Production of cartilage degrading activity by human synovial tissues

M F BROWN,1 B L HAZLEMAN,2 J T DINGLE,1 D J DANDY,3 AND A H G MURLEY3

From the 1Strangeways Research Laboratory, Worts Causeway, Cambridge; the 2Rheumatology Research Unit, E6 Addenbrooke’s Hospital, Cambridge; and the 3Orthopaedic Departments, Addenbrooke’s and Newmarket Hospitals

SUMMARY  Human synovial tissues have been assayed for the production of cartilage degrading activity (CDA). This activity is thought to be homologous with catabolin/interleukin 1 (IL1) produced by porcine synovium and leucocytes and by human leucocytes. The CDA of 26 rheumatoid (RA) and 41 non-rheumatoid synovia was measured on a dry weight basis. The rheumatoid synovia showed a threefold increase in activity over the non-rheumatoid, but there was no significant overall difference on a deoxyribonucleic acid (DNA) basis. The rheumatoid synovia appeared to consist of two populations; in over half the samples CDA was not related to cellularity, but eight patients had a high CDA and a high cellularity.

Key words: catabolin, interleukin 1, resorption.

Using cocultivation of porcine cartilage and synovial tissue, Fell and Jubb showed that the degradation of the cartilage was not entirely explicable on the basis of diffusion of proteolytic enzymes from the synovial tissues.1 Dingle suggested that the breakdown of the cartilage might be caused by a diffusible factor secreted by the damaged synovial tissue.2 A search for this factor, which was later named ‘catabolin’, led to the isolation3 and subsequent purification of catabolin.4 5 Catabolin acts on living chondrocytes to stimulate increased catabolic activity and to diminish the synthesis of proteoglycan and collagen. Its action is mediated by specific receptors on the chondrocyte surface; it does not have any action on dead cartilage since it has no hydrolytic activity for matrix molecules. Most of the work on the cartilage degrading activity of this factor has been carried out on the material obtained from porcine tissues, and relatively little research into the nature and properties of the human synovial analogue of porcine catabolin has been undertaken. This is largely due to the difficulties in obtaining human synovial membrane in sufficient quantities to attempt purification. Nevertheless there has been much speculation as to whether catabolin-like cartilage degrading activity (CDA) is produced in human articular tissues and involved in the cartilage erosion characteristic of arthritis.6

It seems likely that catabolin is part of the interleukin 1α series of messenger molecules.7 8 Although the precise relationship must await the completion of sequencing, the molecular weight and pI, as well as certain biological properties, would seem to indicate that there is a degree of overlap of activity sufficient to suggest homology, though whether the various activities are a single gene product seems doubtful. It is in our view unwise to extrapolate from one biological test, such as thymocyte proliferation, to the role of cytokine activity in, for example, cartilage or bone resorption. It is preferable to assay for cytokine activity in the particular biological system which is relevant to the mechanisms under study. Thus although interleukin 1 activity has been demonstrated in rheumatoid synovial fluid,9 this work does not necessarily show that CDA was being produced by synovial tissue in vivo, since synovial fluid thymocyte activity could have been produced by indigenous cells in the synovial fluid, by activation of a precursor, by changes in inhibitor levels (no inhibitors have been demonstrated for CDA measured in the cartilage bioassay), or the thymocyte activation could even be due to the presence of other cytokines. Experience of the cartilage bioassay has shown that it is free

Accepted for publication 15 October 1986.
Correspondence to Dr J T Dingle, Strangeways Research Laboratory, Worts Causeway, Cambridge CB1 4RN.
from most interfering activities, tumour necrosis factor being one of the few other messenger molecules that react in this system (Saklatvala, personal communication). We have investigated human synovial tissue for the production of CDA using short term in vitro assays in an attempt to determine whether significant activities are produced by RA and non-RA tissues.

CDA has been partially purified from human synovial tissue and shown to be similar to porcine catabolin and to IL1 produced by mononuclear leucocytes, but this study did not measure the CDA activity of tissues from a population of traumatic and arthritic synovia. The work described in this paper is the first attempt to examine the production of CDA from numbers of human synovial samples and to attempt to correlate such production with the cellularity and metabolic activity of the diseased tissue.

Materials and methods

Synovial samples from human patients suffering from rheumatoid arthritis, other non-inflammatory joint disease, or mechanical trauma were obtained at surgery—either during arthroscopy or open operation for joint replacement, synovectomy, or ligament repair. The source of tissue is shown in Table 1. Nine of the 26 rheumatic patients were receiving corticosteroid therapy at the rate of 5-0-7.5 mg prednisolone a day.

Synovial tissue was obtained from 41 patients who did not have rheumatoid arthritis: 28 at arthroscopy of the knee following trauma, 10 patients required surgery for osteoarthritis, and three patients had seronegative arthritis with involved synovitis.

The tissues were transferred immediately after surgery to Dulbecco’s modified Eagle’s minimal essential medium (DME). Within three hours samples were dissected using full sterile precautions in a class II vertical flow cabinet to give pieces of synovium free as far as possible from underlying tissue, and as near as possible of uniform size (roughly 2x2 mm). These were washed in DME and cultured in the same medium buffered for 5% CO2 with bicarbonate for 48 hours at 37°C in a 5% CO2 incubator, changing the medium after 24 h. Four to six replicate cultures were set up from each patient’s synovium.

