Original Article

Histopathological changes of the antigen-induced chronic arthritis in the knee joint of the rabbit

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ABSTRACT

Background and Objectives: **The cause and pathophysiology of rheumatoid arthritis has not been fully understood and an experimental model of this disease is essential for research on the problem. In this research study, establishment and histopathological changes of chronic arthritis due to intra-articular antigen injection was used as a model of experimental rheumatoid arthritis.**

Materials and Methods: **Thirty three New-Zeeland white rabbits were sensitized by subcutaneous injection of combination of methylated bovine serum albumin (MBSA) and Freund's complete adjuvant (FCA) at days 1 and 14. Sensitized animals at day 28 received intra-articular injections of MBSA. At days 7, 14, 21, and 28 post-injection, excised knee joints were investigated for routine light microscopic changes.**

Results: **It was found out that at day 7 there are fibrinous exudates in the joint space and pericapsular soft tissue, edematous synovial villi, and an intact cartilaginous site of joint. At day 14, lymphoid follicle formation at pericapsular area, short and widening of synovial villi, superficial erosion of joint cartilage (perichondritis) was observed. Thereafter, at day 21 increased secondary lymphoid follicles with active germinal centers at pericapsular areas, papillary hyperplasia of the synovial villi, thinning of the cartilaginous site of joint with mononuclear cellular infiltrates (chondritis) was noted. In addition, day 28 was demarcated by continuation of the chondritis and beginning of osteitis, granulation tissue formation (Pannus) at cartilaginous site of joint, and fibrotic changes of the synovial villi. Rare findings including pseudocyst space and palisading granuloma at the pericapsular area was also observed.**

Conclusion: **Antigen-induced chronic arthritis in the knee joint of the rabbit is a good experimental model to evaluate the pathogenesis and/or effects of drug interferences in the rheumatoid arthritis.**

Key words: **Chronic inflammation, Rabbit, Immune-arthritis, Histopathology, Rheumatoid arthritis**

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Introduction

Rheumatoid arthritis is a prototype of progressive chronic arthritis in human due to antigen deposition in the joints. It is composed of the extensive angiogenesis, synovial cellular hyperplasia, infiltrations of the different types of the leukocytes and changes of the CAM's (cell-adhesion molecules) and inhibitors of the proteases, and many different cytokines (1,2). Tissue edema and fibrinous exudates depositions of the first week are replaced by the mononuclear cellular infiltrates. Finally, infiltrations of the synoviocytes type B in the cartilage and pannus formation with destruction of the cartilaginous-osseous areas are due to the effects of the release of the metalloproteinases, which are released by the mononuclear cells and fibroblasts (3). Although many researches have been conducted to find etiology and even treatment mode of this debilitating disease, however, its exact pathogenesis is obscure and also specific drug therapy has not been recommended yet. The main goal of most routinely-used drugs is pain relief in inflamed joints. For these reasons; an experimental animal model for this disease is mandatory for better evaluation of inflammatory changes and also studying the effects of various drugs on the process of inflammation and repair.

Some previous studies have been done in temporomandibular joint of rabbit with focusing on the release of different interleukins (4). Therefore, in this study it was tried to focus on light microscopic findings of the simple approach knee joints to find the histopathology of infiltration of acute exudation, lymphoid cells, macrophages, plasma cells, and also pannus formation. These data could be expanded on their immunohistochemical patterns to the future studies.

Materials and Methods

Experiments were conformed to the national guideline for performing animal studies and conducted on 33 New-Zealand male white rabbits (with a weight range of 2.2-2.8 kg) divided into 5 groups: control (n=5), days 7, 14, 21, and 28 (n=7 for each of them) groups. Animals were anaesthetized with ip injection of 50 mg/kg of sodium thiopental. The back of the neck was shaved and 1 ml of a combination (1:1) of MBSA (4 mg/ml in distillated water) and FCA was injected intradermally at five points (0.2 ml at each point). For making this solution, MBSA was added drop-by-drop to FCA and vigorously shacked until a semi-viscous homogenized solution was made. To test the stability of antigen, a drop was added to the surface of a beaker containing distilled water, if the drop was spread on water a few drops of FCA was added and shacked until the drop did not spread over the water. The injection was repeated at day 14, and then at day 21 sensitization of the animal was confirmed by MBSA skin test. For this, 0.2 ml of MBSA solution (0.2 mg/ml) was injected subcutaneously in a shaved area of leg skin. The skin thickness was measured pre- and 24 hour post-injection using a caliper. An increase in skin thickness of at least 100% was a confirmation of sensitization to antigen.

