Cellular immunity to cartilage proteoglycans: relevance to the pathogenesis of ankylosing spondylitis

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Abstract

Cellular immunity to cartilage proteoglycans may be responsible for sustaining chronic inflammation in ankylosing spondylitis. This hypothesis was examined by measuring peripheral blood and synovial fluid mononuclear cell proliferation in five preparations of human cartilage proteoglycan monomer in vitro. Peripheral blood mononuclear cells from 25 patients and synovial fluid mononuclear cells from five patients were compared with those from normal and disease control subjects matched for age. No significant differences were found between the three groups. This suggests that autoimmune responses to cartilage proteoglycans are unlikely to play a significant part in the pathogenesis of ankylosing spondylitis.

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Autoimmunity to elements of the cartilage matrix may be responsible for sustaining chronic inflammatory arthritis and has been reported for type II collagen in rheumatoid arthritis (RA).[1 2] Cellular immunity to human cartilage proteoglycans, the other major constituent of articular cartilage, is recognised in relapsing polychondritis[3] and may play a part in RA and ankylosing spondylitis.[4–7] Other studies have suggested a specific role for cartilage proteoglycans in the pathogenesis of ankylosing spondylitis and T lymphocytes specific for cartilage proteoglycans have been described in the peripheral blood of patients with ankylosing spondylitis.[8] Ankylosing spondylitis is characterised clinically by extra-articular sites such as the anterior uvea, entheses, and the aortic root being affected. Other proteoglycans immunologically related to cartilage proteoglycans occur at these sites[9] providing further support for the idea that autoimmunity to cartilage proteoglycans is important in ankylosing spondylitis. Arthritis occurs in experimental animals following the injection of cartilage proteoglycans in complete Freund's adjuvant.[10] This is of particular interest in adjuvant arthritis, a model with some clinical similarities to ankylosing spondylitis.[11] Relatively few patients with ankylosing spondylitis have been studied and there are no reports of synovial fluid mononuclear cell responses to cartilage proteoglycans. This paper reports in vitro lymphocyte proliferation to cartilage proteoglycans in patients with ankylosing spondylitis, normal controls, and control subjects with arthritis using five different preparations of cartilage proteoglycans.

Patients and methods

PATIENTS AND CONTROLS

Patients were recruited from the rheumatology outpatient clinics of Guy's and Lewisham Hospitals. Twenty five patients with definite ankylosing spondylitis as defined by the modified New York criteria[12] took part. Paired synovial fluid and peripheral blood samples were available for four patients and three control subjects with rheumatic disease: one with RA, one with psoriatic arthritis, and one with Reiter's syndrome. Peripheral blood samples from seven other control subjects with rheumatic disease were also tested (three subjects with RA, three with psoriatic arthritis, and one with osteoarthritis. Twenty five healthy subjects served as controls, six of whom were known to be HLA-B27 positive. This group was recruited with the help of the tissue typing laboratory (Guy's Hospital) and had been typed for other reasons. The table gives the clinical characteristics of the patients with ankylosing spondylitis at the time of testing.

SEPARATION OF MONONUCLEAR CELLS

Peripheral blood and synovial fluid were collected under aseptic conditions into sterile universal tubes containing 0·2 ml heparin. Mononuclear cells were isolated by density gradient centrifugation on Ficoll-Metrizoate (Lymphoprep, Nyegaard, Norway). Cells were washed three times in Hanks's balanced salt solution (HBSS) and adjusted to 2×10^6 cells/ml in tissue culture medium which consisted of RPMI 1640 (Gibco, UK) supplemented with 10% heat inactivated fetal calf serum (Serolab, UK), 2 mM glutamine, and penicillin and streptomycin at 200 U/ml.

ANTIGEN PREPARATIONS

Five preparations of the central protein core of the monomer believed to be the immunologically

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Characteristics of the 25 patients with ankylosing spondylitis studied in this work

Median (range) age (years)	46 (24–64)
Sex ratio (M/F)	22/3
Median (range) disease duration (years)	10 (1–44)
Mean (range) ESR (mm/hour)*	22 (3–50)
Patients with history of	
Peripheral arthritis	11
Iritis	6
Treatment	
NSAIDs only†	19
Disease modifying drugs	5‡

*Data from 19 patients; ESR=erythrocyte sedimentation rate.
†NSAIDs=non-steroidal anti-inflammatory drugs.
‡Two patients were receiving methotrexate, two sulphasalazine, and one corticosteroids by mouth.

