PATHOLOGY ONCOLOGY RESEARCH
Vol 2, No 4, 1996

Science Press Publishing Ltd. TUDOMÁNY KIADÓ

REVIEW

Aggrecan: A Target Molecule of Autoimmune Reactions

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Aggrecan in cartilage forms aggregates with hyaluronan and link protein, embedded in a collagen network. It accounts for the compressive stiffness and resilience of the hyaline cartilage. Many forms of inflammatory arthritis were shown to be accompanied with aggrecan degradation and loss from the cartilage. The loss of this major component of cartilage renders the tissue more vulnerable when exposed to abrasive forces. Therefore, aggrecan degradation may significantly contribute to cartilage destruction in arthritis. Furthermore, fragments of degraded aggrecan are released during joint inflammation. Thus, molecules of an avascular, immune-privileged tissue (hyaline cartilage) may become accessible to the cells of the immune system. Similarly, there is a "leakage" of aggrecan fragments from cartilage during aging and after joint injury, which may also lead to autosensibilisation. Autoimmune reactivity to aggrecan can be detected in human joint diseases, as well as in animal models of arthritis. The epitopes involved in these processes are currently being identified. Recent data from work with mice suggest a strong immune response focused to the N-terminal G1 domain of aggrecan that leads to arthritis and spondylitis. (Pathology Oncology Research Vol 2, No 4, 219–228, 1996)

Key words: proteoglycan, aggrecan, arthritis, spondylitis, autoimmune, cartilage

Introduction

The large aggregating proteoglycan, aggrecan, is currently considered an important target molecule of autoimmune attack in hyaline cartilage. Aggrecan might also act as a causal factor in human autoimmune joint disorders. Autoimmunity directed toward type II collagen (the other major component of cartilage matrix) has been extensively studied both in humans and in experimental models of arthritis. The relative delay in the study of proteoglycan (PG) specific autoimmunity was a consequence of several factors. One factor was the lack of availability of an appropriate technique for the isolation of intact proteoglycan monomers from cartilage until the work of Hascall and Sajdera in 1969.[48] Their method combines the use of dissociative conditions (solvent) and isopicnic density gradient centrifugation. This method is being used, with slight modifications, worldwide to prepare PG monomers. Another hampering element of studying cartilage proteoglycan is the high glycosylation of the molecule (protein content varies between 6-10% of the total molecular mass). Immune reactions to protein antigens have been more extensively studied and are far better understood. The time-lag in the development of glycoimmunology is reflected in the relative delay in understanding the precise nature of proteoglycan specific responses of the immune system. Further problems arise from the polymorphism of aggrecan: the age and site related variability in the number, size, sulfation pattern and charge density of the glycosaminoglycan side chains. There is an age-related shift from proteoglycans of high buoyant density in young tissue to proteoglycans of medium buoyant density in older tissue. This shift is accompanied by the accumulation of truncated aggrecan molecules many of which are lacking their chondroitin sulfate attachment region.[93] Furthermore, there are at least three forms of aggrecan transcripts, generated by alternative exon usage.[24] In addition, the heavy glycosylation may also interfere with amino acid sequencing. Glycosylation may protect proteolytic cleavage sites[71] complicating the study of fragments produced by enzymatic digestion of the core protein. Finally, the large molecular

Received: Aug 23, 1996, accepted: Oct 12, 1996

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mass (1-3x 10^6 D) doesn't permit the use of some simple biochemical analysis techniques, e.g. SDS PAGE.

The rapid progress of molecular biology, crosstalks with other fields of science and the work carried out in proteoglycan-immunology, however, clearly helped overcome the drawbacks and accelerate the development of this field. Many important questions remain to be answered including the role of possible cross-reactivity with bacterial peptidoglycans or with other proteoglycans. Also, the question of whether the immune response to aggrecan is causal, a consequence of cartilage destruction, or a reflection of a molecular mimicry still awaits exploration. Answering these questions may significantly contribute to a better understanding of the pathomechanism in several human joint disorders.

Molecular structure of aggrecan

Hyaline cartilage covering articular surfaces is characterized by an abundant extracellular matrix that is produced and maintained by chondrocytes. The matrix components synthesized by these relatively sparse cells are collagens, proteoglycans and non-collagenous matrix proteins. The major structural PG in hyaline cartilage is the large aggregating proteoglycan that has served as the prototype of proteoglycans.