Sensitized animals at day 28 received intra-articular injection of MBSA. This was performed using a 1 ml syringe containing 0.5 ml MBSA solution (2 mg/ml). The needle (28 G) was inserted into the middle of mid-patellar tendon into the joint cavity. Half of the solution was injected deeper into posterior space and the other half into anterior space. At days 7, 14, 21, and 28, excised knee joints along with normal (control) knees were put in 10% formaldehyde solution for 48 hours to be fixed. The bony joints were then decalcified in 10% nitric acid for 3-5 days by daily checking of its softness. The fixed tissues were processed in tissue processor and after paraffin embedding they were cut into 5-10 micron thick slices by tissue slicer. They were stained by routine Hematoxyline and eosin (H&E) method. Slide preparations were investigated by two different pathologists independently and blinded to animal groups by Olympus and Seitz microscopes.

The histopathological findings were scored according to the following arbitrary scores (4): synovial hyperplasia: 0 = one to three layers of

cells (synoviocytes), 1= four-six, 2= seven or more layers of cells; villous hyperplasia; 0= absence, 1= few, dispersed and short, 2= prominent and tall, 3= prominent and diffuse; mononuclear cellular infiltrates: 0=absence, 1= mild (25% of the field), 2 = (50%), 3 = (>50%), 4 = diffuse with lymphoid follicle formation.

Results

It was found out that normal joint space reveals intact chondroidal tissue overlying the bony trabeculae. In addition, synovium showed one or two layers of synoviocytes and loose stroma of the supporting chorion with scattered lymphocytes. The time course of histopathological changes during 4 weeks post intra-articular injection of antigen was as follows: at day 7 the findings were fibrinous exudate in the joint cavity and also extending to the pericapsular areas with pseudocyst formation. The infiltrate extends to the chorion of the synovium. Mononuclear cells infiltrate was mostly macrophages, lymphocytes and a few fibroblasts. Chondroid surface of the joint appears normal (Figure 1). At day 14, the changes were towards decreasing of the exudative reaction in the joint space and pericapsular areas. Villous hyperplasia as increased layers of the surface synoviocytes with tall and wide appearance was also noticed. The mononuclear cellular infiltrates were diffuse collections of the lymphocytes, macrophages and plasma cells in the congested stroma. Chondroid surface showed dispersed seeding of the fibrohistiocytic cells (Figure 1). At day 21, the changes were villous hyperplasia of the synovium of the joint space as tall and multiple proliferations. In the pericapsular areas, the infiltration of the mononuclear cells was diffuse along with multiple lymphoid

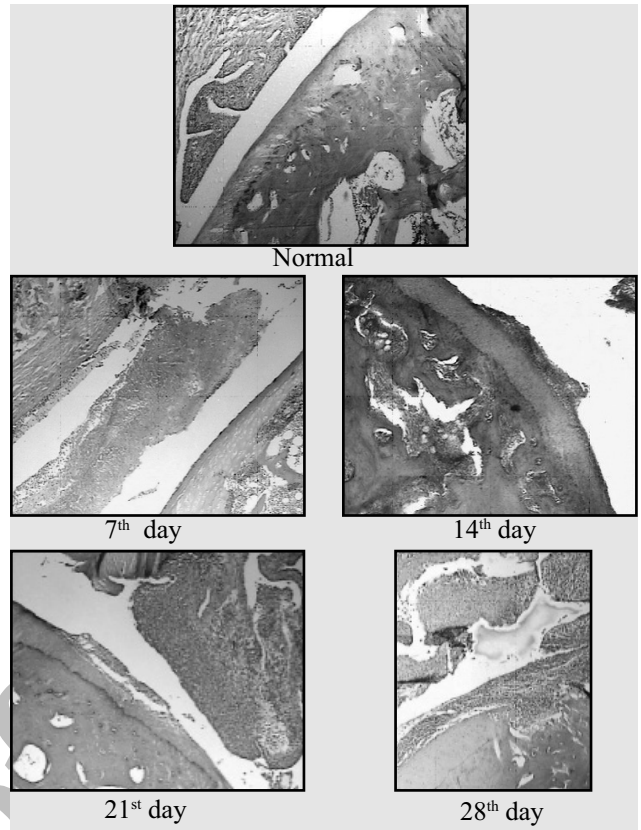

Figure 1: In comparison with normal joint, the histopathological changes during different days of experiment were as follow: at day 7 fibrinous exudates in the joint cavity with extension to the lamina propria of the synovium and non-inflammed chondroid surface were seen. At day 14, chondroidal surface showed dispersed seedings of the fibrohistiocytic cells. At day 21, the changes were villous hyperplasia along with diffuse mononuclear cellular infiltrate into the synovium. Erosion of the chondroidal surface with proliferation of the fibrohistiocytic cells as chondritis were also observed. At day 28, the changes were prominent villous hyperplasia in the synovium and extension of the fibrohistiocytic cells to deep layers as osteitis and pannus formation.

