Immunoochemical markers of joint inflammation, skeletal damage and repair: where are we now?

Although the destruction of diarthrodial joints has long been a focus of arthritis research, we still know extremely little about the metabolism of joint tissues in vivo and how this may change in arthritis. The use of skeletal imaging, particularly radiographic analysis, and more recently, magnetic resonance imaging, has provided much insight into the consequences of the disease process, namely the destruction of cartilage in rheumatoid arthritis (RA) and osteoarthritis (OA), juxta-articular bone destruction (erosions and osteopenia) in RA, and extensive bone remodelling (sclerosis) in OA. But such analyses tell us little about the disease processes that lead to these skeletal changes.

We need to predict outcome of injury to joints that may cause degenerative changes and joint disease. We have clinical measures of joint swelling and pain but we must be able to assess joint inflammation in more quantitative ways and the accompanying cartilage and bone damage as it relates to alterations in the turnover of these tissues long before these changes are detectable by more traditional approaches. We need to know whether therapy can shut down cartilage and bone destruction, as well as controlling inflammation. If therapy is to be used to stimulate repair of these tissues which, in the case of articular cartilage, is only occasionally observed in the adult, we must have ways of detecting and following reparative events in vivo. Thus we need specific markers of synovial inflammation and of cartilage and bone synthesis and degradation. Ideally, these should be serum/plasma markers. These could have enormous benefit, if used prognostically, to help decide on appropriate therapy and in evaluating the capacity of therapy to control disease activity and promote repair.

We are moving towards a realisation of these goals as a result of ever-growing research on 'markers of skeletal metabolism', specifically joint and bone disease, but also including new markers of joint inflammation (table).

With the advent of recent new knowledge of the compositions and metabolism of these tissues, very sensitive immunoassays have been developed to detect, in body fluids, minute amounts of often tissue-specific molecules, the release of which, usually in a degraded state, can be measured. These molecular fragments reflect specific rate-limiting events such as bone or cartilage synthesis or degradation and joint inflammation (fig). Synovial inflammation in RA and OA can now be detected by the measurement of hyaluronan or hyaluronic acid (HA). This molecule, which is synthesised by many cells and tissues, is produced by synovial cells in large amounts, acting as a boundary lubricant in synovial joints. In RA, its release into the circulation is increased, possibly due to stimulation of synthesis by cytokines such as interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α). Elevated levels of serum HA in early disease are indicative of joint inflammation and the progression of erosive joint diseases. The milder, less-pronounced intra-articular inflammation of OA is also reflected by increased serum hyaluronan, which correlates with joint damage and functional capacity.

Cartilage metabolism is also extremely sensitive to cytokines, which can arrest (TNF-α, IL-1) or stimulate (TGF-β and IGF-1) synthesis of specific cartilage molecules. The chronic inflammation of RA appears to depress synthesis of the cartilage proteoglycan aggrecan systemically since levels of aggrecan degradation products are inversely related to those of acute phase proteins and TNF-α. It appears that impairment of cartilage matrix synthesis is coupled to increased degradation in the pathogenesis of RA. Destruction of articular cartilage is reflected by increased proteoglycan content in synovial fluids and may, like hyaluronan, be prognostic for disease outcome. These degradation products probably include fragments of newly synthesised proteoglycans. These can be detected by epitopes on chondroitin sulfate chains of the proteoglycan aggrecan which, in the case of epitope 846, resides on the largest and probably intact newly synthesised molecules. Their increased detection in arthritic

### Markers for Joint Metabolism

<table>
<thead>
<tr>
<th>Marker</th>
<th>Primary Indication</th>
<th>Osteoarthritis Serum</th>
<th>SF</th>
<th>Rheumatoid Arthritis Serum</th>
<th>SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synovial Hyaluronan (hyaluronic acid, hyaluronate, HA)</td>
<td>Synovitis, HA synthesis is stimulated by IL-1 and TNF-α</td>
<td>ND</td>
<td>I</td>
<td>ND</td>
<td>I</td>
</tr>
<tr>
<td>Cartilage C-propeptide of type II collagen</td>
<td>Synthesis of cartilage type II collagen</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Type II collagen C chain fragments</td>
<td>Degradation of cartilage type II collagen</td>
<td>Studies in progress</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteoglycan aggrecan: (a) keratan sulphate</td>
<td>Synthesis/degradation of aggrecan</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>(b) core protein</td>
<td>Degradation</td>
<td>ND</td>
<td>I</td>
<td>ND</td>
<td>I</td>
</tr>
<tr>
<td>(c) intact chondroitin sulphate epitopes for example, 846, 3B3</td>
<td>Probably synthesis</td>
<td>I</td>
<td>I</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Cartilage oligomeric protein (COMP)</td>
<td>Synthesis and/or degradation?</td>
<td>ND</td>
<td>I</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cartilage matrix protein</td>
<td>Synthesis and/or degradation? only present in non-articular cartilages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone Bone sialoprotein</td>
<td>Synthesis/degradation</td>
<td>Studies in progress</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>Synthesis</td>
<td>ND</td>
<td>I</td>
<td>ND</td>
<td>I</td>
</tr>
<tr>
<td>3-hydroxyproline (pyridinoline and deoxypyridinoline) cross-links</td>
<td>Degradation</td>
<td>urine only</td>
<td>urine only</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND = not determined
SF = synovial fluid
* = acute disease only
cartilages\textsuperscript{12} and very much increased contents in synovial fluids over levels in blood\textsuperscript{16} suggest that increased synthesis followed by degradation of newly synthesised molecules is a key feature of both these diseases. These markers may also be useful in detecting decreased synthesis, such as that associated with joint immobilisation.\textsuperscript{14}

Cartilage collagen synthesis is reflected by the release of the C-propeptide of type II procollagen into serum in development,\textsuperscript{15} and synovial body fluids in arthritis.\textsuperscript{16} Degradation of type II collagen can now also be measured.\textsuperscript{17} These markers are now available for studies in arthritis.

