Rheumatoid arthritis and ankylosing spondylitis – pathology of acute inflammation

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ABSTRACT

Histomorphological analysis of inflammatory lesions in rheumatoid arthritis (RA) and ankylosing spondylitis (AS) display similarities but also major differences. Ankylosing spondylitis is characterised by two key pathological findings: sacroiliac joint and spinal inflammation and new bone formation with the possible consequence of bone fusion, usually in the axial skeleton. In AS the primary site of inflammation is located at the enthesis or subchondral bone marrow with bone marrow oedema, lymphocytic infiltrates, increased osteoclast density and increased microvessel density are typical findings in acute inflammation. In RA joint inflammation has its origin in the synovial membrane of peripheral joints. Osteitis in the subchondral bone marrow reveals similar findings compared to AS and it is suggested to occur secondary to inflammation in the synovial membrane. Structural damage defines the outcome in both diseases. However, in AS it is defined by new bone formation and in RA by the destruction of cortical bone.

Introduction

RA and SpA are the two most common forms of inflammatory arthritis. Chronic inflammation, both systemically and affecting joints, is the most important clinical feature found in the two conditions. Accordingly, the use of non specific anti-inflammatory drugs has provided important clinical results. At the same time, the two conditions are different regarding a number of key features: localization of the disease; genetic background; auto antibodies; consequence of inflammation on joint structure ranging from bone and cartilage destruction in RA to new bone formation in SpA.

RA is a chronic inflammatory joint disease affecting peripheral joints in a symmetrical fashion, but not affecting the thoracic and lumbar spine (1). The presence of highly disease specific anti-CCP antibodies is characteristic of a large subset of RA, where an association with the HLA-DR 1 and DR 4 subtypes is found (2). The consequence of chronic inflammation of the synovium is the release of proinflammatory cytokines and the activation of proteases (3). The net result is the destruction of bone and cartilage combined with the complete inhibition of any repair activity.

The spondyloarthrtides (SpA) comprise ankylosing spondylitis (AS), reactive arthritis (ReA), arthritis/spondylitis with inflammatory bowel disease and arthritis/spondylitis with psoriasis. The main link between each other is the association with HLA-B27, the same clinical symptoms such as inflammatory back pain, a similar pattern of peripheral joint involvement with an asymmetrical arthritis predominantly of the lower limbs, and the possible occurrence of sacroiliitis, spondylitis, enthesitis and uveitis (4). The SpA can also be split into SpA with predominant axial and into SpA with predominant peripheral involvement, both forms overlap in about 20-30% of the cases. The disease starts in more than 90% of patients with a sacroiliitis. Further in the course of the disease the whole spine can be affected by spondylitis, spondylodiscitis and arthritis of the small intervertebral joints. Magnetic resonance imaging (MRI)-technique has been a major progress for an earlier diagnosis of AS, and for the detection and monitoring of inflammatory activity (5). It is suggested that new bone formation with subsequent ankylosis occurs as a reaction to inflammation which can affect the entire spine. Structural damage such as erosive changes and new bone formation of SI-joints and spine are normally monitored by x-rays (6).

Histomorphology in AS and RA

RA is characterized by inflammation of the synovium of peripheral joints (3).

Although the reason remains unclear, immune cells migrate from blood to the synovium and accumulate around vessels. These mononuclear cells represent all the cells involved in cell mediated immunity, including monocytes, T cells, B cells, dendritic cells (7). A similar picture is found in juxta-articular bone. From the resulting cell interactions with resident synoviocytes, monocyte derived cytokines such as TNF and IL-6 are produced (8). Such production is further enhanced by the secretion of T cell derived factors, among which IL-17 has been the recent focus (9). This inflammatory milieu induces activation and formation of osteoclasts leading to bone destruction and at the same time new bone formation by osteoblasts is completely inhibited (10). Accordingly, RA is defined as an inflammatory disease characterized by a profound lack of bone and cartilage repair activity. Recent studies have also focussed on the histopathology in bone samples from RA patients. Hereby, it could be shown that beside osteoclasts, infiltration by lymphocytes and macrophages and a high microvessel density were predominant in inflammatory lesions (11, 12).

Ankylosing spondylitis (AS) is characterised by two key pathological findings: acute inflammation and new bone formation in sacroiliac joint and in the spine with the possible consequence of bone fusion, most frequently found in the axial skeleton. Both inflammation induced pain and stiffness and new bone formation, possibly resulting in ankylosis of vertebral segments, cause and contribute to functional disability in AS patients.

