INTRODUCTION: Rheumatoid arthritis (RA) is characterized by a variety of pathologic changes in joints, including synovial hyperplasia, inflammation, and alterations in the cellular immune responses. The pathologic changes observed are associated with synthesis and degradation of components of the extracellular matrix (ECM) in RA joints as well as osteoarthritis (OA). Tenascin-C (TN-C) is a hexameric glycoprotein component of ECM. A subunit of TN-C includes heptad repeats, epidermal growth factor (EGF)-like repeats, fibronectin type III-like (FNIII) repeats, and a carboxyl-terminal globular domain shared with fibronectins. The FNIII repeats can undergo alternative splicing. The large variants, containing the alternatively spliced FNIII domains in various combinations, play key roles in many pathologic conditions of joints, including tumorigenesis, regeneration, and inflammation. In RA and OA, TN-C is highly expressed in both synovium and cartilage in diseased joints. We have recently reported that, using an enzyme-linked immunosorbent assay (ELISA) with a monoclonal antibody specific to the large TN-C variant, TN-C levels of synovial fluid (SF) with OA were significantly increased, comparing those with healthy adults. The main purpose of the present cross-sectional study was to compare SF levels of the large variants from patients between RA and OA.

METHODS: SF samples were obtained during total knee arthroplasty from 18 patients with RA and 56 patients with OA. Both patient groups showed an advanced stage of the disease. All RA patients fulfilled the revised criteria of the American College of Rheumatology. The study group suffering from RA consisted of 4 men and 14 women with a mean age of 64.4 years and a mean body mass index (BMI) of 21.8 kg/m². The mean age of OA patients (7 men and 49 women) was 73.1 years and their mean BMI was 26.0 kg/m². None were treated with intraarticular injection of steroid or hyaluronic acid. C-reactive protein (CRP) was measured in sera as a marker of inflammation. Synovial membrane samples were obtained at the time of operation in patients with RA (n=4) and with OA of the knee (n=5). All patients gave informed consent, and this study was approved by the local ethics committee. Joint fluid was centrifuged at 15,000 x g for 15 minutes, and the supernatants were stored at -80 °C until analyzed. Western blot analysis of synovial fluids was performed using a previously described method to examine the presence of large splice variants of TN-C. Briefly, samples of 8-fold diluted SF were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis with a polyacrylamide gradient. The electrophoresed proteins were blotted onto Immobilon membranes, blocked with blocking buffer, and incubated with monoclonal anti-TN-C antibody (4F10TT against the EGF-like domain or 6C6MS against the FNIII C domain). After being washed, sections were treated with peroxidase-conjugated anti-mouse IgG Fab', followed by color development with diaminobenzidine / H₂O₂ solutions. Light counterstaining with hematoxylin was performed to aid orientation. Statistical analyses were performed using the Mann-Whitney U-test and the Fisher’s exact test. Correlation was estimated using Spearman’s rank correlation test. Statistical significance was set at p < 0.05.

RESULTS: We assayed for the presence of large TN-C variants, which include the FNIII C domain, in SF of knees with both RA and OA. The antibody 4F10TT reacted with all TN-C variants with molecular weights of 350 to 210 kDa in SF. The main band at 350 kDa co-migrated with the largest variant of human glioma TN-C. The antibody 19C4MS, which is specific for the large variants, reacted with the bands at 350 and 240 kDa, but did not label the 210 kDa band. These results indicate that the levels of large TN-C variants are considerably elevated in SF with RA and OA.

ELISA results indicated that the TN-C levels in the SF of RA samples were roughly threefold higher when compared with OA (p < 0.01, Figure). All OA patients showed serum CRP within the normal range, whereas all RA patients showed elevated serum CRP with the mean of 5.3 µg/ml. However, no correlation was found between the synovial levels of TN-C and the serum CRP.

Immunohistochemistry of synovium represented the positive labeling in the synovial lining cells and subintimal tissues from patients with RA and OA. Increased numbers of blood vessels were found in the inflamed synovium and TN-C deposited around the vessels.

DISCUSSION: Our study showed local synthesis of TN-C was increased during rheumatic disease. Immunohistochemistry indicated that TN-C of SF in RA might reflect synovial cell activation and inflammation. In RA, elevated TN-C levels in SF were higher than those in OA, implying a diagnostic usefulness to distinguish RA from OA.


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