The resulting media from the 24 h and 48 h cultures were assayed for CDA by a modification of the assay procedure of Dingle et al using smaller (2.5 mm) discs of bovine nasal cartilage and culturing in one uninterrupted period of six days. Dead cartilage was used as a control; no significant enzyme activity was found in the control medium. The results were converted by the Logit transformation of the data obtained from a porcine catabolin standard dose-response curve into equivalent concentrations of a porcine synovial catabolin standard preparation, as described by Dingle and Qi (1 ul is equivalent to 2 ng pure porcine leucocyte IL1). The same media were also assayed for glucose to allow an estimation of the overall metabolic rate of the synovial tissue.

The synovial samples were freeze dried and weighed before being digested with papain and analysed for DNA. Small pieces were removed before culture and examined histologically after staining with haematoxylin and eosin.

Results

The metabolic activity and cellularity of the rheumatoid and non-rheumatoid synovia populations are shown in Table 2. There was only sufficient tissue for 14 of the 40 non-rheumatoid (traumatic) synovia to be assayed for DNA and glucose utilisation, but

<table>
<thead>
<tr>
<th>Source</th>
<th>No of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthroscopy</td>
<td>31</td>
</tr>
<tr>
<td>Total hip arthroplasty</td>
<td>12</td>
</tr>
<tr>
<td>Metatarsal arthroplasty</td>
<td>5</td>
</tr>
<tr>
<td>Total knee arthroplasty</td>
<td>6</td>
</tr>
<tr>
<td>Shoulder arthroplasty</td>
<td>1</td>
</tr>
<tr>
<td>Ligament repair (knee)</td>
<td>3</td>
</tr>
<tr>
<td>Tendon repair (hand)</td>
<td>3</td>
</tr>
<tr>
<td>Synovectomy (knee)</td>
<td>2</td>
</tr>
<tr>
<td>Synovectomy (wrist)</td>
<td>1</td>
</tr>
<tr>
<td>Synovectomy (hand)</td>
<td>1</td>
</tr>
<tr>
<td>Synovectomy (elbow)</td>
<td>1</td>
</tr>
<tr>
<td>Wrist arthroplasty</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2  Cellularity and metabolic activity of RA and non-RA synovia

<table>
<thead>
<tr>
<th>Source</th>
<th>No of patients</th>
<th>RA</th>
<th>Non-RA</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthroscopy</td>
<td>31</td>
<td>14</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Total hip arthroplasty</td>
<td>12</td>
<td>64</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Metatarsal arthroplasty</td>
<td>5</td>
<td>DNA (µg/mg dry wt)</td>
<td>2.09 (0.36)</td>
<td>6.65 (1.43)</td>
</tr>
<tr>
<td>Total knee arthroplasty</td>
<td>6</td>
<td>Glucose used (µg/mg dry wt/24h)</td>
<td>67.6 (13.0)</td>
<td>233 (14.2)</td>
</tr>
</tbody>
</table>

*Values are mean (SEM).

Synovial tissue was obtained at surgery. The tissue was cultured for two periods of 24 h in DME at 37°C. Samples were dried and assayed for dry weight and DNA. Glucose utilisation in the synovial cultures was measured as described by Brown. The maximum glucose utilisation was 15% of the initial level (4 mg/ml).
all tissue samples from 24 rheumatoid patients examined for CDA were so assayed. It is apparent that the cellularity (as measured by DNA/mg dry wt) of the rheumatoid synovium is some threefold greater than that of the non-rheumatoid tissue and that the glucose utilisation is about fourfold higher. Measurement of the CDA production by rheumatoid and non-rheumatoid tissues is shown in Table 3.

It can be seen that on a dry weight basis there is an approximately threefold increase in CDA from rheumatoid tissue compared with non-RA samples, but on a DNA basis the mean CDA of the RA tissues does not differ from the mean of the 64 patients examined. In this part of the study we usually examined six samples of tissue from each rheumatoid tissue and three from each non-rheumatoid tissue.

Attempts to correlate the CDA production with the cell content on an individual patient basis are shown in Fig. 1, and similar comparisons between metabolic activity (glucose utilisation) and CDA production are shown in Fig. 2. As can be seen from Fig. 1 most of the non-rheumatoid population had a CDA of <1 μl std/mg dry wt; most of these tissues had a low cellularity (<1·5 μg DNA/mg dry wt). In the few tissues with a higher cellularity there was no associated increase in CDA.