In the past decade, it became apparent that proteoglycans are not just space-filling components of the ground substance but structurally and functionally diverse molecules produced by most eukaryotic cells. The enormous variety of proteoglycans necessitated the introduction of the specific term, *aggrecan* for the predominant PG in articular cartilage.[21] The name reflects the ability of this molecule to form macromolecular aggregates with hyaluronan and link protein by non-covalent association (*Fig.1*). Aggrecan, as any proteoglycan, is a glycoprotein containing glycosaminoglycan (earlier termed as mucopolysaccharide) covalently attached to a core protein. This proteoglycan is substituted by chondroitin sulfate and keratan sulfate in separate domains as well as N- and O-linked oligosaccharides interspersed along the protein core (Mr ~200 000). The core protein of the aggrecan has been cloned and sequenced in several species.[illegible] The large, multi-domain protein core of aggrecan contains three globular domains, G1, G2 at the N-terminus, G3 at the C-terminus, as well as extended central regions that carry the bulk of the glycosylation (~1200-1500 amino acids long). (For review see [1]).

- *G1 domain (hyaluronic acid binding domain, HABr):* it consists of 3 loops stabilized by disulfide bridges (an immunoglobulin fold A loop and proteoglycan tandem repeat B and B' loops). Both aggrecan G1 and link protein are members of the immunoglobulin superfamily,[73] and their interaction is considered to be mediated by the immunoglobulin folds. Binding to hyaluronan is considered to be mediated by the tandem repeat loops.[46] The B and B' loops are substituted with some keratan sulfate and N-linked oligosaccharides.
- *Interglobular domain (IGD):* this region is highly sensitive to enzymatic cleavage[47] and is substituted with keratan sulfate.
- *G2 domain*: contains tandem repeat loops (B, B') but does not bind hyaluronan or with link protein. It is also substituted with keratan sulfate.
- *Keratan sulfate attachment region:* in human aggrecan, it contains 11 repeats of hexameric amino acid sequence E-E-P-(S, F)-P-S[24] and carries the majority of the keratan sulfate side chains.

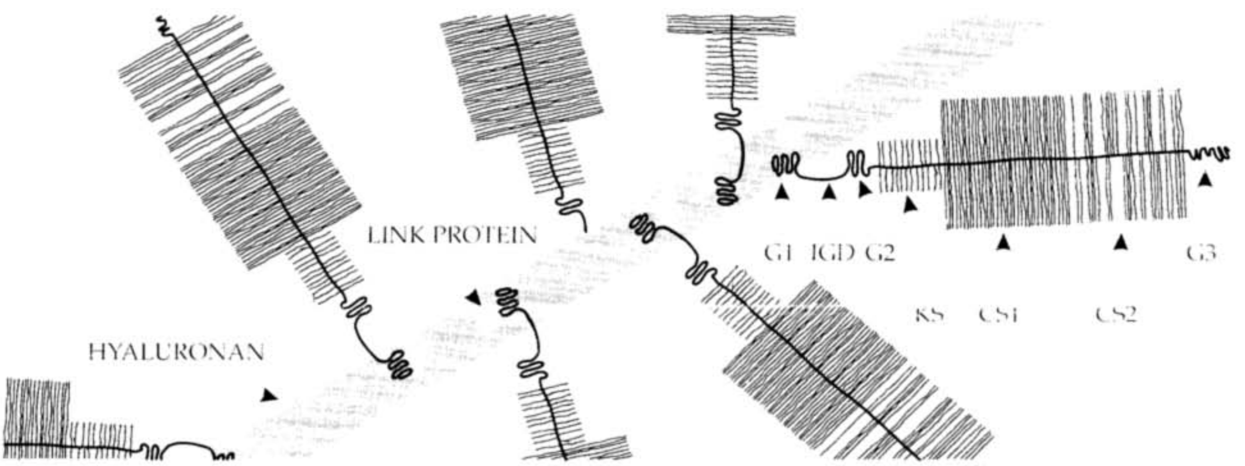

Figure 1. *Schematic representation of the proteoglycan aggregate of hyaline cartilage. The domain structure of the aggrecan monomer is indicated. KS domain is substituted with keratane sulfate side chains. CS1 and CS2 carry chondroitin sulphate glycosaminoglycans. The G1 globular domain of the aggrecan core protein is attached to both hyaluronan and link protein with non-covalent association.*

