Absence of autoimmunity to type II collagen in generalised nodal osteoarthritis

R B Clague, K Morgan, I Collins, M Patrnick, M Doherty

Abstract
A cardinal feature of generalised nodal osteoarthritis is the loss of articular cartilage. To determine if autoimmunity to these cartilage collagen occurred, serum antibodies to native and denatured type II collagen were measured by enzyme linked immunosorbent assay (ELISA) in 96 patients (90 women, six men, aged 47-91 years) with generalised nodal osteoarthritis. Generalised nodal osteoarthritis was diagnosed by typical clinical and radiological features. Serum samples from 42 blood donors were assayed as controls. No significant difference was found between the patients with generalised nodal osteoarthritis and the controls. Furthermore, the 20 patients who were HLA-A1, B8 positive had similar antibody levels to the group as a whole. One woman patient with generalised nodal osteoarthritis (HLA-A1, B8 negative) had markedly increased antibody levels to native and denatured type II collagen in a pattern similar to that seen in patients with rheumatoid arthritis. This patient did not develop super added rheumatoid arthritis during this three year follow up period. Autoimmunity to type II collagen is therefore rare in generalised nodal osteoarthritis. A lack of collagen antibodies in a condition characterised by hyaline cartilage loss suggests that the presence of such antibodies in rheumatoid arthritis is more than a secondary event to joint damage.

Generalised nodal osteoarthritis is well established as a subset of osteoarthritis and is characterised by polyarticular interphalangeal and thumb base involvement with the formation of Heberden (and Bouchard) nodes.1 Its aetio-pathogenesis is unknown, but because of its marked predominance in women, frequent perimenopausal onset, and increased incidence of the histocompatibility antigens (HLA) A1, B8, it has been suggested that an autoimmune diathesis to a normal joint component may occur.2 This is further supported by the histology of the synovium, which may resemble rheumatoid arthritis,3 and an increased prevalence of rheumatoid factor reported in one early study.4 Autoantibodies to the major cartilage collagen, type II, have been found in serum samples from some patients with rheumatoid arthritis, and may be involved in its pathogenesis.5 A cardinal feature of generalised nodal osteoarthritis is the loss of articular cartilage with the release of type II collagen. For this reason we investigated the incidence of serum autoantibodies to native and denatured type II collagen in patients with this disease.

Patients and methods
PATIENTS
Serum samples were available from 96 (90 women, six men) unrelated patients born in the United Kingdom with typical clinical and radiographic features of symptomatic generalised nodal osteoarthritis. These patients were attending the rheumatology unit at the City Hospital, Nottingham and have been described previously.2 Their ages ranged from 47 to 91 years (mean 67 years). Forty two serum samples from normal blood donors were used as controls. Aliquots of serum samples were stored at −20°C until required.

COLLAGENS
Bovine type II collagen was extracted from bovine nasal septa by pepsin digestion and was extensively purified by differential salt precipitation and dialysis against phosphate buffers.6 The lyophilised collagen was stored at −20°C until required. The collagen was checked for purity by sodium dodecyl sulphate/polyacrylamide gel electrophoresis and by immunoblotting with specific antisera. No uronic acid could be detected by the method of Bitter and Muir,7 indicating that there was no proteoglycan contamination. The collagen was dissolved at a concentration of 2 mg/ml in 0.45 M sodium chloride/0.02 M TRIS buffer (pH 7.5) before use.

DETECTION OF ANTICOLLAGEN ANTIBODIES BY ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)
Serum samples were tested at a dilution of 1/100 for anticollagen antibodies in a solid phase double antibody ELISA as previously described.6 Samples, controls, and known positive standards (patients with rheumatoid arthritis) were screened in one assay using alkaline phosphatase labelled antihuman IgGAM (Dako) as the second antibody. The results were expressed in arbitrary units.

HLA TISSUE TYING
HLA-A and B antigens were determined in 93 patients by standard lymphocytotoxic techniques.8

STATISTICAL ANALYSIS
An analysis of the results showed an underlying
Poisson like distribution. The analysis was therefore repeated using the square roots of the observed antibody levels.9 The levels of variation associated with the means were determined by
dextranformation and are represented by 99% confidence limits. The differences between the study
groups were tested using an F-ratio from a standard
analysis of variance table with the
GLIM 3·77 statistical computer program.10

Results
Table 1 shows the results of determining
antibodies to native and denatured type II
collagen in the controls and patients with
generalised nodal osteoarthritis. There was no
significant difference in the results obtained in
each of these groups (p>0·05). Table 2 shows that
there was also a lack of correlation between
the level of antibodies to native and denatured
type II collagen and the presence or absence of
HLA-A1, B8 (p>0·05) in the group of patients
with generalised nodal osteoarthritis.

