Eosinophilic Fasciitis

Increased Collagen Production and Type I Procollagen Messenger RNA Levels in Fibroblasts Cultured From Involved Skin

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- Two patients with eosinophilic fasciitis were studied to elucidate the activation of collagen production in this disorder. Histologic examination of biopsy specimens from the involved area of skin revealed the presence of inflammatory cell infiltrates and various degrees of collagen accumulation in the dermis, subcutis, fascia, and underlying muscle. Fibroblast cultures initiated from the involved skin exhibited 2.0- to 3.7-fold increased collagen production when compared with control fibroblast cultures established from the uninvolved skin of the same patients. Eosinophilic fasciitis fibroblasts also displayed 2.4- to 6.2-fold higher steady-state levels of type I procollagen messenger RNA than did the control cells, indicating pretranslational activation of type I procollagen gene expression. In addition, cellular fibronectin messenger RNA steady-state levels were elevated 1.9- to 3.3-fold in eosinophilic fasciitis fibroblasts. These results suggest that fibroblasts in the involved skin of patients with eosinophilic fasciitis exhibit an activated phenotype, similar to that of scleroderma fibroblasts, leading to accumulation of collagen in the skin and the underlying structures. (Arch Dermatol. 1990;126:613-617)

Eosinophilic fasciitis is a connective-tissue disorder clinically characterized by symmetrical swelling, induration, and thickening of the skin primarily affecting the middle parts of limbs, ie, forearms and legs, but sometimes also extending to the trunk. The skin involvement characteristically spares the fingers and toes. The induration of skin in the limbs may lead to impaired mobility. Typical laboratory findings include peripheral blood eosinophilia, hypergammaglobulinemia, and increased erythrocyte sedimentation rate, whereas rheumatoid factor and antinuclear antibodies are usually absent. Histologic examination of the involved skin and the underlying structures reveals the presence of inflammatory cell infiltrates and accumulation of collagen in dermis, subcutaneous fat, fascia, and the underlying muscle. It has been demonstrated that fibroblast cultures established from the involved skin of patients with localized and systemic scleroderma exhibit an activated culture phenotype, including elevated types I and III procollagen messenger RNA (mRNA) steady-state levels. This elevation has been demonstrated to be a result of enhanced rate of transcription of the corresponding genes. Similarly, activation of collagen synthesis of individual fibroblasts may also play a role in the pathogenesis of eosinophilic fasciitis.

In this study, we have examined procollagen gene expression in dermal fibroblasts cultured from involved skin of two patients with eosinophilic fasciitis to elucidate the role of activation of collagen synthesis in the pathogenesis of this disorder. The results indicate that eosinophilic fasciitis fibroblasts are characterized by an increased rate of collagen synthesis and elevated type I procollagen mRNA levels, suggesting that these cells exhibit an activated phe-
notype similar to that found in fibroblasts from the involved skin of patients with scleroderma.

REPORT OF CASES

Case 1. A previously healthy 23-year-old man was seen at the dermatologic clinic of University Central Hospital, Kuopio, Finland, 3 months after experiencing arthralgias involving multiple joints, progressive thickening of the skin over the arms and legs, weight loss of 26 kg, and persistent low-grade fever. Clinical examination revealed that the arms and legs were symmetrically swollen and that the skin was thickened and firmly bound to the underlying tissue, leading to considerable limitation of motion in both wrists, elbows, knees, and ankles. The fingers, toes, and face were spared. There was no evidence of systemic involvement (chest roentgenogram, electrocardiogram, and esophagomanscopy showed normal findings), and there was no history of Raynaud's phenomenon. Laboratory tests showed the following clearly elevated values: total peripheral blood leukocyte count, 13.2 x 10^3/L; with 0.46 eosinophils; IgE, 226 to 271 µg/L (normal range, 0 to 34 µg/L); erythrocyte sedimentation rate, 14 mm/h; C-reactive protein, 28 mg/L; and serum aldolase, 13.0 U/L (normal range, 1 to 6.5 U/L). Laboratory tests for serum levels of creatinine kinase, urea, iron, transferrin, folic acid, vitamin B12, angiotensin-converting enzyme, albumin, and calcium were within the normal range, and the titers for rheumatoid factor, antinuclear antibody, anti-DNA, and Borrelia burgdorferi antibodies were negative.

Case 2. A previously healthy 31-year-old woman was seen at the dermatologic clinic of University Central Hospital, Kuopio, Finland, 3 months after fever and noticeable tightness and swelling of the legs and forearms had developed. The skin of the extremities was found to be symmetrically indurated and edematous, but the fingers and toes were spared. There was no evidence of systemic involvement or Raynaud's phenomenon. The laboratory tests revealed eosinophilia of the peripheral blood (up to 0.50 of the total leukocyte count); other laboratory tests, as in case 1, were within the normal range or showed negative findings.

MATERIALS AND METHODS

Histopathology

Full-thickness skin biopsy specimens, including subcutaneous fat, fascia, and underlying muscle, were obtained from the affected skin sites of the patients. The specimens were fixed in 10% formaldehyde, processed routinely, and embedded in paraffin. Five-micron-thick sections were cut and stained with Masson trichrome stain.

Cell Cultures

For cell cultures, a full-thickness skin biopsy specimen was obtained from the palpable edge of a lesion of the forearm of patient 1, and, similarly, two adjacent punch biopsy specimens were taken from the edge of the skin lesion in the arm of patient 2. These locations were chosen to represent the most active site of the disease process, as judged clinically. Control cell cultures were taken from clinically unaffected skin on the same limbs of these patients. Fibroblast cultures were initiated by the explantation method and maintained in Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum (Gibco Biocult, Paisley, Scotland), streptomycin (50 µg/mL), and penicillin (100 U/mL) under an atmosphere of 95% air and 5% carbon dioxide. After reaching confluence, the primary cell cultures were subcultured by trypsinization. The cells used in the experiments were in the third or fourth subculture.