- *Chondroitin sulfate attachment region (CS):* the major glycosaminoglycan binding region, it carries ~100 chondroitin sulfate side chains. The first part (CS1) in human aggrecan contains a 19-amino acid sequence, repeated 19 times with 77 (S-G) repeats. Chondroitin sulfate side chains linked to serine are relatively evenly distributed in CS1, separated by the 19 amino acid long "spacer sequences". In the second part (CS2), the chondroitin side chains are found in clusters.
- *G3 domain:* in the case of human aggrecan, two exons in the G3 are alternatively spliced. The encoded epidermal growth factor (EGF) and complement regulatory protein (CRP)-like domains predominate in fetal and young aggrecan. A lectin-like domain is located in between the latter two domains. It is homologous with type C animal lectins and was shown to bind fucose and galactose.[45]

Aggrecan-induced autoimmune arthritis of BALB/c mice

Hyperimmunization of BALB/c mice with heterologous, partially deglycosylated aggrecan induces a chronic, progressive polyarthritis of joints of the limbs and the spine.[36,68] The clinical, histological and radiological symptoms highly resemble human rheumatoid arthritis and ankylosing spondylitis. The incidence is around 100% when PG is injected into adult female BALB/c mice intraperitoneally in an emulsion with Complete Freund Adjuvant (CFA) or Incomplete Freund Adjuvant (IFA).

The initial external symptoms of arthritis appear after the third or fourth intraperitoneal injection of aggrecan, depending on the BALB/c colony used for immunization. The arthritis starts as an acute polyarticular synovitis and includes spondylitis. During the early phases, perivascular accumulation of mononuclear cells and polymorphonuclear cells can be seen. After the primary inflammation, remissions and spontaneous exacerbations alternate. These lead to cartilage and bone erosion within the joint, under a villous pannus. Stiffness and gross deformities develop (e.g. radial deviation in the wrist joint), as well as ankylosis of the spine and resorption of growth plates and intervertebral discs.

The arthritis starts most frequently in the joints of the hind limbs. With time the other limbs and the axial skeleton become involved. Lumbar spine and proximal intervertebral disks of the tail are most frequently exposed to inflammatory and degenerative changes. In the case of the spine, the inflammatory process starts adjacent to the intervertebral disk and may involve periostitis, tendonitis and myositis. This is followed by the invasion of the disk by inflammatory cells. About 50% of sacroiliac joints are also involved. Other cartilages, such as those in the ribs, ears, or the respiratory tract, are never exposed to the inflammatory process. The only exception is the growth plate, which is often eroded. However, slight tendonitis and nodular lesions on periarticular areas, skin rashes, and transient diarrhea are common.[37] The development of the disease is dependent on the expression of both cellular and antibody mediated autoimmune reactivities to mouse aggrecan.[5,68] Abnormalities detected by functional tests (such as strength of grip, the maintaince of posture on different surfaces at different tilt angles and gait analysis) could predate by weeks the appearance of the first clinical symptoms and precede the appearance of autoantibodies in serum.[68]

Mouse aggrecan specific autoantibodies can be detected from the fifth to sixth week of immunization, whereas antibodies against immunizing human fetal aggrecan appear during the second week of immunization.[68] Titer of these antibodies reaches a maximum level at 8 to 12 weeks of immunization and then slowly decreases as joint damage develops.[40] Autoantibodies were found cytotoxic to both human and mouse chondrocytes in the presence of complement.[36,68] A small population of autoantibodies disappeared from the circulation of arthritic animals, but could be retrieved by low pH elution from articular tissues. This finding suggests that the cartilage undergoing degradation may bind autoantibodies. Joint-eluted autoantibodies were at least 100 times more cytotoxic on murine chondrocytes than those retained in the circulation. Antibodies to collagen type II were detected in 25% of arthritic animals, but only in advanced arthritis. Occasionally, rheumatoid factor (either IgM or IgG or both) could be detected in the arthritic mice. Also immune deposits were detected in kidneys, mainly in rheumatoid factor positive animals.