One patient had levels of serum antibodies to
native type II collagen (765 units) well above the
normal upper limit (134 units). The same patient
also had more than the normal upper limit (794 units) of serum antibodies to denatured
type II collagen (1315 units). This patient also
had increased levels of antibodies to native type
V, VI, and XI collagens (minor cartilage colla-
gens) in her serum samples (data not shown).
She therefore seemed to be reacting to several
collagens found in the joint, not just to type II
collagen. None of the serum samples from
blood donors had increased levels of antibodies
to native or denatured type II collagen, nor to
the minor cartilage collagens.

The patient with generalised nodal osteo-
arthritis with increased antibody levels was
HLA-A2, B12 44, B15 positive with an α1
antitrypsin phenotype of M at a normal concen-
tration. She was negative for rheumatoid factor
with no clinical or radiographic features to
distinguish her from the other patients. This
patient had not developed superadded rheuma-
toid arthritis during a three year follow up
period.

Discussion
Only one patient out of 96 with generalised
nodal osteoarthritis had serum antibodies to
native and denatured type II collagen, which
indicates that autoimmunity to this cartilage
collagen is rare, despite the loss of articular
cartilage. This contrasts with a previous study
of 94 patients with large joint osteoarthritis, in
which 67 patients (71%) had serum antibodies
to type II collagen.11 However, that study used
the passive haemagglutination technique, which
has an inherent susceptibility to many anomalous
reactions12 and a lack of specificity for type
II collagen was seen in the results. The negative
results in generalised nodal osteoarthritis
reported here compare with an incidence of 10%
for antibodies to native type II collagen and 25%
for antibodies to denatured type II collagen in
patients with rheumatoid arthritis.6 Furthermore,
antibody levels to type II collagen in
generalised nodal osteoarthritis serum samples
were no higher in patients possessing the so-
called autoimmune genes (HLA-A1, B8). This
finding contrasts with that in rheumatoid arthri-
tis where we have previously shown that HLA-
A1, B8, DR3 is associated with higher levels of
serum antibodies to native type II collagen.13
These findings suggest that the release of native
and denatured type II collagen from degenerat-
ing articular cartilage fails to stimulate auto-
munity in generalised nodal osteoarthritis.
Thus the release of these autoantigens into the
joint is not a sufficient stimulus for the produc-
tion of autoantibodies to type II collagen, and
suggests that the presence of such autoantibodies
in rheumatoid arthritis is more than just a
secondary event to joint damage.

The one patient with generalised nodal osteo-
arthritis who had antibodies to native and
denatured type II collagen was HLA-A1, B8
negative. She also had high levels of antibodies
to the minor cartilage collagens; these antibodies
are only rarely found in patients with rheumatoid
arthritis. It is possible that her antibodies may
be directed against different epitopes on the
collagen molecules than the autoantibody found
in rheumatoid arthritis patients, and so may not
be pathogenic.14 We hope to investigate this and
will also follow her progress to determine
whether she develops rheumatoid arthritis.

The presence of serum antibodies to type II
collagen is therefore not a generalised feature in
generalised nodal osteoarthritis, although our
study has not ruled out the possible role of local
autoantibody production and fixation within
the joint. Such immune complexes have been
demonstrated in articular collagenous joint
tissues in 51% of patients with idiopathic osteo-
arthritis.15 An investigation of autoimmunity to
other cartilaginous antigens such as proteogly-
cans would be of interest.

<table>
<thead>
<tr>
<th>Table 1 Presence of serum antibodies to native and denatured type II collagen (units) in 96 patients with generalised nodal osteoarthritis and 42 controls</th>
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</thead>
<tbody>
<tr>
<td>Native type II</td>
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<tr>
<td>collagen</td>
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<td>Controls</td>
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<tr>
<td>Positive</td>
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<tr>
<td>Range</td>
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<td>Mean</td>
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*Number of sera above the 99% confidence limits of controls (native type II >134 units; denatured II>794 units).
†Range of values obtained for the serum samples in each group.

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<tr>
<th>Table 2 Range and correlation between presence or absence of HLA-A1, B8 antigens and antibodies to type II collagen (units) in patients with generalised nodal osteoarthritis. Values given are range (mean)</th>
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</thead>
<tbody>
<tr>
<td>HLA-A1, B8</td>
</tr>
<tr>
<td>positive (n=20)</td>
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<tr>
<td>Native type II collagen</td>
</tr>
<tr>
<td>Denatured type II collagen</td>
</tr>
</tbody>
</table>

*F=0·030, p=0·971.
†F=0·906, p=0·591.
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