modification of the method described previously. Antibody binding was expressed as absorbance at 405 nm. Consistent antigen binding was assessed by the Pierce protein assay.

Antibodies to keratan sulphate, chondroitin sulphate, dermatan sulphate, and hyaluronic acid
Flat bottomed microtitre plates (Dynatech Laboratories) were coated with buffer containing 2·0 μg/well of either keratan sulphate, chondroitin 4-sulphate, chondroitin 6-sulphate, dermatan sulphate, or hyaluronic acid (Sigma) or without antigen (control). Binding of chondroitin 4-sulphate, chondroitin 6-sulphate, dermatan sulphate, and hyaluronic acid to microtitre plates was assessed by spectrophotometric analysis of the antigen coating buffers before and after coating. Plates were washed, blocked, and used for the assay of serum samples from immunised mice as described earlier.

Rheumatoid factor
IgM rheumatoid factor levels were determined by an ELISA described elsewhere. Flat bottomed microtitre plates (Dynatech Laboratories) were coated overnight at 4°C with 0·3 μg/well rabbit IgG (Fisher Scientific) or with buffer alone. After washing with PBS Tween the plates were blocked with PBS/milk for 24 hours at 4°C. The plates were again washed and 0·1 ml of 1·40 dilutions of serum samples from mice injected with low density proteoglycans and high density proteoglycans was added to the wells. Plates were incubated overnight at 4°C, washed, and alkaline phosphatase labelled goat antimouse IgM was added to each well. After overnight incubation at 4°C the plates were washed, developed, and read as described earlier. Rheumatoid factor binding was expressed as absorbance at 405 nm.

WESTERN BLOT IMMUNOASSAY
Sodium dodecyl sulphate polyacrylamide gel electrophoresis of proteoglycan core proteins from low and high density proteoglycans and control fractions was performed as described by Laemmli. A 5% sodium dodecyl sulphate running gel and a 3% sodium dodecyl sulphate stacking gel were used. Tracks 9 and 10 containing low and high density proteoglycans were cut out, placed in fixing solution (250 ml deionised water, 50 ml glacial acetic acid, and 200 ml methanol) overnight and silver stained as described by Merrill et al. The remaining portion of the electrophoresis slab (tracks 1–8) was electrotransferred onto nitrocellulose paper in electrode buffer (0·0 1 of 0·2 M TRIS-acetic acid, pH 8·8, 0·1% sodium dodecyl sulphate) at 150 mA overnight. After washing the nitrocellulose twice with 200 ml PBS for 30 minutes it was cut into three strips, labelled with 1% methyl green and dotted on the lower portion of the appropriate gel track with core protein of high and low density proteoglycans and the control (enzyme alone). The strips were blocked with PBS/5% milk at 4°C for 2·5 hours and placed in appropriate serum sample pools diluted 1:100 in PBS/5% milk at 4°C overnight. The strips were washed three times with PBS/0·3% Tween for five minutes at 5 ml/track and then washed once with just PBS for five minutes at 5 ml/track. The strips were placed in 5 ml/track of the secondary antibody solution (39·0 ml PBS, 1·0 g milk, 1 ml normal goat serum, 0·08 ml goat antimouse IgG conjugated to alkaline phosphatase (Fisher Scientific) at room temperature overnight. They were again washed three times with PBS/0·3% Tween for five minutes at 5 ml/track and once with PBS for five minutes at 5 ml/track. The strips were placed into the aqueous substrate solution (0·2 mg/ml nitroblue tetrazolium, 100 mM TRIS-HCl, pH 9·7, 0·5 mM magnesium chloride, 0·001 mM zinc chloride, 0·2 mg/ml 5-bromo-4-chloro-3-indolyl phosphate (US Biochemicals), 1·2% (v/v) dimethyl sulfoxide) at 5 ml/track for 2·5 minutes to develop. The development was stopped with water for 20 minutes at 10 ml/track, air dried on parafilm, and photographed.