PG-induced arthritis could be transferred to irradiated, naive BALB/c mice with mononuclear cells.[67] However, successful transfer required the injection of lymphocytes stimulated *in vitro* or *in vivo* with either human fetal or mouse aggrecan. IL-2 or immune sera from animals with arthritis significantly reduced the time of onset of transferred arthritis. The transfer was blocked by pretreatment of donor lymphocytes with either anti T cell or anti B cell antibodies and complement.[67] Migration of antigen-specific T cells to the synovium seems to be the initial essential step in the transferred arthritis.[69]

PG arthritis is associated with an increased IL-1 production by synovial cells. Another inflammatory cytokine, IL-2 was found to be secreted in a higher amount by T cells from arthritic animals than those obtained from animals immunized with non-arthritogenic PG, such as PGs isolated from rat chondrosarcoma or fetal bovine cartilage.[5] Monoclonal antibody (BE 626, IgM), when injected intravenously into mice, induced a transient synovitis without cartilage destruction (unpublished data). Other aggrecan specific monoclonal antibodies were shown to cause depletion of cartilage proteoglycan with little or no synovitis.[20]

T lymphocytes play key roles in the pathological mechanism of aggrecan induced arthritis. This is supported by several observations.

- The susceptibility to arthritis is associated with certain MHC genes.
- There is an increased IL2/IL4 production of spleen and especially joint draining lymph node cells upon *in vitro* stimulation with "arthritogenic" PGs.[8]
- There is a PG-specific selective proliferation of $CD4^+$ cells during the development of arthritis.[8]
- Prevention of arthritis is achieved after *in vivo* treatment with anti CD4 antibody.[2]
- The transfer of the disease requires the presence of T cells from arthritic animals.
- The failure to induce PG-arthritis in homozygous BALB/c nude mice also argues in favour of the significant role of T cells in PG-arthritis (unpublished data).
- Recently, we have shown that an aggrecan-specific T cell hybridoma clone was capable of inducing arthritic symptoms in BALB/c mice; providing direct evidence that PG-specific T helper cells may play a crucial role in autoimmune arthritic processes. In this T hybridoma-transferred arthritis, a massive swelling and redness of the paws were the leading clinical symptoms. The histological features include a reactive synovial cell proliferation and the accumulation of hybridoma and inflammatory cells in the joint space, the loss of PG from the superficial layer of the articular cartilage and cartilage erosion.[9]

Genetic predisposition to the disease is determined by MHC and non-MHC genes.[1] Although cartilage aggrecans from various species have many biochemical and immunological similarities, only a select group of proteoglycans from fetal and newborn human, fetal pig and canine articular cartilages, human osteophytes and human chondrosarcomas were able to induce arthritis in BALB/c mice. The human fetal aggrecan monomer used for immunization has to be enzymatically depleted in chondroitin sulfate side chains. Human adult aggrecan was also found to induce arthritis after the removal of both chondroitin sulfate and keratan sulfate side chains (TT Glant, manuscript submitted).

Recently, an anti-CD44 antibody was shown to have a therapeutic effect upon joint inflammation in both aggrecan and type II collagen induced arthritis.[63] Such approaches in animal models may provide valuable experimental systems to develop new human therapeutic strategies.

Epitopes of the aggrecan recognized by antibodies

The antigenicity of aggrecan was proved by landmark studies in the 1960s.[22,58,96] In the next decades, many papers were published describing immunoreactivity to cartilage proteoglycans. The development of monoclonal antibody technology has opened new vistas in proteoglycan research and enabled investigators to isolate antibodies with monospecifity to certain determinants of the aggrecan molecule.

The disaccharide unit (N-acetyl glucosamine and galactose) is a common structure in mammalian glycoproteins and proteoglycans. However, immunization of mice with aggrecan induces a strong response to keratan sulfate. Several monoclonal antibodies have been raised to keratan sulfate (single or clustered keratan sulfate chains in which the sulfation pattern may be different) e.g. 5-D-4,[62] AN9P1,[99] EFG11.[74] These monoclonal antibodies have been widely used to detect or measure keratan sulfate in tissues and to study aggrecan structure and metabolism.[13,88,89,99] Chondroitin sulfate was considered to be non-immunogenic until the isolation of hybridomas which secrete monoclonal antibodies to chondroitin 4 sulfate or chondroitin 6 sulfate stubs attached to the core protein of aggrecan.[12,13,74] The pretreatment of chondroitin sulfate with chondroitinase ABC, chondroitinase AC, or testicular hyaluronidase in most cases was essential for the antibody binding. An especially strong humoral immune response can be provoked by the unsaturated glucuronic residues created by chondroitinase ABC digestion.[16]