The rheumatoid patients examined appeared to consist of two distinct populations. In somewhat over half the samples tested the CDA activity was between 1·5 and 2·5 μl std/mg dry wt and this was not related to the cellularity of the tissue, which ranged from 1 to 8·5 μg DNA/mg dry wt. In eight rheumatoid patients, however, the CDA was between 5·5 and 10 μl std/mg dry wt and all of these

Table 3 CDA production by RA and non-RA (traumatic) synovia

<table>
<thead>
<tr>
<th></th>
<th>Non-RA</th>
<th>RA</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients</td>
<td>40</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>CDA (μl std/mg dry wt)</td>
<td>1·60 (0·44)*</td>
<td>4·02 (0·84)</td>
<td>&lt;0·001</td>
</tr>
<tr>
<td>(n=113)</td>
<td>(n=153)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDA (μl std/μg DNA)</td>
<td>1·15 (0·31)</td>
<td>0·94 (0·10)</td>
<td>NS</td>
</tr>
<tr>
<td>(n=82)</td>
<td>(n=153)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values are mean (SEM).

Synovial tissue was cultured in DMEM (approximately 10 mg wet wt/ml medium) for 24 h. Usually three replicate samples of non-RA and four to six replicate samples of each RA synovium were set up. The medium was harvested and assayed for CDA activity using bovine nasal cartilage discs, as described in the text. Results are expressed as μl of a std catabolin preparation/mg dry wt or μl std/μg DNA.

Fig. 1 CDA and cellularity of synovial tissues. CDA was assayed as described in the text. DNA was measured by the method of Royce and Lowther.15 The figure shows the relation between CDA/mg dry wt and DNA/mg dry wt for 50 individual patients.
had a high cellularity (>4 μg DNA/mg dry wt). The difference between these two populations was statistically significant (p<0.01). There would appear to be a number of patients whose synovial tissue was highly cellular and produced significantly higher levels of CDA than was present either in patients with relatively inactive rheumatoid arthritis or in the non-rheumatoid population.

It was thought worthwhile determining whether the increased CDA was related to an increased metabolic activity of the tissues. There was no significant correlation, however, either in the non-rheumatoid or in the rheumatoid population between the metabolic activity (expressed as glucose utilised/mg dry wt) and the CDA of the synovial tissue (Fig. 2).

In addition to the above results three osteoarthritic patients were examined; their CDA production was not appreciably different from that of normal (traumatic) synovia. Three intensely proliferative synovia of non-rheumatoid aetiology were also examined. In these cases there was a high production of CDA (7.95 μl std/mg dry wt).

It has been known for some time that in vitro treatment of porcine synovial tissue with steroids depresses CDA production.\(^1\)\(^4\) We have found that in vitro treatment of rheumatoid arthritic synovia (n=12) with prednisolone (0.25 μg/ml) gave 62–68% inhibition of CDA production.

**Discussion**

The problem of assessing the biological activity of a tissue that is extremely heterogeneous in cell type and activity is very difficult. The problems of such variability were discussed as long ago as 1955 by Page Thomas and Dingle.\(^1\)\(^5\) who concluded that it was probably necessary to make differential cell counts of individual tissue samples, a technique that can only be applied on a very limited scale. Other estimates, such as dry weight, wet weight, DNA, are all, at best, partial representations of the cellular state of the pathological material.\(^1\)\(^3\) Many authors who have measured various parameters of synovial activity, e.g., production of prostaglandins and enzymes,\(^1\)\(^7\) have used wet weight, which is perhaps the least useful basis; in this particular study we have used both tissue dry weight and DNA. It is clear that on a dry weight basis rheumatoid tissues produce approximately three times the CDA of non-rheumatoid tissue, but when a DNA basis is used there is no significant difference. There is of course in the patients with RA a greatly increased synovial mass and a large increase in cell number, and one
could argue that the total production of CDA in the rheumatoid joint is therefore much greater than in the normal. This is probably less important than local changes in CDA that may occur.

In this study there are two populations of RA tissues—those in which CDA is not directly related to cell number and others where there is clearly an increased CDA and an increased cellularity of the tissue. This active cell population may represent a local stimulation of CDA production by other cytokines. It would be particularly interesting to study the CDA production in the area of the cartilage/pannus junction, but this was not possible in the present work. Future studies using specific anti-IL1 antisera will allow immunofluorescent localisation of this cytokine and will be of value in determining the cellular pattern of activation.

The well reported inhibition of CDA production in animal synovial tissue treated with steroids in vitro has been shown to occur in experiments with human synovial membrane in vitro. Such inhibition may occur in vivo. The use of steroids to suppress CDA production in vivo has not been explored to any great extent. If we can judge by in vitro work very low levels of these agents can suppress CDA production—a fact which may be of importance in preserving the integrity of articular cartilage.

Although the work reported in this paper concentrated on the catabolic effects of the human synovial tissue CDA, it must not be forgotten that recent work has shown that this catabolin/IL1 can also inhibit the synthesis of matrix components.18 19 The combined effect, i.e., the inhibition of repair processes combined with an increased catabolic activity, shows that the synovial production of this cytokine could be important in controlling the development of the degenerative lesions in arthritic disease.

References


Production of cartilage degrading activity by human synovial tissues.
M F Brown, B L Hazleman, J T Dingle, D J Dandy and A H Murley

Ann Rheum Dis 1987 46: 319-323
doi: 10.1136/ard.46.4.319

Updated information and services can be found at:
http://ard.bmj.com/content/46/4/319

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/