important fraction of cartilage proteoglycans[13] were prepared as described previously by Heinegård and Sommarin[14] using 4 M guanidine-HCl gradients. Three samples were prepared from knee condyles, one from the patella (all A1 D1 fraction, purified in two sequential gradients, first associative then dissociative; lowest fractions), and one from tracheal cartilage (D1 D1 fraction, purified by two sequential dissociative gradients). Preparations were dissolved in HBBS, filter sterilised, and stored in aliquots at −20°C. Tuberculin purified protein derivative (PPD) (Central Veterinary Laboratory, Tunbridge Wells, UK) served as a control antigen.
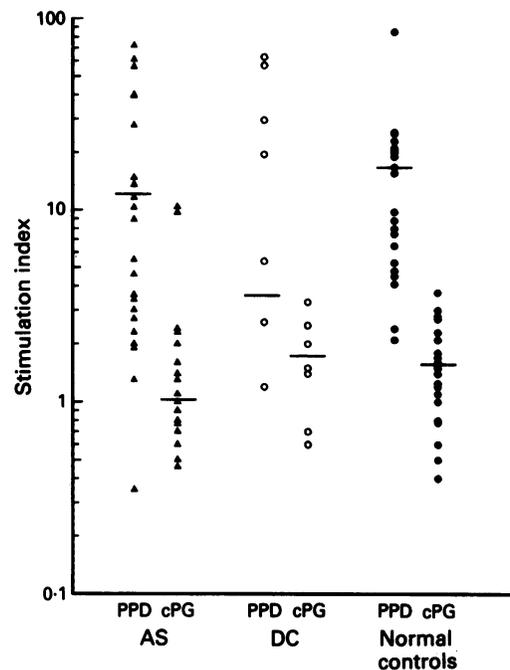

Figure 1 Stimulation indices of peripheral blood mononuclear cells to tuberculin purified protein derivative (PPD) and cartilage proteoglycan (cPG) for the three groups studied. AS=patients with ankylosing spondylitis; DC=disease controls. Horizontal bars show median values. Note the logarithmic scale; a stimulation index greater than three was considered to be significant.
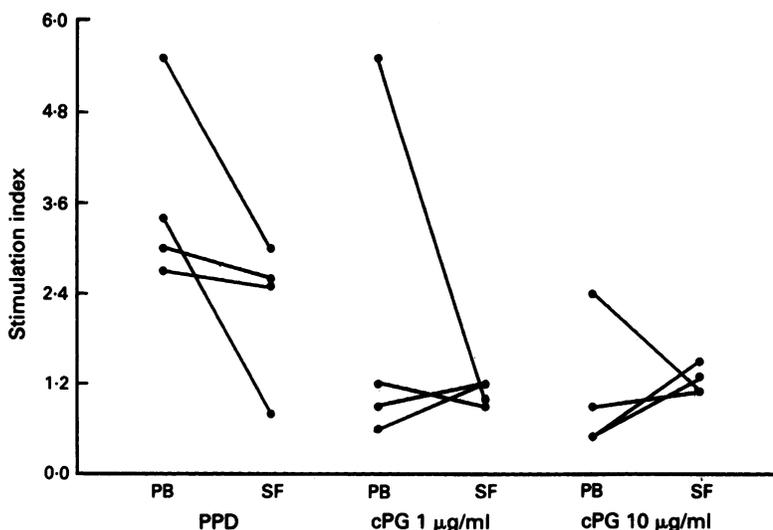

Figure 2 Paired responses of peripheral blood (PB) and synovial fluid (SF) mononuclear cells from patients with ankylosing spondylitis to tuberculin purified protein derivative (PPD) and cartilage proteoglycan (cPG) at two concentrations.

STIMULATION OF MONONUCLEAR CELLS

Mononuclear cells from patients and controls were stimulated with the core protein of cartilage proteoglycans. The concentrations of cartilage proteoglycans tested ranged from 0·2 to 20 µg/ml. Typically 2×10^5 cells/well were cultured in 96 well flat bottomed plates (Nunc, Kamstrup, Denmark). Tuberculin PPD at a concentration of 10 µg/ml established in previous experiments (data not shown) served as a positive control antigen. Cells were cultured in triplicate in a 5% carbon dioxide incubator for six days with the addition of 7·4 kBq of tritiated thymidine ([3]HTdR: Amersham International, Amersham, UK), specific activity $1·9 \times 10^8$ kBq/mmol, for the final 18–20 hours of culture. Cells were harvested on a semiautomated harvester (Skatron, Lier, Norway) and [3]HTdR incorporation measured as disintegrations per minute (dpm) by counting in a liquid scintillation counter.

STATISTICAL ANALYSIS

The results are expressed as stimulation index (SI): the ratio of radioactivity incorporated (dpm) into stimulated cells compared with that incorporated into control cells. Groups were compared by Student's t test.

Results

There was no significant difference in the ages of patients with ankylosing spondylitis (median age 46 years (range 24–67)), normal control subjects (median 37·5 years (range 24–64)), and control subjects with rheumatic disease (median 41·5 years (range 29–66)). Figure 1 shows the results of antigen stimulation with cartilage proteoglycans and tuberculin PPD in peripheral blood in all three subject groups. There was no statistical difference between the three groups in their SI to tuberculin PPD or cartilage proteoglycans. Significant proliferation occurred when cells were stimulated with tuberculin PPD in contrast to the lack of proliferation to cartilage proteoglycans for all three groups.