A detailed investigation of the immunopathology in AS at the site of inflammation was of importance to get more insights into the pathogenesis of AS. There is rising evidence that, in contrast to RA, the synovium is not the primary site of inflammation, as discussed recently in great detail (13, 14, 15). It was suggested that all of the immunopathology could be explained by an enthesitis, an inflammation at the insertion site of tendons, ligaments or capsules to the bone, which are composed out of fibrocartilage. Currently, it is discussed whether all aspects of the clinical and pathological picture can be explained by this (13, 14, 15), or whether enthesitis is only part of a more general picture. Based on a review of the available literature including histopathological analysis and data from MRIs Maksymowych has presented evidence (15), that the primary site of inflammation occurs at the interface of bone and cartilage including, but not exclusively, the enthesis. In support of this, there are histopathological studies - for example from intervertebral discs (16), femoral heads (17), sacroiliac joint (18, 19), and manubriosternal junction (20) – suggesting that a subchondral inflammation at the interface between bone and cartilage – a subchondral osteitis - could be the primary site of the AS immunopathology, even without involvement of an enthesis. Among these, an interesting publication from Bywaters and Olsen back in 1968 reported on a post mortem case of a 21-year-old AS patient with hip arthritis (17). Radiography of the hip showed central erosions of the femoral head and acetabulum and a histopathological analysis described granulation tissue penetrating the cartilage of both acetabulum and femur head from the subchondral bone marrow site.

Immunohistochemistry in AS and RA

Osteitis in peripheral joints

In RA recent studies have also focused on immunohistochemical analysis of bone specimens. Hereby, bone erosions with cortical bone penetration was associated with lymphocytic infiltrates, increased vascularity and bone marrow oedema (7, 21). It is believed that bone marrow oedema in affected joints of RA patients occurs secondary to primary inflammation in the synovial membrane (Fig. 1).

More recent immunohistochemical analyses made several interesting and important findings, different from RA, by analysing acute inflammatory lesions of subchondral areas in femoral heads of AS patients (11, 22). Enthesial sites of femoral heads and knees from AS patients undergoing endoprothetic surgery displayed inflammation driven by mononuclear cells (11). In the subchondral bone marrow and at the bone cartilage interface of femoral heads of AS patients the number of subchondral T cells was significantly higher in areas which had still cartilage on the surface compared to areas without cartilage, while, in contrast, such a difference was not found in RA patients (11). Angiogenesis as measured by microvessel density was significantly increased in AS patients compared to RA and OA. The number of osteoclastic foci in areas of bone resorption at the bone cartilage interface of femoral heads with cartilage on the surface was also significantly higher in AS compared to RA and OA (11). Thus, a very important conclusion from this study was that inflammation is linked to the presence of cartilage and that inflammation seems to disappear once cartilage is destroyed. This concept is further supported by the use of MRI for the investigation of acute inflammation in AS. Several studies consistently reported subchondral bone marrow oedema as a consequence of inflammation at different sites such as sacroiliac joints (23, 24), femoral head (15), manubriosternal joints (25), knee (26) and shoulder (27) of AS patients.

Synovitis in peripheral joints

In RA the accumulation of immune cells inside the synovium is not organized at random. Since they migrate through blood vessels, accumulation is predominant around vessels with high endothelial venules (28). Expression of complementary adhesion molecules by migrating cells and synovium vessels controls the phenotype and function of the accumulating cells (29). In some instances, cells organizing and forming follicles are observed in activated lymph nodes. Although rather similar, key differences are observed regarding the expression of chemokines such as CCL19 (30). In line with these observations, subsets of dendritic cells show differences with the accumulation of rather immature DC when compared with lymph nodes and tonsils (31). B cells have been the focus of renewed interest. In addition to mature CD20 B cells, plasma cells are highly present