The 145 kDa cartilage matrix protein is absent from articular cartilages and therefore provides the opportunity to study selectively systemic cartilage metabolism.\textsuperscript{18} Moreover, the systemic osteoporosis of RA and the severe juxta-articular osteopenia, a consequence of enhanced bone resorption resulting from an imbalance in RA, can be measured by immunoassays\textsuperscript{19,21} or high performance liquid chromatography\textsuperscript{22} of the urinary hydroxyproline cistral collagen cross-links, derived from cartilage and bone matrix collagens. Even skeletal changes in OA are detectable by elevated cross-links in urine.\textsuperscript{23} The dominance of bone mass means that these cross-links are probably mainly derived from this tissue. New immunoassays should soon be able to permit collagen cross-link detection in blood.

In contrast, bone synthesis is reflected by the release of osteocalcin, synthesised by osteoblasts. Serum osteocalcin is hardly changed in RA,\textsuperscript{1} probably due to the suppression of synthesis by inflammation. But in OA, the extensive hypertrophy and juxta-articular bone turnover is accompanied by elevated serum osteocalcin (A R Poole, M Ionescu, A Swan and P Dieppe, manuscript in preparation).

Degeneration of cartilages, such as frequently results from post-traumatic injuries, can eventually lead to clinically-defined OA. Research at the University of Lund in Sweden, headed by Drs Dick Heinegård, Frank Wolheim, Stefan Lohmander and Tore Saxne, together with the work of Drs Eugene Thonar and Klaus Kuettner in Chicago, has pioneered many developments in this field. The early work of Dick Heinegård's group was the first to use immunoassays to study cartilage marker molecules.\textsuperscript{23} The Lund group drew attention to alterations in cartilage turnover in RA\textsuperscript{11} and following traumatic injuries to articular tissues such as cruciate ligaments and menisci.\textsuperscript{24,25}

In this issue of the journal they describe a study of the cartilage oligomeric protein (COMP),\textsuperscript{26} a member of the thrombospondin family, first identified by Fife and Brandt\textsuperscript{27} and cloned by Heinegård's group.\textsuperscript{28} Like aggrecan, this molecule is significantly elevated in synovial fluid after traumatic joint injury. The elevations of both aggrecan and COMP persist, albeit often at lower levels, and are a characteristic feature of post-traumatic OA. The reported elevations in serum aggrecan in OA,\textsuperscript{29,30} although not detected by others\textsuperscript{8,10} may also reflect differences in cartilage metabolism that predispose to OA, rather than being a consequence of disease.

COMP is interesting since its synthesis may be differently regulated to that of other cartilage molecules, such as type II collagen and aggrecan. Whereas in inflammation, cytokines such as, IL-1 and TNFα, can inhibit synthesis of cartilage aggrecan and collagens,\textsuperscript{7,8-10} COMP synthesis may be preferentially stimulated by the cytokine TGF-β (Dr A D Recklies, Joint Diseases Laboratory, Shriner's Hospital, Montreal, personal communications), which is released in increased amounts in inflammation. Moreover, and more importantly, COMP levels in serum may reflect those in synovial fluids.\textsuperscript{31} In RA, levels of COMP are elevated in patients with rapidly progressive erosive joint disease over those with much slower erosive disease.\textsuperscript{32} This may result
directly from the elevated levels of TGF-β in these inflamed joints and the lack of inhibition of synthesis by cytokines such as IL-1 and TNF-α.

The development and use of these new immunological assays to identify ‘markers’ of joint metabolism and disease is still in its infancy. They hold much promise in studying skeletal development. In arthritis research, we need to rigorously assess these markers by their use in carefully-controlled clinical trials. We must study sub-groups characterised by clearly recognisable differences in disease activity. Establishment of the molecular bases of their production, correlations with well-defined clinical parameters and with each other are essential if we are to understand their true significance and explore their value fully. Too little has so far been done in these areas. Too much emphasis has been placed in earlier work on the measurement of levels of single markers, often with little regard to clinical changes and to each other, and to the molecular mechanisms regulating their release.

But their use is already helping us to a better understanding of the pathobiology of these diseases in vivo.

The bone terminal markers have been shown to have considerable potential in the study of osteoporosis and metastasis to bone. Other markers may be of considerable benefit in identifying more appropriate therapy, especially in early disease. As in other areas of clinical research, they should help us not only evaluate the effectiveness of therapy but provide a basis for the development of new therapies, designed to control inflammation and damage to skeletal tissues, stimulate repair, and overall, help us achieve improved management of these diseases.

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