

Increased Levels of IGF-I and IGFBP-3 in Synovial Fluids of Patients with Rheumatoid Arthritis

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INSULIN-like growth factors (IGFs), which are the most potent factors influencing proliferation and differentiation of chondrocytes [1], act on articular cartilage via synovial fluids. IGFs exist in the circulation and in many biological fluids associated with specific IGF binding proteins (IGFBPs). Although the physiological role of these IGFBPs in various biological fluids remains to be clarified, IGFBPs may modify the systemic and local activity of IGFs. Little is yet known about the IGF axis in synovial fluid. In this study, we have measured the concentrations of IGF-I, IGF-II and IGFBP-3 in normal synovial fluids, as well as pathological synovial fluids obtained from rheumatoid arthritis (RA) patients. We also identified and characterized the IGFBPs in normal synovial fluids by Western ligand blotting (WLB) and Western immunoblotting (WIB), and have compared them with those in samples from RA patients. IGFBP-3 protease in synovial fluids also has been identified.

Materials and Methods

Synovial fluids from 10 normal healthy volunteers (range 20-34 years old) and from 10 patients with RA (range 20-58 years old) were obtained after informed consent was given. Recombinant human IGF-I was obtained from

Bachem (Torrance, CA) and rh IGF-II was provided by Eli Lilly Research Laboratories (Indianapolis, IN). Rh IGFBP-3 was generously donated by Celtrix (Santa Clara, CA). IGF-I, IGF-II and *E. coli*-derived IGFBP-3 were iodinated by a modification of the chloramine T method [2].

IGF-I and IGF-II were measured by RIA after size-exclusion chromatography in 1% formic acid. IGFBP-3 was measured with a two-site immunoradiometric assay (IRMA) kit generously provided by D.S.L. (Diagnostic Systems Laboratories, Webster, Texas). Synovial fluids were subjected to Western-ligand blot analysis, as described by Hossenlopp [3] and modified as previously described [4]. IGFBPs were also characterized by Western immunoblot analysis by immunoprecipitation methods or chemiluminescence detection [4]. Synovial fluids were assessed for IGFBP-3 protease activity as previously described [5]. Significance was determined by the Student's unpaired 2-tailed *t*-test. *P* values < 0.05 were considered significant.

Results

The concentrations of IGF-I, IGF-II and IGFBP-3 are shown in Fig. 1. The IGF-I levels in synovial fluids of patients with RA (86 ± 34 ng/ml) were significantly higher than normal (19 ± 3 ng/ml) ($P < 0.05$), but IGF-II levels in normal (197 ± 14 ng/ml) and RA synovial fluids (259 ± 47 ng/ml) differed little. IGFBP-3 levels in synovial fluids from patients with RA (847 ± 104 ng/ml) were much higher than those in normal synovial fluids

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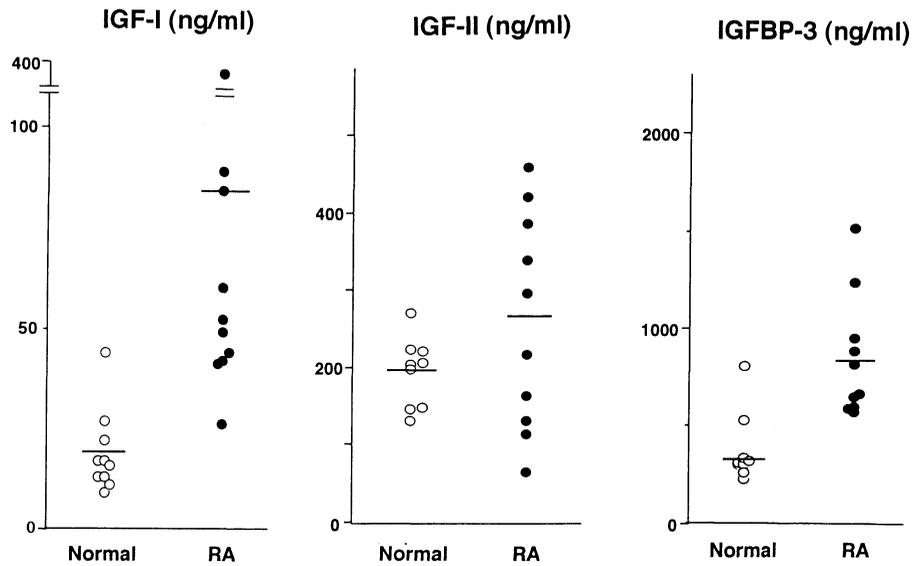


Fig. 1. The concentrations of IGF-I, IGF-II, and IGFBP-3 in human synovial fluid from normal and patients with rheumatoid arthritis (RA). Each point represents the mean of 3 replicates determined by radioimmunoassay. Each bar shows the average value in each group. IGF-I: Normal vs. RA: $P<0.05$, IGF-II: Normal vs. RA: not significant, IGFBP-3: Normal vs. RA: $P<0.001$.

