

Differential release of molecular markers in joint disease

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Cartilage and bone, the principal tissues of the diarthrodial joint, are dynamic tissues with continuous matrix turnover. These tissues, like all connective tissues, contain few cells surrounded by an abundant matrix. The cells, e.g. the chondrocytes in cartilage, regulate both synthesis and degradation of the matrix constituents in response to various environmental factors, such as hormones, nutrients, mechanical load or cytokines. Under normal conditions, the balance between matrix catabolism and anabolism is well regulated, and the tissue integrity is maintained. However, in pathological conditions, such as rheumatoid arthritis (RA), arthrosis (OA) or traumatic conditions, changes in the tissue turnover occur which disturb the balance. Thus in RA the inflammatory process induces cytokine-mediated matrix degradation of cartilage with concomitant depression of synthesis gradually leading to loss of the entire matrix (Harris 1990, Heinegård and Saxne 1991).

During the normal turnover and in increased amounts in pathological conditions, joint matrix macromolecules or fragments thereof are released into synovial fluid and may subsequently reach the blood stream and some fragments are eventually found in the urine (Heinegård and Saxne 1991). This forms the rationale for the efforts to quantify matrix macromolecules in body fluids by immunoassay to define non-invasive methods to monitor pathological tissue processes, allowing repeated measurements in the same individual. A further objective is to use these analyses for diagnostic and prognostic purposes and for monitoring effects of therapy on the tissue (Saxne and Heinegård 1995). Major advances in the understanding of changes in the tissue turnover in arthritis have been made using this novel technology and important steps have been taken towards the introduction of the methods in the clinic (Heinegård and Saxne 1991, Thonar et al. 1993, Poole and Dieppe 1994).

It is however important to realize that many, still unsolved, problems are associated with this approach to studies of tissue involvement in arthritis which has been discussed in recent reviews (Brandt 1989, Heinegård and Saxne 1991, Lohmander et al. 1992).

Differential release of tissue markers in RA and OA

A principle, which is becoming feasible is the use of quantification of a number of tissue markers in the same fluid sample to distinguish and characterize the process in the tissue and to assess the tissue damage at the molecular level. Both theoretical and experimental data form the basis for this approach to evaluating tissue involvement in RA, in particular new knowledge of the anatomical distribution in the tissue of various macromolecules. The applicability has also been substantiated by results of patient studies where several markers have been quantified in synovial fluids and sera of patients with varying progression of radiographic changes. In OA, information is emerging, which suggest that this principle is feasible also in this disease.

Theoretical and experimental rationale

The ultimate aim of the molecular marker technology is to develop methods to delineate processes in different layers of the tissue, i.e. for cartilage in superficial, middle and deep layers as well as in relation to the cells, i.e. in pericellular, territorial and interterritorial matrix (Szirmai 1968). The cartilage structure is very different in various layers or compartments and selective expression of matrix macromolecules in the superficial, middle and deep layers have been found (Aydelotte et al. 1992, Schumacher et al. 1994, Lorenzo and Heinegård, unpublished). Selective expression of certain proteins in relation to the cell has also been shown (Heinegård and Oldberg 1993). The different and specific organization of various parts of the cartilage suggests variable functions and thus the consequences of a disease process may vary considerably depending on where in the tissue it is located. Obviously different parts of the tissue may be involved at different time points in a progressive disease process, with different functional and structural consequences. It is likely that structural disturbances close to the cell may be more easily dealt with than those occurring in the interterritorial matrix

since there the distance to the cell is large and the assembly of the structural elements will be more difficult to regulate.

Selective expression of cartilage proteins have also been shown in terms of differences between cartilages. Cartilage matrix protein, CMP, is not detectable in articular cartilage but is present in extraarticular cartilages (Paulsson and Heinegård 1982). Serum CMP is thus a promising marker for non-articular cartilage involvement in disease (Saxne and Heinegård 1989).