The peripheral blood mononuclear cells of three patients showed significant responses to cartilage proteoglycans (SI 5·5, 9·7, 10·4). Two of these patients had peripheral arthritis and these two had active disease at the time of testing. Synovial fluid was available from one of these patients but synovial fluid mononuclear cells from this patient did not proliferate in response to cartilage proteoglycans. In addition repeated testing of peripheral blood mononuclear cells in these subjects did not show a consistently increased SI (data not shown). One patient with RA from the disease control group responded to cartilage proteoglycans (SI 3·3) and two of the normal control subjects also responded to cartilage proteoglycans (SI 3·0, 3·7), one of whom was HLA-B27 positive.

Figure 2 shows the proliferation response of paired peripheral blood and synovial fluid mononuclear cells to cartilage proteoglycans and tuberculin PPD. The proliferative responses of synovial fluid mononuclear cells to tuberculin PPD were reduced compared with the response

of the corresponding peripheral blood mononuclear cells. There was no evidence of an enhanced response to cartilage proteoglycans with synovial fluid mononuclear cells. Four further extracts of cartilage proteoglycans were used as antigens in proliferation assays with peripheral blood mononuclear cells from five patients and synovial fluid mononuclear cells from one patient. Concentrations of 1–20 µg/ml were tested. No significant stimulation occurred with any preparation of cartilage proteoglycans in the presence of significant stimulation to tuberculin PPD (data not shown).

Discussion

Proteoglycans are released from cartilage early in the development of experimental models of arthritis[15] and in patients with acute forms of arthritis such as reactive arthritis.[16] The antigenic central protein core of these molecules is unlikely to have been encountered by T lymphocytes during their development and this may result in autoimmunity to cartilage proteoglycans leading to prolonged synovitis. Cross reactivity between microbial antigens and self antigens such as cartilage proteoglycans[17] may account for autoimmune responses to cartilage. It has also been suggested that as free fragments of cartilage proteoglycans within a joint are cationic and have a high molecular weight they may produce synovial changes and their persistence may lead to joint destruction independently of immune responses.[18] Cartilage proteoglycans, however, although they are released in high concentrations in acute joint inflammation and in trauma, are found in lower concentrations in chronic joint disease and are not disease specific. Investigations of cell mediated immunity to cartilage proteoglycans in inflammatory arthritis have shown a variety of responses,[4-7] though some experimental evidence has shown T lymphocytes specific for cartilage proteoglycans in patients with ankylosing spondylitis,[8] implicating such cells in the pathogenesis of ankylosing spondylitis. Our results with five preparations did not show any specific response to cartilage proteoglycans in patients with ankylosing spondylitis; in the patients showing a response this was not reproducible on repeated testing.

Proteoglycans are complex polydisperse macromolecules with a central protein core which binds a large number of negatively charged carbohydrate side chains. Cartilage proteoglycans vary with the anatomical origin and depth of cartilage and the age of the subject from whom they were extracted.[19 20] The core protein of larger cartilage proteoglycans (molecular weight 210 000) shares many antigenic sites and peptide similarities, however, though greater variation has been shown for smaller proteoglycans.[9] Monomers of cartilage proteoglycans from different sources were used as an antigen in this study and were purified and stored under similar conditions to those used by other workers. Some studies appear to have used pooled sources of cartilage proteoglycans as antigens.[5] Concentrations of cartilage proteoglycans used in studies reporting a response to

cartilage proteoglycans were higher than in our study[4]—typically 30–50 µg/ml compared with our range of 0·1–20 µg/ml, though in view of the heterogeneity of cartilage proteoglycans direct comparisons may not be valid. This heterogeneity of cartilage proteoglycans may provide an explanation for the variety of responses described in inflammatory arthritis. These factors and the ubiquitous release of cartilage proteoglycans in joint diseases makes it difficult to envisage a role for cartilage proteoglycans as autoantigens. In addition studies implicating cartilage proteoglycans in the pathogenesis of ankylosing spondylitis do not account for the genetic basis of this disease. There appear to be no other methodological differences in the cell proliferation assays performed here compared with those of other studies. Earlier studies have measured the production of leucocyte inhibitory factor as indicative of antigen responses. It has been suggested that these methods may measure different aspects of cellular immunity and may be more sensitive.[6]

In summary we have tested a large number of patients with ankylosing spondylitis for evidence of cellular immunity to purified monomers of cartilage proteoglycans using a range of preparations and have compared the responses with healthy control subjects matched for age. There was no evidence of a specific response in patients with ankylosing spondylitis. Variations in preparations of cartilage proteoglycans may account for the differing responses described in previous studies due to the heterogeneity of human cartilage proteoglycans.

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