DELAYED TYPE HYPERSENSITIVITY TO PROTEOGLYCANs
BALB/c mice were injected intraperitoneally with 100 μg of native high or low density proteoglycans emulsified in Freund's complete adjuvant, with Freund's complete adjuvant only, or with sterile saline. Two weeks after immunisation the mice were challenged by an intradermal injection of 10 μg high density proteoglycans solubilised in PBS into the pinna of the right ear; the left ear was injected with 10 μg low density proteoglycans. Twenty four and 48 hours after challenge, the delayed type hypersensitivity response was assessed by measuring the ear swelling using a micrometer caliper equipped with a constant tension slitting clutch. The delayed type hypersensitivity response was expressed as the difference in the measured thickness between the ears before and after challenge.

Results

PROTEOGLYCAN ANALYSIS
The native high density proteoglycans isolated from human fetal articular cartilage migrated as a single band on agarose-acrylamide electrophoretic gels. The band stained with toluidine blue and less so with silver. On 5% polyacrylamide gels the native high density proteoglycans did not penetrate the gel and appeared as a dark band at the top of the gel (fig 1, track 1). A lack of penetration of 4% polyacrylamide gels is consistent with a size
estimate of 1000–3000 kilodaltons for the proteoglycan monomer alone. Also present in the native high density proteoglycans were two faint molecular weight bands (L1 and L2) corresponding to the link proteins (51 and 42 kilodaltons). After digestion of the native high density proteoglycans with chondroitin ABC lyase, electrophoresis showed the conversion of the high molecular weight band at the top of the gel to the core protein of high density proteoglycans which migrates as a very faint and diffuse band (191–338 kilodaltons). The two link proteins which are not digested by the lyase also remain (fig 6, track 10).

The high density proteoglycans contain only the intact native molecule consisting of the proteoglycan monomer, hyaluronic acid, and the link proteins. It has been reported that if the caesium chloride gradient fraction used to obtain the low density proteoglycans is not carefully separated from the proteins at the top of the gradient, it may contain components other than the dermatan sulphate proteoglycans, such as cartilage matrix protein or collagen. These proteins, however, were not detected in the electrophoretic patterns or by hydroxyproline analysis in any of our proteoglycan fractions. No other bands were detected when gels were overloaded or overstained, or both (data not shown).

Electrophoresis of native low density proteoglycans showed a diffuse band (84–300 kilodaltons) corresponding to the native dermatan sulphate proteoglycans. Low density proteoglycans appear as a diffuse band (84–300 kilodaltons) corresponding to the native dermatan sulphate proteoglycans; DS-PGI and DS-PGII. M1 shows the migration corresponding to a 134 kilodalton marker, whereas M2 indicates the migration corresponding to a 67 kilodalton marker (not shown).

Figure 1 Sodium dodecyl sulphate polyacrylamide gel electrophoresis of high density proteoglycans (track 1) and low density proteoglycans (track 2) on 5% acrylamide under reducing conditions. Migration is from top to bottom. High density proteoglycans appear as a dense band at the top of the gel. L1 and L2 correspond to link proteins present in the native high density proteoglycans. Low density proteoglycans appear as a diffuse band (84–300 kilodaltons) corresponding to the native dermatan sulphate proteoglycans; DS-PGI and DS-PGII. M1 shows the migration corresponding to a 134 kilodalton marker, whereas M2 indicates the migration corresponding to a 67 kilodalton marker (not shown).

**FEATURES OF PROTEOGLYCAN INDUCED ARTHRITIS**

Figure 2 illustrates the typical appearance of arthritis in a mouse injected with native high density proteoglycans compared with the normal appearance of a mouse injected with native low density proteoglycans. The table gives the clinical signs of arthritis in mice and the temporal evolution of the disease. Eight out of 10 mice injected with high density proteoglycans had arthritis whereas none of the mice injected with low density proteoglycans or Freund's complete adjuvant alone developed the disease. This difference was highly significant (p<0.001).

Arthritis developed between the sixth and the fourteenth week after injection of the high density proteoglycans. Three arthritic mice progressed from redness and swelling (clinical score 1) to deformities (clinical score 2) in the affected limbs but no mice developed joint ankylosis. The disease in two mice was seen to enter remission with the clinical appearance of the affected limbs returning to normal after five days.

The histological assessment showed an aggressive joint disease in BALB/c mice immunised with high density proteoglycans. Extensive synovial proliferation, pannus formation, and erosive changes were present at the end of the study, and invasion of the articular cartilage by proliferative pannus at the joint margins was a consistent finding (fig 3).