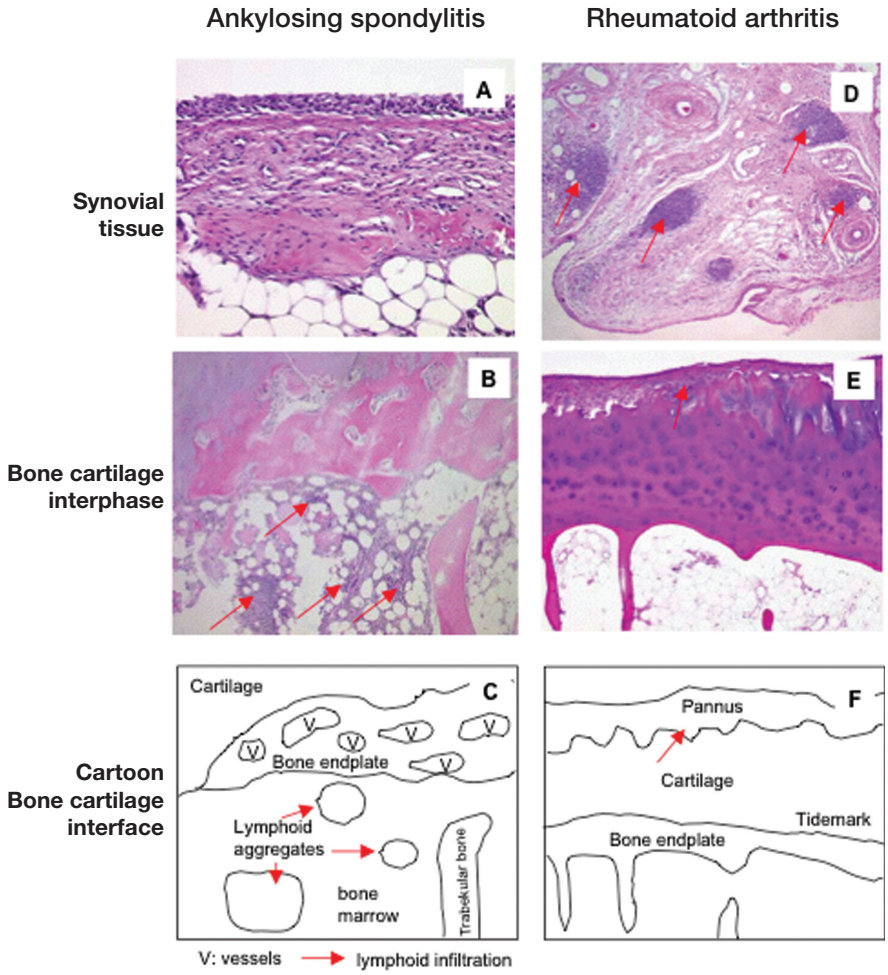

Fig. 1. Synovial membrane and subchondral bone marrow in a patient with ankylosing spondylitis (**A**-**C**) or rheumatoid arthritis (**D**-**F**): the primary site of inflammation is located at the bone cartilage interface or in the subchondral bone marrow. In RA inflammation primarily occurs in the synovial membrane.

including long lived plasma cells (32). Synovitis in SpA will be discussed in detail by the manuscript of Dominique Baeten within this edition.

Axial skeleton

At the onset of the disease the sacroiliac joints of AS patients are often primarily affected. Within the last decades numerous histomorphological descriptions of sacroiliac joints have been published while immunohistochemical analyses are rare. Inflammation in the subchondral axial and synovitis are the earliest histomorphological changes. In contrast to RA the hypertrophic synovium displays only little synovial hyperplasia of the synovial layer cells and the subsynovial tissue consists of macrophages and T lymphocytes (18, 24).

For further immunohistochemical analysis of inflammation in the axial skeleton bone tissue samples from facet joints of AS patients who underwent a polysegmental correction of rigid hyperkyphosis were collected and studied to describe histopathological and immunohistochemical features of acute inflammation in zygapophyseal joints of the lumbar spine (33). In this study it could be demonstrated that zygapophyseal joints are directly involved in inflammatory processes in AS. It was striking that even after long standing disease, inflammatory activity can still be present in the spine of some patients with AS with advanced ankylosis. The finding of T cells, B cells and neoangiogenesis in lesions with persistent inflammation gave evidence that several immunological mechanisms might be involved in the pathogenesis of the disease (33). Similar to findings in femoral heads bone marrow oedema neighbouring such inflammatory infiltrates and also high micro vessel density was observed.

RA as a rule does not affect the axial skeleton. The major exception is the upper cervical spine where C1/C2 dislocation is the consequence of local synovitis leading to bone and ligament damage and destruction. Similar to peripheral joints, new bone formation is not seen.

Does inflammation as detected by bone marrow oedema in MRI analysis in AS and RA patients reflect histopathological findings?

An analysis which compared the histopathological findings of osteitis in AS facet joints with MRI revealed a good correlation between histological and MRI oedema in AS patients, although MRI seemed to be less sensitive in comparison to histopathological analysis (34). It could be shown that oedema was a product of inflammatory reaction but these histopathological studies also indicated that inflammation reflected by cellular infiltrates did not always cause the same degree of oedema. It was suggested that this might be an explanation for the sometimes observed discrepancy between high disease activity and negative MRI in AS. Such correlation analysis has also been performed in metacarpophalangeal and proximal interphalangeal joints from RA patients with similar results. Bone erosion as detected by MRI correlated well with cortical bone penetration and bone marrow oedema correlated well with lymphocytic infiltrates, increased vascularity and water content (21) which could be confirmed by a more recent analysis (12).