Monoclonal antibodies were also isolated to epitopes of native chondroitin sulfate (e.g. 3B3, 7D4, 6C3) although many of these epitopes proved to contain oversulfated or unusual disaccharides in the chondroitin sulfate chain.[14,87,100] Core protein epitope-specific monoclonal antibodies were also isolated. Some of them (e.g. 1C6, 2A5, 5-C4, 8C1) recognize epitopes in the G1 domain of the aggrecan[13] and some may cross-react with the homologous link protein.[11] Monoclonal antibodies recognizing epitopes in the chondroitin sulfate attachment region have also been isolated.[25]

Recently, a new generation of polyclonal and monoclonal antibodies has been raised against aggrecan. These antibodies recognize neoepitopes created by "proteoglycan degrading enzymes, like aggrecanase, or stromelysin.[3,54] Monoclonal antibody BC3 recognizes the new N-terminus (*ARGSV...*) produced by the action of the yet uncharacterized "aggrecanase".[51] Antibody BC4 recognizes the new C-terminus (*...DIPEN*).[51] Antibody AF28 reacts with a neoepitope on polypeptides with FFGVG sequences of G2, generated by the digestion of G1-G2 substrate by stromelysin, collagenase, gelatinase, matrilysin.[29] These antibodies may provide valuable tools for identifying enzymes involved in the pathomechanism of cartilage aggrecan degradation. Such a cleavage-site specific antibody was successfully used to demonstrate the matrix metalloproteinase-dependent catabolism of aggrecan at sites of chondrolysis in collagen-induced and in proteoglycan-induced arthritis.[86]

T cell epitopes of aggrecan

The epitope recognized by the arthritogenic 5/4E8 Th1 hybridoma clone maps to the *G1* of human aggrecan.[9] Furthermore, two T cell hybridomas, isolated by Leroux,[55,56] react with two different epitopes also located within the G1 domain of aggrecan. All three T cell hybridoma clones were isolated from mice immunized with partially deglycosilated aggrecan. Their epitopes are recognized after natural processing and presentation of aggrecan by various antigen presenting cells.

In several proteins, repeat sequences were shown to possess important antigenic properties. On this basis, Goodacre investigated the immunogenic features of the multiple repeat sequence of CS1. Two, partially overlapping cryptic T cell epitopes were identified (*EVLETAAPGVED* and *GVEDISGLPSG*).[43] They are not recognized by T cells upon immunization with the aggrecan monomer, suggesting that fragments containing these epitopes are not formed by natural processing. However, extracellular degradation of the aggrecan prior to antigen processing might lead to the recognition of these cryptic structures.

Recently, T cell reactivity to pooled synthetic peptides spanning the chondroitin sulfate domain of aggrecan was demonstrated in patients with rheumatoid arthritis.[44] These data suggest that human autoantigenic T cell epitope(s) may lie within the chondroitin sulfate domain of aggrecan. Important data are provided by studies on enzymatic cleavage sites within the core protein[27,53,59,60,78,79] and may help in locating relevant T cell recognition sites within the long protein core of aggrecan.

Arthritogenic epitope(s) in aggrecan induced murine arthritis

The peptide nature of arthritogenic epitope(s) of aggrecan is supported by several observations.

- The arthritogenic epitopes are protease sensitive, as human fetal and mouse aggrecans loose their capacity to induce arthritis after proteolytic cleavages with trypsin, papain, pronase or cyanogen bromide. However the arthritogenic structure is resistant to clostripain and stromelysin cleavages.[40]
- Isolated glycosaminoglycans react neither with autoreactive monoclonal antibodies nor autoantibodies from arthritic animals[18,35,40] and do not stimulate aggrecan specific T cells isolated from arthritic mice.[40,56,67]
- Cleavage of aggrecan with testicular hyaluronidase, chondroitinase ABC, chondroitinase AC, Hg-Acetate and ß-galactosidase results in the formation of different chondroitin sulfate stubs.[10,97] However, these glycolytic treatments do not alter arthritogenicity of the aggrecan (unpublished observation).
- Chondroitin sulfate side chains have to be removed from the core protein in order to reveal arthritogenic capacity of the human fetal aggrecan.[36,68] Human adult aggrecan contains large amounts of keratan sulfate[89] and needs further deglycosylation with endo-ß-galactosidase to achieve an "arthritogenic" character.[38]

The aggrecan specific T helper hybridoma clone, 5/4E8, recognizes a peptide sequence in the G1 of the human aggrecan and shows a joint specific cell migration when injected to irradiated naive mice.[9] Aggrecan specific B cells,[6] interferon-γ stimulated synovial cells and chondrocytes were shown to present antigen very effectively to this hybridoma clone.[5] Removal of the keratan sulfate side chains from the native aggrecan with keratanase I markedly increased recognition of the PG by this hybridoma.[7] The epitope structure recognized by the 5/4E8 hybridoma clone was identified (manuscript in preparation). The capacity of this epitope to induce arthritis is currently being investigated.