In the second experiment, mice injected with core protein from either high or low density proteoglycans, or native low density proteoglycans did not develop arthritis (0/6, all groups). All animals injected with native high density proteoglycans in this experiment developed arthritis (6/6).

**ANTIBODY RESPONSE TO PROTEOGLYCAN**

Figure 4 shows the total serum antibody response to native low and high density proteoglycans in mice injected with native proteoglycans after 0, 4, 6, and 8 weeks. Strong antibody responses were observed in all immunised mice, but the highest antibody binding was seen in mice injected with native low density proteoglycans assayed against the immunising antigen (low density proteoglycans). Serum samples from mice injected with low density proteoglycans binding high density proteoglycans, and serum samples from mice injected with high density proteoglycans binding low density proteoglycans were approximately equal in antibody activity. Mice

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![Figure 1](http://ard.bmj.com/)

**Prevalence of arthritis in BALB/c mice injected with proteoglycans**

<table>
<thead>
<tr>
<th>Immungene</th>
<th>No of mice with arthritis</th>
<th>Severity of arthritis</th>
<th>Time of onset (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native HDPG</td>
<td>8/10</td>
<td>Erythema (8)</td>
<td>6–14</td>
</tr>
<tr>
<td>Native HDPG</td>
<td>0/10</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Native HDPG</td>
<td>0/6</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Native HDPG</td>
<td>0/6</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

*HDPG = high density proteoglycan; LDPG = low density proteoglycan; FCA = Freund's complete adjuvant.*
Proteoglycan induced arthritis

Proteoglycan induced pannus may be seen invading proteoglycans. Control mice injected with Freund's complete adjuvant were negative for antibodies binding to either low or high density proteoglycans. Statistical analysis of the results indicated that the level of binding of antibodies to low density proteoglycans was significantly higher than the remaining antibody binding levels (p<0.0025) which were not statistically different from each other.

The ELISA in serum samples from mice injected with native high and low density proteoglycans showed high binding to the respective core proteins, suggesting that most of the response was directed against the protein component. Consistently higher binding to native molecules was seen, however, when serum titrations were assayed against native high or low density proteoglycans, and the respective core proteins concomitantly (data not shown).

The ELISA in serum samples from mice injected with core proteins of high or low density proteoglycans showed the presence of antibodies reactive against native proteoglycans and core proteins (data not shown). Although a pattern of antibody binding similar to that seen in mice injected with the native proteoglycans was observed (core protein of low density proteoglycans induced a higher antibody response than core protein of high density proteoglycans), the difference between the response to core proteins of high and low density proteoglycans was not statistically significant.

Immunglobulin isotypes and subclasses in the antibody response to proteoglycans

Figure 5 shows the distribution of Ig classes and isotypes of antibodies to proteoglycans in mice injected with native low and high density proteoglycans. The predominant class of antibodies to proteoglycans (either low or high density proteoglycans) eight weeks after immunisation was IgG. IgM antibodies to proteoglycans were present at lower titrations and were similar in the two groups of mice. IgA antibody levels to high and low density proteoglycans were minimal. The IgG1 isotype makes up the greatest portion of the antibodies in groups of mice injected with either native fraction or core protein (data not shown). The results indicate that mice injected with native low density proteoglycans develop the highest IgG1 and total IgG antibody response. The distribution of complement fixing isotypes was equivalent in antibodies binding either high or low density proteoglycans.

Western blot analysis

Figure 6 shows the electrophoretic and western blot analysis results. Bands in tracks 9 and 10 from the electrophoresis are the silver stained low and high density proteoglycan core protein fractions respectively, corresponding to different molecular weights within the core protein.
fractions digested by chondroitin ABC lyase. Bovine serum albumin (band B1) with a molecular weight of 67 kilodaltons and migrating at the position of the lower arrow, and bovine serum albumin dimer (band B2) with a molecular weight of about 134 kilodaltons and migrating at the position of the upper arrow, were not injected into the mice, and provide a specificity control for the blot. Core proteins of low and high density proteoglycans in tracks 1 and 2 respectively were treated with pooled serum samples from the Freund’s complete adjuvant control mice. There were no bands present on either track, indicating no antibodies to low or high density proteoglycan core proteins in mice injected with Freund’s complete adjuvant. Likewise no binding activity was detected in the track with only chondroitin ABC lyase and bovine serum albumin for serum samples either injected with low or high density proteoglycans (fig 5, tracks 3 and 6 respectively).