***In situ* analysis of cytokines in AS and RA**

Both diseases are associated with high proinflammatory cytokine expression at the site of inflammation. The demonstration of their clinical relevance has started in RA with inhibitors of TNF, which have shown a very significant effect in a substantial proportion of patients (35). Although results in the mouse predicted a key role for IL-1, inhibition of IL-1 with IL-1RA has not provided similar clinical response as for

anti-TNF inhibitors (36). Reasons are still unclear. More recently inhibition of IL-6, another non specific cytokine, has shown efficacy when targeting the IL-6 receptor for both the systemic and local manifestations of RA (37).

Immunohistochemical analysis of sacroiliac joints in AS patients could detect TNF as one important cytokine mediating inflammation (19), leading to the first clinical trials with TNF-blocking agents in AS patients (38, 39).

While it is believed that T cells play a relevant role in the pathogenesis of SpA the type of effector T cells is not clear. Several antigens, both bacterial and auto-antigens, have been described in AS to be stimulatory for Th1 cells, but their relevance for the disease process is still not defined. The role of Th17 cells, another potentially important T effector subpopulation, is not clear and not yet well investigated in SpA/AS.

We are currently investigating the role of TH17 cells in AS and could already obtain preliminary data showing that IL-17 secreting cells are present in an increased number in synovial fluid and histological sections (40) from the spine in SpA patients.

Regulatory T cells in AS and RA

Regulatory T cells (T regs) are crucial for the inhibition of effector T cells and a relative imbalance in the effector/regulatory T cells ratio and/or a resistance of effector T cells to Treg could contribute to an ongoing (auto-) immune response in chronic inflammatory rheumatic diseases such as SpA and RA.

Although the presence of T regs has been shown at the site of RA inflammation, in vitro studies have indicated functional defects. Inhibition of TNF has been shown to improve some of these defects without reaching a full correction (41). Since IL-6 and IL-17 are involved in the balance between regulatory T cells and proinflammatory Th17 cells (42), it is possible that inhibition of IL-6 and in the future of IL-17 may be a better way to restore these regulatory defects.

A further characterization of these T cells with regard to their activation and memory status has not been performed so far in SpA, which would be crucial for a better understanding of the balance/imbalance between effector and regulatory T cells and for the development of new targeted therapies. We are currently investigating the frequency of such cells in bone specimens of AS patients. In the synovial fluid of SpA patients we could already get some evidence that SpA patients with a more self limiting course of joint inflammation have a higher frequency of FoxP3+ T cells than patients with RA suffering from a chronic persistent joint inflammation (43).

From bench to bedside: new targets for the therapy of inflammation in AS and RA

The number of targeted therapies continues to grow for the treatment of RA and AS. Targeting of the IL-6 receptor is now approved in some countries. Targeting of cell cell interactions can be performed by two ways. The first way is to act on the interaction between T cells and antigen presenting cells by acting on the CTLA4 pathway. The other way is the depletion of B cells through CD20 targeting in order to eliminate the interaction between T cells and B cells. Among the list of cytokine targets, IL-17 could represent an interesting candidate through its effects on inflammation and destruction, often through synergistic interactions with TNF (44). Trials with monoclonal antibodies against the cytokine and its receptor are now starting.

The treatment with TNF blockers has meant a breakthrough in the management of active AS patients refractory to conventional therapy. About half of the patients treated with any of the approved TNF-blocking agents show a 50% improvement of their disease activity which goes up to 70-80% in patients with shorter disease duration (45, 46). Other treatment strategies using biological drugs have already been performed or data from such trials are currently collected. In a study conducted in 20 AS patients a subpopulation has been defined to benefit from treatment with antiCD20 antibody rituximab (47). Studies with abatacept are currently ongoing and studies with an anti-IL17 antibody have been initiated recently.

Conclusion

Acute inflammation either in RA or in AS is driven by similar cellular components consisting of T and B lymphocytes, high microvessel density and osteoclasts. In RA the synovial membrane is the primary site of inflammation from which bone destruction takes its origin (Fig. 1). In AS inflammation and bone destruction occurs primarily at the enthesis, at the bone cartilage interface and subchondral bone marrow and synovitis is secondary (Fig. 1). The course of inflammation is highly different in both diseases. While RA shows a chronic persistent inflammation AS patients often experience self limiting inflammation. It is very suggestive that this difference is of great importance for the explanation why arthritis in RA leads to bone destruction and in AS finally to new bone formation. In both diseases cytokines like TNF-alpha are strongly involved in pro-inflammatory processes which could be proven either by immunohistochemistry or by our daily experience of treating RA and AS patients successfully with TNF-alpha blocking agents. It will be interesting to see in the future if similar observations will be made with abatacept or other anti-inflammatory biologicals.

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