In a recent study, after the removal of keratan sulfate, the G1 domain of aggrecan was shown to induce erosive polyarthritis and spondylitis in BALB/c mice.[55] These data provide evidence for the presence of arthritogenic epitope(s) within the N terminal G1 domain of aggrecan. Whether this is the only region capable of inducing arthritis remains to be explored.

Data from a study using overlapping recombinant proteins seems to counterproof the assumption that autoantigenicity is restricted to the G1 domain. In this work, short sequences, predominantly in the chondroitin sulfate-attachment region of the mouse aggrecan, were shown to induce T cell proliferation.[61] This finding raises the possibility that the chondroitin sulfate attachment region of aggrecan may also contain auto/arthritogenic epitopes.

These works had the essential goal of mapping antigenic and/or arthritogenic segments of the core protein of aggrecan. They raise many important questions. It is not clear what role the keratan sulfate and chondroitin sulfate chains play in the uptake, processing and presentation of aggrecan by antigen presenting cells. Are the epitopes masked by steric hindrance, or are they recognized as new determinants after the modification of the tertiary structure of the core protein by removal of the negatively charged glycosaminoglycan side chains? The indisputable importance of glycosilation in determining the arthritogenicity of aggrecan urges further studies to resolve these questions.

Immune responses to aggrecan in human rheumatoid joint diseases

Autoimmune reactivity to aggrecan was detected in three human diseases including rheumatoid arthritis, ankylosing spondylitis and relapsing polychondritis. Ankylosing spondylitis is characterized by the most prominent aggrecan-specific reactivity. Cellular responses to aggrecan have been detected in >85% of the patients with ankylosing spondylitis.[34,42,90] Moreover, T-helper cell

lines and clones, specific to aggrecan, have been isolated from the peripheral blood of patients with ankylosing spondylitis.[65]

Several reports described aggrecan specific cellular immune responses in patients with rheumatoid arthritis[34,41,42,85] and in juvenile rheumatoid arthritis.[34] As mentioned above, in a recent work, pooled synthetic peptides spanning the chondroitin sulfate attachment region were used. In a portion of rheumatoid arthritic patients, such peptides could elicit a T cell proliferative response.[44] In rheumatoid synovial fluid samples, anti-proteoglycan antibodies were detected only if they were dissociated from immune complexes.[35] All positive (6/197) synovial fluids were collected from patients with definite rheumatoid arthritis 1-3 weeks following an acute exacerbation of the disease. These autoantibodies reacted with different epitopes of the core protein. Antibody reaction usually required the depletion of chondroitin sulfate side chains, indicating that their epitope(s) was located on the chondroitin sulfate attachment region. However antibodies in two synovial fluid samples reacted with intact aggrecan as well. Aggrecan specific autoantibodies were detected in sera of patients with rheumatoid arthritis in 11 out of 29 cases. [34] These autoimmune responses do not necessarily identify aggrecan as a primary autoantigen in rheumatoid arthritis. These reactions could also be consequences of secondary immune responses raised against fragments of aggrecan released by local inflammatory processes.

Relapsing polychondritis is a rare disorder and its etiology is unknown. Most of the patients with relapsing polychondritis have a preceding or coexistent rheumatic or autoimmune disorder. These patients have cellular or humoral immune reactions to collagen or aggrecan.[26,28,50,75]

Concluding remarks

Aggrecan is one of the major structural elements of hyaline cartilage. Several components of the hyaline cartilage were shown to be capable of inducing an organ specific experimental autoimmune disease (arthritis) in genetically susceptible animal strains. Some of these arthritogenic matrix components are major constituents of cartilage, like type II collagen[10,17,91] or aggrecan, whereas others are present in cartilage in a significantly smaller amount, such as minor collagens (types IX and XI).[4,70] Data from human studies, as well as from work with murine aggrecan induced arthritis, strongly suggest that autoimmune recognition of aggrecan may be a significant pathway which may lead or contribute to cartilage destruction. Interestingly, supportive data for the importance of aggrecan-specific (auto)reactivity are accumulating from other experimental systems, too.