The binding activity of serum samples from mice injected with native low or high density proteoglycans against the core protein of high density proteoglycans (fig 6, tracks 5 and 8 respectively) indicates a response to bands of molecular weight higher than 130 kilodaltons, but no difference was seen between the two groups of mice. In contrast, serum samples from mice injected with native low density proteoglycans showed binding activity to several bands of the core protein of low density proteoglycans (fig 6, track 4) which the serum samples from mice injected with native high density proteoglycans did not bind (fig 6, track 7).

RHEUMATOID FACTOR AND ANTIBODIES TO CONNECTIVE TISSUE COMPONENTS

Serum samples from all mice injected with native high and low density proteoglycans or core proteins did not contain serum IgM rheumatoid factor, or antibodies binding type II collagen, chondroitin 4-sulphate, chondroitin 6-sulphate, dermatan sulphate or hyaluronic acid. Positive antibody titres to keratan sulphate were observed in mice immunised with native high density proteoglycan (data not shown). AUTOANTIBODIES TO MOUSE PROTEOGLYCANS

Figure 7 shows antibody binding to autologous (mouse) native proteoglycans. Only mice injected with native high density proteoglycans developed autoantibodies to murine proteoglycans, and these antibodies were specific for mouse high density proteoglycans. Autoantibodies to the native molecule were not detected in mice immunised with core proteins of high density proteoglycans, or low density proteoglycans (native or core). Antibodies binding mouse low density proteoglycans were not present in any proteoglycan immunised group.

DELAYED TYPE HYPERSENSITIVITY

Figure 8 shows the delayed type hypersensitivity response to native low and high density proteoglycans. Mice injected with high density proteoglycans responded equivalently to low and high density proteoglycans, whereas mice injected with low density proteoglycans showed a higher delayed type hypersensitivity response to low density proteoglycans than to high density proteoglycans. Positive responses to high density proteoglycans were observed in a number of mice injected with Freund’s complete adjuvant alone and in the saline injected...
mice shows many similarities to rheumatoid arthritis. This study agrees with the clinical description of this model and examined immunity to proteoglycan fractions of different densities in the induction of arthritis. The size of the native high density proteoglycans and that of the core proteins from human articular cartilage found in our study are consistent with estimates previously reported for bovine articular cartilage\(^2\) and rat chondrosarcoma.\(^3\) The link proteins present in our native high density proteoglycans have bands with molecular weights that are consistent with those reported for link proteins associated with high density proteoglycan monomer from human chondrosarcoma.\(^4\) articular cartilage,\(^5\) and costal cartilage.\(^6\) The 84 to 300 kilodalton size of the DS-PGI and DS-PGII in the native low density proteoglycans from human articular cartilage in our study is in agreement with the size previously reported for bovine nasal,\(^7\) bovine articular,\(^8\) human articular,\(^9-12\) and rat cartilage low density proteoglycans. The diffuseness of the electrophoretic band is also in agreement with previous reports as the DS-PGI and DS-PGII exist as polydisperse monomers with overlapping molecular weight ranges.\(^13\) The molecular weight ranges vary with the electrophoretic conditions\(^13\) and when conditions similar to ours were used a molecular weight range of 99 to 435 kilodaltons was reported,\(^14\) in agreement with our measurements. The size of the core proteins of low density proteoglycans for human articular cartilage in our study is also consistent with that reported previously for core protein of low density proteoglycans from bovine\(^8-10\) and human\(^11-13\) cartilages.

BALB/c mice injected with native high density proteoglycans developed clinical signs of arthritis, whereas mice injected with native low density proteoglycans did not. The disease invariably began in the forepaw and progressed to affect hind limbs in most of the arthritic animals. The disease progressed from an inflammatory phase (erythema and oedema) to joint deformity in several animals and histopathological examination consistently showed a mononuclear cell infiltrate, pannus formation, and marginal cartilage erosion. Proteoglycan induced arthritis appears to be a less aggressive disease than collagen induced arthritis in susceptible mice,\(^17\) as joint ankylosis did not develop in arthritic mice after a ten week disease course.