Table 1. Pathological scores of rabbit knee joint during 28 days post intra-articular injection of antigen

Groups	Mononuclear Cellular Infiltrate	Villous Hyperplasia	Synovial Hyperplasia	Pannus Formation	Total
7th day	1.4	1.6	0.2	0	3.2
14th day	1.8	1.6	0.8	1	5.2
21st day	3.4	1.8	0.4	1.6	7.2
28th day	2.8	2.2	0.6	2	7.6

follicle formation. Chondroid surface showed erosion with proliferation of the fibrohistiocytic cells and focal granulation tissue at the surface as chondritis (Figure 1). At day 28, the changes were prominent villous hyperplasia in the joint cavity. Dense mononuclear cellular infiltrates with focal palisading granuloma formation were occasionally seen at the pericapsular areas. Extension of the fibrohistiocytic cellular proliferation to the deep chondroid surface and even to the underlying bone tissue was also seen as pannus formation (Figure 1).

Table 1 shows the pathological scores of the above changes in the four groups of animals. In this respect, score of all indices increased with time with a high rate of increase during the first 21 days and reached an almost plateau later on.

Discussion

In this research study we established a good model of experimental arthritis focusing on histopathological changes during the best golden-time (28^{th} day). Also, these findings could be correlated with the rheumatic disease in human and in the future it is possible to look for its pathogenesis and effects of different drugs on the inflammatory processes of the affected joints.

Acute transient inflammatory changes at the first week of our research may be due to release of acute inflammatory mediators. In this respect, IL-1 beta and IL-1α ra are special cytokines and the key mediators of joint inflammation and cartilage destruction by inducing production and release of proteases from fibroblasts or chondrocytes, while reducing the production of the proteoglycans (4). Consden et al have shown that the injected antigen in antigen-induced arthritis accumulates in synovial cells and chondroytes (5). Stebulis also showed that products of fibroblast-like synovial cells such as propyl-4-hydroxylase, procollagens I and III, and TNF-α increase synthesis of IL-6, IL-8, and COX-2 mRNA (6). In addition, Buckley mentioned the effects of fibroblasts in the switching from resolving to persistent disease (7).

The infiltrate of the small lymphocytes in the stroma of the synovium and also pericapsular areas are the most prominent of our findings during 28-day time course of the study. Leipe has shown that CD_4^+, CD_{25}^+ T cells have a role in controlling and pathogenesis of the rheumatic diseases (8). Appel has noted a relative high secretion level of IL-10 and a low secretion of TNF-α in the synovial fluid and peripheral blood of patients with reactive arthritis as compared to patients with rheumatoid arthritis (9). Formation of lymphoid follicles as primary and/or reactive secondary ones was observed after the first week in our study. In this regard, B cells play a central and important role in the pathophysiology of rheumatoid arthritis (10). Carlsen has shown that newly recruited monocytes/macrophages play a role for lymphoid neogenesis in human inflammatory diseases (11). Braun showed that fibroblast-like synoviocytes express lymphotoxin beta receptors following its production by T and B cells. Release of this cytokine plays a central role in the development of lymph nodes and is critical for the formation of ectopic germinal center reactions in rheumatoid synovitis (12). Finally, the formation of the pannus as early seeding of the fibrohistiocytic cells on the surface of the cartilage, erosion with granulation tissue formation and extending of the inflammatory cells to the deep layer and underlying bone were observed after second week in our study. In this respect, Longato has shown that pannocytes do not originate from monocytes-macrophages or from fibroblasts but they belong to the family of the residue of the primitive mesenchymal tissue (primitive embryonal connective tissue-forming cells) (13). Wang also has shown that increased proteolytic activity of matrix metalloproteinases (MMPs) may promote articular destruction such as occurs in rheumatoid arthritis and osteoarthritis. Furthermore, MMP-12 enhances the arthritic lesions, resulting in sever synovial thickening, pannus formation, and prominent macrophages infiltration at an early stage and a marked destruction of articular cartilage associated with loss of proteoglycan at a later stage (14).

Conclusion

this experimental model is very useful to evaluate the inflammatory process during antigen- induced arthritis. The difference between scores of days 21 and 28 was slight, showing that 28 days was enough for the study. For further research, it is also a valuable model to investigate the effects of various drugs and treatments on inflamed joint.

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