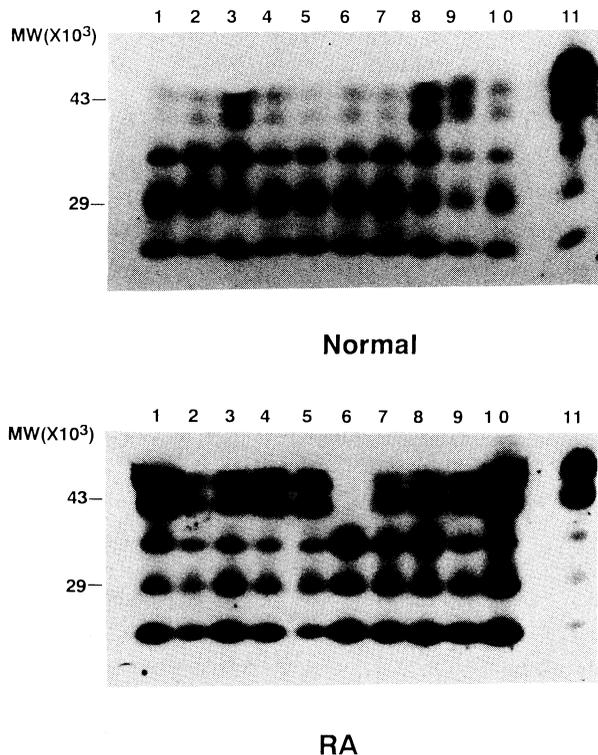


Fig. 2. Western ligand blotting (WLB) of synovial fluid. The molecular weight markers are indicated. Lane 11 on each autoradiograph shows IGFBPs of normal human serum. Upper panel: IGFBPs in synovial fluid (20 μ l) from ten normal young adults. Lower panel: IGFBPs in synovial fluid (10 μ l) from ten patients with RA.

(372 \pm 55 ng/ml) ($P<0.001$). To characterize the IGFBPs, normal synovial fluids (20 μ l) were subjected to WLB (Fig. 2, upper panel), which showed three distinct IGFBP bands at 32 kDa, 29–26 kDa and 24 kDa. A 42–39 kDa doublet band was seen in all samples but band intensity varied (lanes 1–10). To characterize the IGFBPs in pathological states, synovial fluids (10 μ l) from patients with RA were analyzed by WLB (Fig. 2, lower panel). All IGFBPs in synovial fluids from RA patients were higher than normal synovial fluids (lanes 1–10). The 42–39 kDa doublet of IGFBP-3 was particularly prominent (lane 1, lanes 3–5 and lanes 7–10).

To characterize IGFBPs, synovial fluids were subjected to immunoprecipitation and it was found that 42–39 kDa doublet was IGFBP-3, the 32 kDa band was IGFBP-2, the 26–29 kDa band was IGFBP-1, and the 24 kDa band was IGFBP-4 (data not shown).

Western-immunoblot for IGFBP-3 showed only the 30 kDa immunoreactive form of IGFBP-3 in all normal synovial fluids (Fig. 3, upper panel, lanes 1–10). Similar results were obtained from synovial fluids of RA patients (Fig. 3, lower panel), but the 42–39 kDa doublet was also evident in some RA patients (lanes 1 and 10).

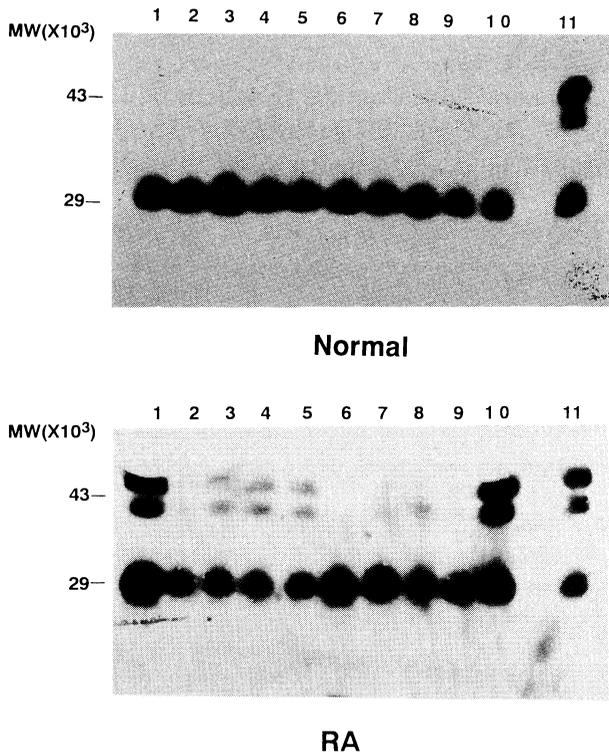


Fig. 3. Western immunoblot analysis with the antibody α IGFBP-3 in human synovial fluid from normal adults (upper panel), patients with rheumatoid arthritis (lower panel). Lane 11 shows normal human serum and Lanes 1-10 represent individual samples of synovial fluid.