In a given compartment the organization of the matrix is finely regulated and the cells may be capable of repairing structural damage. Thus, it has been shown that proteoglycans may be lost and replaced without influencing the long-term function of the joint (Saxne et al. 1993, Palmoski et al. 1979). In other circumstances, with more widespread structural derangement including impaired function of the collagen network, progressive joint destruction may ensue. Thus in early stages of disease, we may perhaps find one type of fragmentation of macromolecules and in later stages the pattern of fragments released into synovial fluid will change, indicating that different compartments and different levels within a compartment of the molecular organization are affected.

The theoretical rationale for sequential release of matrix macromolecules during processes of cartilage destruction has recently been corroborated by results in explant culture systems (Goldberg et al. 1995). Thus after exposure to interleukin-1, explants of bovine nasal cartilage initially lost aggrecan, subsequently the release of COMP increased and finally increased release of collagen into the medium occurred (Goldberg et al. 1995).

Experience from clinical studies in RA

In the first clinical study that explored the concept of grading of the cartilage damage at the molecular level we examined synovial fluid concentrations (and total content) of aggrecan by an immunoassay using a polyclonal antiserum recognizing epitopes along the core protein (PG), predominantly in the chondroitin sulfate rich region in patients with RA (Saxne et al. 1985). The patients had different degrees of radiographic damage of the examined knee joint and we could show that the synovial fluid content of aggrecan was high even before changes were visible radiographically. In patients with advanced cartilage damage the synovial fluid content of aggrecan was significantly lower. The explanation could be that in the latter cases the cartilage mass was reduced and consequently fewer fragments were released into synovial fluid. We have subsequently confirmed this finding in

two other separate cohorts of RA patients studied in a cross-sectional fashion (Saxne and Heinegård 1992, Saxne and Slott-Jensen unpublished). Moreover, we have also found decreasing synovial fluid aggrecan content in a longitudinal study of RA patients (Saxne et al. 1995a). Interestingly, this pattern was only seen in those patients who rapidly developed joint destructions, whereas the aggrecan levels were low and remained unchanged in the patients with slower progression. This corroborates earlier findings indicating a prognostic potential of synovial fluid aggrecan levels in RA (Saxne et al. 1987).

A primary cleavage site of aggrecan appears to be located between the G1 and G2 domain of the core protein (Sandy et al. 1991). Since the G1 domain (the hyaluronan-binding region, HABr) is bound to hyaluronan, we raised the hypothesis that the HABr is initially retained in the tissue during cartilage breakdown. Increased release of HABr into synovial fluid would then indicate more severe derangement of the matrix integrity, perhaps a stage beyond repair. We therefore examined in a cross-sectional fashion patients having RA with different degrees of joint damage as visualized radiographically by an immunoassay for the HABr. We were able to show that the synovial fluid content of HABr is highest in patients with advanced tissue damage in line with the hypothesis (Saxne and Heinegård 1992). We could also demonstrate that RA patients with no or few radiographic changes had similar synovial fluid HABr levels as patients with non-destructive reactive arthritis (Saxne and Heinegård 1992). Thus, increased release of HABr into synovial fluid may be a sign of irreversible cartilage damage.

Increased release of COMP was in a cross-sectional study seen somewhat later than release of PG (Saxne and Heinegård 1992a). The lowest synovial fluid concentrations were, however, as for PG, seen in patients with advanced joint damage. In contrast, in the longitudinal study we did not find any changes in the synovial fluid COMP levels over time and they did not differ between the groups with rapid or slow progression of joint destructions (Saxne et al. 1995a).

