PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **186**, 384–389 (1987)

GROWTH FACTORS WITH HEPARIN BINDING AFFINITY IN HUMAN SYNOVIAL FLUID

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Abstract. Synovial effusions were obtained from the knees of 15 subjects with joint trauma, menisceal or ligamentous injury, or osteoarthritis. Heparin-Sepharose affinity chromatography of these synovial fluids revealed, in general, three major peaks of mitogenic activity as measured by incorporation of ^{3}H-thymidine into 3T3 cells. Gradient elution patterns showed activities at 0.5M NaCl, which is characteristic of platelet derived growth factor, and at 1.1M NaCl and 1.6M NaCl, indicative of acidic and basic fibroblast growth factors, respectively. The identities of these mitogenic fractions were confirmed by specific immunologic and receptor-binding assays. The presence of platelet derived, acidic and basic fibroblast growth factors in the synovial fluid may contribute to wound healing in the arthritic joint.

Growth factors that promote proliferation and migration of cells have been isolated from cartilage (1,2) and bone (3). These growth factors may play a role in the reparative processes in the osteoarthritic joint where capillaries and mesenchymal cells migrate from subchondral bone to the joint surface and produce new cartilage and bone. A cartilage derived growth factor (CDGF) (2), a cationic 18,000 molecular weight polypeptide that appears to be similar to basic fibroblast growth factor, produces in cell cultures many of the changes that occur within the osteoarthritic joint in vivo, such as chondrocyte and synovial cell proliferation with enhanced hyaluronate synthesis, and angiogenesis (4).

Chromatography on heparin-Sepharose has been used as a method for characterizing and separating growth factors (5). Platelet derived growth factor (PDGF) binds weakly to heparin and eluates at 0.5M NaCl. Acidic and basic fibroblast growth factors (aFGF, bFGF) have stronger affinities for heparin, eluting at about 1.1M NaCl and 1.6M NaCl, respectively. We used heparin-Sepharose chromatography to demonstrate growth factors in effusions obtained from subjects with knee complaints related to injury or to osteoarthritis (OA). Although their distribution varied from patient to

0037-9727/87 $1.50

patient, we report the identification of PDGF, aFGF and bFGF in many of these joint fluids.

Subjects and Methods. A total of 15 subjects with knee complaints provided informed consent for an indicated knee aspiration, and were divided into 3 groups.

Group I - those with acute spontaneous or traumatic knee effusions of a few days duration.

Group II - those undergoing arthroscopy for knee complaints that were recurrent for months to a year or more (a), or for whom only joint aspiration was performed (b).

Group III - those with knee complaints of several years duration with classical clinical and radiographic findings of OA who were undergoing total knee replacement.

Collection and fractionation of synovial fluids. Synovial fluid was collected in chilled sterile tubes containing citrate, gentamycin, and protease inhibitors (benzamidine, EDTA, iodoacetic acid and PMSF). In no instance was any blood observed grossly in the fluid. Cell counts were done and the fluid centrifuged (10,000 x 20 min) and stored at 4°C until used. Between 12-15 ml of fluid were treated with 10 TRU of streptomyces hyaluronidase (Miles Labs, Naperville, IL) and incubated for 15 min at 37°C. Heparin-Sepharose (Pharmacia, Piscataway, NJ) affinity chromatography (2) was carried out at 4°C (column 0.7 x 4.0 cm) with gradient elution (30 ml 0.2M - 2M NaCl, 0.01M Tris HCl, pH 7.4).

Assays for growth factors. Samples (10 ul) of each fraction were tested for mitogenic activity by measuring incorporation of ^{3}H-thymidine into DNA of confluent quiescent BALB/c 3T3 cells (2). One unit of growth factor activity is defined as that inducing half maximal DNA synthesis.

Fractions obtained from the 0.5M eluates from heparin-Sepharose chromatography were tested directly for PDGF by a radioreceptor assay (6). The 1.1M and 1.6M eluates from heparin-Sepharose chromatography were further purified by reverse phase HPLC (2) to permit immunological assays: Western blot analysis for aFGF (7), and immuno-slot-blot analysis for bFGF (8).