We assessed synovial fluids for IGFBP-3 protease activity (Fig. 4). Iodinated IGFBP-3 appeared as a 29 kDa band with slight proteolysis (lane 0), but in synovial fluid from normal healthy adults, iodinated fragments of IGFBP-3 at 22 kDa, 20 kDa and 17 kDa were seen (Fig. 4. upper panel). A similar protease pattern was observed in synovial fluid from RA patients (Fig. 4. lower panel).

Discussion

Synovial fluids are important carrier of nutrients and growth factors to chondrocytes. We have found that the IGF-I levels in normal synovial fluid were about one-tenth of those in normal adult human serum, reported to be 176 ± 49 ng/ml [6] and that IGF-I levels in synovial fluid from patients with RA were much higher than those in normal samples, suggesting that local regulation of IGF-I may occur in synovial fluid. IGFBP-3 is known to be the most abundant IGFBP in serum, forming a

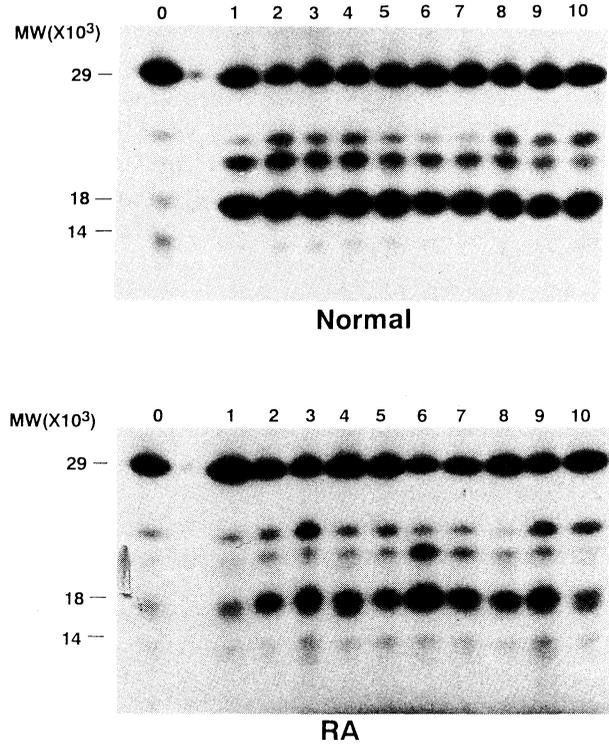


Fig. 4. Autoradiograph of a protease assay for digestion of $[^{125}\text{I}]$ IGFBP-3 with human synovial fluid. Lane 0 shows $[^{125}\text{I}]$ IGFBP-3 alone. Lanes 1-10 show $[^{125}\text{I}]$ IGFBP-3 incubated with human synovial fluid for 5 h at 37 °C. Upper panel: Synovial fluid from ten normal young adults, Lower panel: Synovial fluid from ten patients with RA.

ternary complex (150 kDa), that serves as the major circulating carrier of the IGFs and increases the half-life of the IGFs in the circulation [7]. In contrast to serum, the 42-39 kDa doublet (IGFBP-3) was low in normal synovial fluid, and a prominent immunoreactive 30 kDa IGFBP-3 fragment was detected by Western immunoblot analysis. Despite increased IGFBP-3 bands seen in WLB, intact IGFBP-3 detected by WIB is very low in RA. We can hardly explain these discrepancies between WLB and WIB, however, sensitiveness of antibody may effect on detection of intact IGFBP-3. The existence of an abundant amount of IGFBP-3 fragment was compatible with the presence of IGFBP-3 protease. The pattern of proteolysis of IGFBP-3 is almost the same in normal and in RA, although the proteolysis is decreased in some patients with RA. The reason for the increased levels of intact IGFBP-3 in RA is not clear, but perhaps it may be because of increase in production

of IGFBP-3 with or without decrease in proteolysis of IGFBP-3. Such proteases have previously been detected in serum from pregnant woman [8] and serum from patients undergoing catabolic stress [9, 10]. Proteolysis might increase the bioavailability of IGFs by reducing the affinity of

IGFBP-3 for IGFs in situations where a greater anabolic activity is required, or even under normal conditions to maintain the metabolism of cartilage tissue. But in RA, increased IGFBP-3 and IGF-I in synovial fluid might reflect some disruption of the IGF axis.

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