Adjuvant arthritis is one of the classical, most frequently studied experimental models of inflammatory arthritis. It can be established in susceptible rats with intradermal injection of Mycobacterium tuberculosis.[72] Recently, it was suggested that immunity to cartilage aggrecan plays a major role in the pathogenesis of adjuvant induced arthritis, and can induce specific tolerance to this type of arthritis.[77] Adjuvant arthritis is not the only system in which the (auto)immunogenic potential of aggrecan was demonstrated under conditions where aggrecan was not utilized as the arthritogenic agent. Epitopes of aggrecan elicit an anti-proteoglycan antibody response in chronic IgG-induced synovitis of rabbits. A relatively large proportion of these antibodies recognize a portion of aggrecan containing core protein and associated keratan sulfate.[101]

In type II collagen induced arthritis, a 50% loss of chondroitin sulfate and keratan sulfate from the articular cartilage matrix was revealed.[21] However, aggrecan specific autoimmune reactivity in type II collagen induced arthritis has not yet been reported.

Proteolytic cleavage of the interglobular domain of aggrecan was shown to be a key event in normal and IL-1 stimulated aggrecan turnover.[30,33,49,52,69,80,81] Further cleavage sites were identified within the chondroitin sulfate domain.[60] Furthermore, based on rotary shadowing electron microscopy, only 10-50% of aggrecan in human cartilage extracts have an intact G3 domain.[47] The G1 domain, resistant to proteinases, thermal denaturation and exposure to solvents[47] may retain hyaluronan-binding capacity and accumulate in cartilage.[6,93]

In humans, some data indicate the release of C-terminal aggrecan fragments of various length into the synovial fluid. Quantification of the released chondroitin sulfate rich region of aggrecan in synovial fluid provides a tool to predict future joint destruction in early rheumatoid arthritis[82] or to monitor the effects of therapy on cartilage metabolism.[84] Synovial fluid concentrations of the glycosaminoglycan-attachment region were highest in rheumatoid arthritis patients who had little cartilage damage. On the contrary, the release of the G1 domain predominated in patients with advanced cartilage destruction.[83]

The increased loss of aggrecan from cartilage is not only observed in inflammatory arthritis (e.g. rheumatoid arthritis), but also in post-traumatic conditions. Knee injured patients had twice the average concentrations of aggrecan fragments in joint fluid as healthy volunteers.[19] The physiological (age-related) and pathological release of aggrecan fragments raises the possibility that such fragments might be encountered by elements of the immune system and thus, may elicit an autoimmune response. G1 domain retained in the cartilage may serve as a fixed target directing the immune response to the cartilage. The G2 domain of aggrecan and the link protein carry highly similar structural motifs with the G1 domain and thus may participate in a cross-reactive autoimmune response to G1.

In the murine model, immunization with human aggrecan elicits an immune response to epitope(s) of the immunizing molecule. The T cell reactivity to epitope(s) of

the human PG (recognized either in the primary response or after intramolecular determinant spreading) may, in turn, lead to the recognition of homologous epitope(s) of the self (mouse) aggrecan. This self epitope(s) is presumably cryptic and avoids tolerance induction. However, it could be produced as a result of upregulated self antigen presentation (possibly by the involvement of cells with different processing mechanisms). In the aggrecan-induced murine arthritis, the G1 domain clearly proved to carry an arthritogenic structure.

In humans, the initial priming may occur at the time of a microbial infection. Recognition of epitopes of pathogens structurally related to those of the PG could possibly lead to the presentation of cryptic determinants of the self aggrecan and, as a consequence, result in aggrecan specific autoreactivity. The mechanism by which self antigen presentation is induced still needs to be elucidated. This process is probably the key event in the development of the organ specific autoreactivity. In humans, the biological significance of aggrecan directed autoimmunity is currently being explored. So far, it is not clear which part(s) of the molecule may be involved in the pathomechanism of human joint disorders. However, the growing intensity of work in this field suggests that the answer will not be long awaited.

Acknowledgement

The authors wish to thank Dr. József Dobai for the artwork.

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