Results and discussion. Heparin-Sepharose affinity chromatography was used to isolate and characterize growth factors in human synovial fluids obtained from patients with knee injury or osteoarthritis. A total leukocyte count of 1000/mm^{3} or less in all fluids indicated a low grade state of joint inflammation.

Before analyzing the synovial fluids, known growth factor standards were separated by heparin-Sepharose chromatography and NaCl gradient elution. The column fractions were then tested for mitogenic activity on 3T3 cells (Fig. 1). Human serum (3ml)

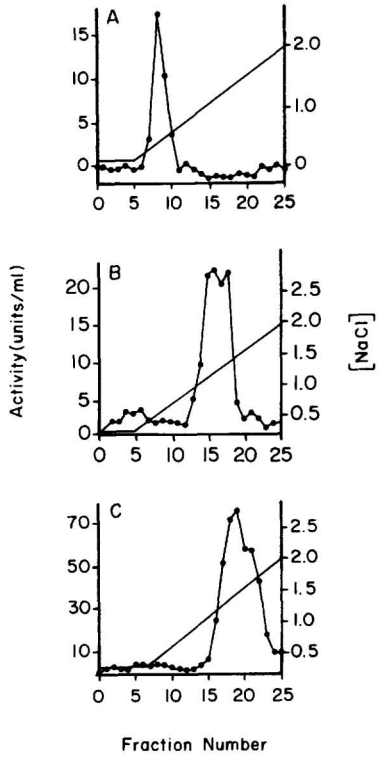

Fig. 1 - Heparin-Sepharose affinity chromatography and NaCl gradient elution of growth factor standards. Each fraction was tested for mitogenic activity by measuring the incorporation of 3H-thymidine into 3T3 cells. A=PDGF in 0.5M eluate fractions; B=aFGF in 1.1M eluate fractions; C=CDGF (similar to bFGF) in 1.6M NaCl eluate fractions.

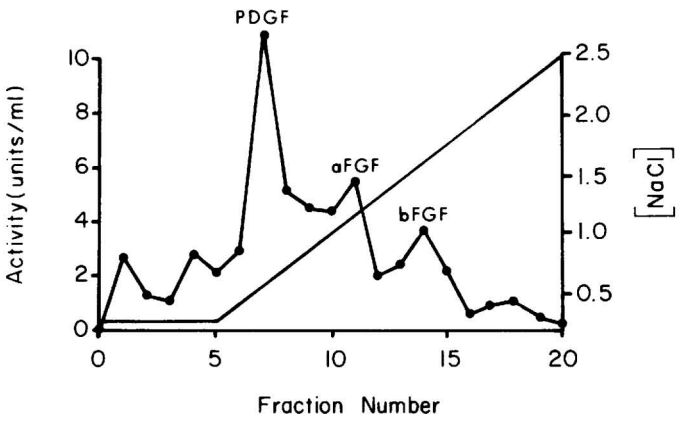

Fig. 2 - Heparin-Sepharose affinity chromatography and NaCl gradient elution of a synovial fluid sample (#8 in the table). Peaks of mitogenic activity correspond to the location of growth factor standards: PDGF, aFGF and bFGF (see Fig. 1).

was used as a source of PDGF since this growth factor is released from alpha-granules of platelets during the clotting process (9). Fractions eluting at about 0.5M NaCl showed mitogenic activity for 3T3 cells (Fig. 1A) and contained PDGF (11.6 ng) as determined by the radioreceptor assay. The other standards used were purified aFGF, and CDGF as a source of bFGF; these were derived from bovine brain (7) and cartilage (2), respectively, and were present in fractions eluting at 1.1M NaCl (Fig. 1B) and 1.6M NaCl (Fig. 1C).

Synovial fluid samples were applied to similar heparin-Sepharose columns. In 12 of 15 samples of synovial fluid studied, one or more peaks of mitogenic activity were observed in the eluate fractions as judged by enhanced ^{3}H-thymidine uptake by 3T3 cells. However, there was considerable variation in the patterns of mitogenic activity in the eluates from each sample of synovial fluid tested. Fig. 2 shows a representative example, as observed in 7 of 12 fluids, in which mitogenic activity was detected at elution position characteristic of PDGF, aFGF, and bFGF.