H-\(^2\) haplotype mice are high responders to type II collagen but are resistant to the induction of collagen arthritis.\(^34\) This immunogenetic regulation and the absence of antibodies to collagen in mice immunised with high density proteoglycans precludes the involvement of an immune response to type II collagen in this model.

The analysis of the immune response identified a distinct marker for the development of disease. Autoantibodies directed against native mouse high density proteoglycans were only found in mice injected with native mouse high density proteoglycans, and this unique specificity correlated with the development of arthritis. Although mice injected with low density proteo-
glycans expressed a higher cellular and humoral response to human proteoglycans than did mice injected with low density proteoglycans, mice injected with low density proteoglycans did not develop arthritis. Similar profiles of immunoglobulin isotypes and subclasses were observed in the two groups of mice, suggesting a similar capacity to fix complement. Analysis by ELISA and western blot indicated that most of the response to native proteoglycans was directed against the core proteins, but binding to the native molecule was consistently higher than binding to the core proteins. This result suggests that antigenic epitopes exist on the native proteoglycan molecule that are not present on core protein. The western blot analysis illustrates that the mice injected with low density proteoglycans develop a more polyclonal antibody response to core protein than the mice injected with high density proteoglycans. Antibodies in mice injected with high density proteoglycans were restricted to a small number of human proteoglycan determinants, and a response to these same determinants was common to mice injected with low density proteoglycans.

Antibodies to keratan sulphate were also present in arthritic mice and experiments by Mikecz et al have suggested that keratan sulphate might contain an arthritogenic epitope.9 This would seem unlikely, however, due to absence of keratan sulphate in murine cartilage proteoglycans.35 Heparan sulphate has been reported in murine cartilage,35 thus one might postulate an unlikely cross reactivity between heparan sulphate and keratan sulphate as an alternative explanation for the antibodies to keratan sulphate. This was not true here as these antibodies failed to cross react with the heparan sulphate.

Our findings provide some contrasts to previous reports on proteoglycan induced arthritis. Mikecz et al proposed that the induction of polyarthritis in BALB/c mice was dependent on the removal of chondroitin sulphate chains which mask a hidden epitope on the core protein.9 Our data suggest that the removal of chondroitin sulphate chains results in reduced antigenic activity. As our core protein of high density proteoglycans did not induce arthritis, we propose that the arthritogenic epitope responsible for the induction of disease is dependent on the native structure of the proteoglycan molecule, resembling the requirement for native type II collagen in CIA.36 Yoo et al27 have reported that a large portion of antibodies to proteoglycans in chronic IgG induced immune synovitis in rabbits recognise a portion of the proteoglycan molecule containing the core protein and associated keratan sulphate side chains intact, and our findings support a similar situation in proteoglycan induced arthritis.

The delayed type hypersensitivity response to native proteoglycans in each group of mice gave an unexpected finding. The Freund's complete adjuvant control group developed a significant delayed type hypersensitivity response to high density proteoglycans, whereas the response to native low density proteoglycans was minimal. Although false delayed type hypersensitivity responses could be the result of water uptake by the glycosaminoglycan rich areas of the molecule, erythema and oedema were sustained for 48 hours, which is more consistent with an immune reaction. These findings are also in agreement with reports by Poole et al using chondroitinase digested core protein.37 Although we did not find antibodies to proteoglycans (either high or low density) in mice injected with Freund's complete adjuvant our results do not eliminate the possibility that the development of arthritis in the mice injected with high density proteoglycans could be related in part to the reaction to a component of Mycobacterium tuberculosis in Freund's complete adjuvant. The involvement of a cross reactive response in this model may be suggested by the studies of Van Eden et al38 and Holoshitz et al.39 T cells reactive against mycobacterial cell wall proteoglycan may cross react with the native high density human proteoglycan and contribute to the pathogenesis of this experimental arthritis model in a similar manner to cross reactive autoimmune mechanisms postulated for experimental arthritis and rheumatoid arthritis.40 41

In conclusion, our observations indicate that although BALB/c mice immunised with native molecule or core proteins from high and low density proteoglycans develop humoral and cellular immune responses, only mice injected with native high density proteoglycans develop autobody reactivity to murine proteoglycan and arthritis. Further work on the molecular target of the arthritogenic response and the possible contribution of the response to proteoglycans induced by Freund's novel complete adjuvant is underway in this novel experimental model of arthritis.

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Proteoglycan induced arthritis


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