Bone sialoprotein (BSP), is a bone-specific protein (Heinegård and Oldberg 1993). It is enriched in the cartilage-bone interface (Hultenby et al. 1994). Synovial fluid BSP was in the cross-sectional study of RA patients increased in the patients having the most advanced radiographic changes (Saxne et al. 1995b). This indicates that in those patients the process also involved the subchondral bone, which probably is a sign of late and irreversible damage. In contrast, osteocalcin, another bone-specific molecule, did not vary according to degree of radiographic

damage (Saxne et al. 1995b). This suggests that release of osteocalcin into synovial fluid reflects a somewhat different aspect of the process in bone. Osteocalcin release may preferentially reflect bone formation, which may be suppressed in these patients, whereas BSP as measured by the currently available antiserum may also reflect degradation.

The results from the cross-sectional studies have been confirmed in a longitudinal study of patients with RA with clear-cut differences in erosiveness in hips and knees (Saxne unpublished). Thus a pattern of initially low synovial fluid concentrations of BSP which subsequently increased in the patients developing large joint destructions was found, indicating progressive involvement of the subchondral bone. The BSP levels did not vary during the 10-year observation period in the slowly progressing group.

Serum concentrations of cartilage and bone macromolecules may also be useful for delineating differences between different stages of RA and between patients with rapid and slow progressive disease. We have recently shown that patients who rapidly develop hip joint destructions necessitating joint replacement very early in their disease course show elevated serum levels of COMP (Forslind et al. 1992). In contrast, the serum levels of a chondroitin sulfate epitope of aggrecan, 846, are increased in the patients with slowly progressive disease (Månsson et al. 1995). The epitope 846 is preferentially expressed on newly synthesized molecules (Rizkalla et al. 1992, Månsson et al. 1995) and the finding is therefore compatible with an attempt at repair of damaged extracellular matrix in the patients with slowly progressive disease.

Experience from clinical studies in OA

Variable release of cartilage and bone macromolecules into synovial fluid at different stages of OA have also been found and will be discussed in the chapter by Lohmander (Lohmander et al 1992, Thonar et al. 1993).

We have also quantified matrix macromolecules in serum in an attempt to identify differential release of tissue markers into the circulation in various stages of OA. In a study of patients with established OA we were able to show that serum COMP measured at entry and after 1 year increased significantly in the patients showing progression (decreased tibiofemoral joint space or knee surgery) after 5 years, whereas serum levels remained unchanged in the patients with no progression after 5 years (Sharif et al. 1995). In a prospective study of individuals aged 35–55 years with long-standing knee pain (> 3 months) recruited by a questionnaire we found that both serum COMP

and serum BSP increased between entry and the 3-year follow-up in the individuals who had radiographic OA at follow-up, but remained unchanged in those with normal radiographs after 3 years (Petersson et al. 1995). Serum keratan sulfate (KS) has been considered a possible marker of OA (Thonar et al. 1992). Interestingly, serum KS did not differ between the groups and did not change over time in any of the groups (Petersson et al. 1995). Changes in serum COMP and perhaps BSP are promising markers for development of OA. The observations suggest early involvement of both cartilage and bone in the pathogenesis of OA.

Conclusions and perspectives for the future

Research in the molecular marker field has now moved from the initial stage of purely descriptive work to a stage of more sophisticated studies. Thus this approach is now one of the tools used to characterize the process in the tissue and to assess the tissue damage at the molecular level, with the ultimate goal to find ways to interfere with the destructive process. In parallel efforts are in progress to delineate the metabolic pathways involved in the elimination of tissue fragments. Knowledge about these pathways and other factors influencing the interpretation of measurements reviewed in this report will be essential for future applicability of the marker technology. Collaborative studies where a panel of immunoassays with different specificities are applied on the same fluid samples will be essential to further substantiate the feasibility. The third stage in the evolution of this technology, i.e. the use of marker analyses for clinical purposes, has not yet been applied, but as pointed out in this report, promising results have been obtained. A prerequisite in future work towards the introduction of this instrument in the clinic is validation against other techniques. Accordance with novel sensitive imaging techniques should strengthen the importance of both techniques. In contrast, inaccordance with older crude and insensitive techniques focused on parameters like pain may be expected since the marker technology adds a new dimension to the evaluation of tissue involvement in joint disease.

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