To establish the identity of these growth factors, fractions eluting similarly to PDGF were monitored by a radioreceptor assay. Those corresponding to aFGF and bFGF were subjected to HPLC and then identified by Western blot analysis or immuno-slot-blot techniques, respectively. The radioreceptor and immunological assays revealed that PDGF was detected in 8 of 15 samples of the 0.5M eluate fractions tested, aFGF was found in 3 of 6 samples of the 1.1M eluates tested, and bFGF was detected in 4 of 4 samples of the 1.6M eluates tested (Table 1).

This report is the first to establish the presence of PDGF, aFGF and bFGF in synovial fluids obtained from patients with joint conditions of varying duration (days to years) and with low grade inflammation secondary to trauma, ligamentous or menisceal injury, or to OA. The number of subjects is too few to document any trends in relation to the clinical condition of the joint. We realize that the examination of synovial fluids from individuals with "normal" knee joints without effusions will be of importance in evaluating the significance of our findings. Limiting factors in such studies will be the amount of fluid obtained (often less than 0.5ml) and the need for a high degree of sensitivity in the specific assays for each growth factor. Another aspect which may complicate evaluation of synovial fluids in a variety of arthritic conditions is the presence of binding proteins, such as described for PDGF (9) which can prevent detection in specific assays. Given such limitations, the findings described here are

TABLE I. PATIENTS STUDIED FOR GROWTH FACTORS IN JOINT FLUID

Group	Patient	Age/Sex	Duration Knee complaints	PDGF ngm/ml*	aFGF+	bFGF++
I						
Arthrocentesis						
	1	40 M	8 days	0.065	pos.	
	2	37 F	3 days	0.5	neg.	pos.
II						
Arthroscopy						
IIa	3	46 F	1 year	neg.		
	4	39 M	1 month	0.18		
	5	57 F	3 years	0.13		
	6	41 M	2 years	neg.		
Arthrocentesis						
IIb	7	59 M	1 year	neg.	pos.	
III						
Total Knee Replacement						
	8	73 F	14 years	0.04		pos.
	9	75 M	15 years	neg.		
	10	67 M	15 years	0.02		
	11	69 F	6 years	1.35	pos.	pos.
	12	74 M	3 years	0.13	neg.	
	13	63 F	12 years	neg.		pos.
	14	76 F	10 years	neg.		
	15	84 F	2 years	neg.	neg.	

* Values for PDGF are calculated on the basis of ng/ml of original synovial fluid. Neg. refers to a level below the detection limit of the assay, generally < 0.02 ng/ml.

+ For aFGF detection based on Western blot analysis, pos. = positive, neg. = negative.

++ For bFGF detection based on immuno-slot-blot analysis.

significant and establish the presence of at least three well-characterized growth factors in human synovial fluid.

A platelet-derived beta-thromboglobulin in synovial fluid (10) may be a remnant of the growth factor termed CTAP-III (11). Other less well defined growth promoting components in joint fluid appear to have angiogenic effects (12,13).

Growth factors with heparin-binding properties in the synovial fluid probably arise locally from joint components. bFGF may arise from endothelial cells and chondrocytes (4), aFGF from bone (3), and PDGF from platelets (9) in the joint fluid, or endothelial cells or macrophage-like cells (9) in the synovial membrane. These growth factors in synovial

effusions may contribute to tissue repair and fibrosis in the joint. In animal studies PDGF (14) and CDGF (15) have been shown to enhance wound healing, and a brain-derived FGF (16) promoted repair in cartilage defects.

Further studies are needed to define the role of these growth factors in synovial effusions and especially in relation to the evolution of OA.

Acknowledgement. We thank Drs. Edward Habermann and Michael DiStefano of the Orthopedics Department who supplied the synovial fluid samples, Andrew Lustbader for the immuno-slot-blot assays, Gerry DiSalvo for the Western blot assays, Darcey Clark and Karen Piccininni for expert technical help.

Supported in part by Teaching Nursing Home Award AG 03949 from the NIA, and a grant from the Charles E. Culpeper Foundation (DH); grant CA 27163 from the NCI (MK); grant HL 18645 from the NHLBI, and a grant from R.J.R. Nabisco (R.R.).

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Received April 27, 1987.
P.S.E.B.M. 1987, Vol. 186.
Accepted October 9, 1987.