Measurement of Procollagen Production

To estimate the rate of procollagen synthesis by skin fibroblasts, the cells were labeled for 24 hours with 5 µCi/mL of tritiated proline (NET-285, 5.0 Ci/mmol; New England Nuclear, Boston, Mass) in culture medium containing, in addition to the supplements indicated above, ascorbate (50 µg/mL) and β-aminopropionitrile (50 µg/mL). At the end of the labeling period, the media were removed, the cells were detached with trypsin, and the number of cells was counted. After precipitation of the proteins with ethanol (final concentration of 80%) (at −20°C overnight), the amount of procollagen in the culture media was determined as peptide-bound tritiated hydroxyproline, as described previously.

RNA Extraction

Total cellular RNA was isolated as described by Chirgwin et al. Briefly, cell cultures were washed with phosphate-buffered saline (0.14 mol/L of sodium chloride and 0.01 mol/L of sodium phosphate [pH 7.4]) and lysed in 3 mL of 4 mol/L of guanidinium isothiocyanate; 25 mMol/L of sodium citrate, pH 7.0; 0.5% sodium lauryl sarcosine; 100 mMol/L of 2-mercaptoethanol; and 0.1% of an antifoaming agent (Antifoam A emulsion, Sigma Chemical Co, St Louis, Mo). The lysate was layered on a 2-mL cushion of 6.7 mol/L of cesium chloride and 25 mMol/L of sodium citrate (pH 6.0) and centrifuged at 35,000 rpm for 20 hours at 30°C in an ultracentrifuge (LS-M, Beckman, Fullerton, Calif) using a propertary rotor (Beckman SW-65 Ti). The pellet was washed with 200 µL of 95% ethanol, dissolved in 300 µL of water, and extracted with an equal volume of phenol/chloroform (1:1). The aqueous phase was precipitated with 2.5 volumes of ethanol and a 1:10 volume of 3 mol/L of sodium acetate. The RNA pellet was dissolved in water, and the RNA concentration was determined by the absorbance at 260 nm.

Measurement of Cellular mRNA Levels

For Northern blot analysis, 12-µg aliquots of each RNA sample were denatured in duplicate with glyoxal and were fractionated electrophoretically on 0.75% agarose gel. The quality of the RNA samples was checked by staining one set of the samples with ethidium bromide to visualize the 18S and 28 S ribosomal RNA subunits under UV light. The other set of RNA samples was transferred to nylon membranes (Pall Biodyne A, Pall Corp, Glen Cove, NY), and the RNA was immobilized to membrane by heating at 80°C for 2 hours. Prehybridization of the filters was carried out at 42°C for 12 to 20 hours in a solution containing 50% formaldehyde, 5 x SSC (standard saline citrate) (1 x SSC is equivalent to 0.15 mol/L of sodium chloride and 0.015 mol/L of sodium citrate), 50 mg/mL of sodium phosphate (pH 6.5), 0.02% Ficoll, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin, 250 µg/mL of denatured calf thymus DNA, and 0.1% sodium dodecyl sulfate (SDS). Hybridizations were performed in the same solution at 42°C for 20 hours using complementary DNA (cDNA) probes nick-translated with α-phosphorus 32 deoxyctydine triphosphate (dCTP) (3000 Ci/mol, Amersham, London, England) to specific activities of approximately 2 x 10^8 cpm/µg. The cDNA probes used were pHCALI, specific for human proα1(I) collagen mRNA, and pFFF1, specific for human fibronectin mRNA. After hybridizations, the filters were washed three times for 5 minutes in 2 x SSC and 0.1% SDS at 25°C and twice for 30 minutes in 0.1 x SSC and 0.1% SDS at 55°C. The filters were exposed to film (X-Omat, Kodak, Rochester, NY) at −70°C using intensifying screens. The hybridization sig-
Table 1.—Procollagen Synthesis by Fibroblasts Cultured From Skin of Two Patients With Eosinophilic Fasciitis

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Hydroxyproline, dpm per 10^6 Cells</th>
<th>Tritiated Proline, dpm per 10^6 Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF-1</td>
<td>79.8 ± 13.1 (2.0)</td>
<td>226.3 ± 36.1 (1.7)</td>
</tr>
<tr>
<td>C-1</td>
<td>40.3 ± 6.6 (1.0)</td>
<td>135.0 ± 25.6 (1.0)</td>
</tr>
<tr>
<td>Patient 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF-2A</td>
<td>146.3 ± 24.8 (3.7)</td>
<td>251.8 ± 35.3 (2.6)</td>
</tr>
<tr>
<td>EF-2B</td>
<td>99.8 ± 10.2 (2.5)</td>
<td>172.5 ± 16.5 (1.8)</td>
</tr>
<tr>
<td>C-2</td>
<td>40.0 ± 5.5 (1.0)</td>
<td>98.3 ± 2.9 (1.0)</td>
</tr>
</tbody>
</table>

*Fibroblast cultures were established as from affected skin (EF-1 from patient 1 and EF-2A and EF-2B from patient 2) and from contralateral, clinically uninvolved skin (C1 and C2) of two patients with eosinophilic fasciitis. Cultures were labeled with tritiated proline for 24 hours, and the amount of peptide-bound tritiated hydroxyproline and the total incorporation of tritiated proline into secreted proteins was measured. The values represent means ± SDs of four parallel cultures. The values in parentheses indicate the fold increase in comparison with the control cultures from the same patient. The abbreviation dpm indicates disintegrations per minute.

Table 2.—Proα1(I) Collagen and Fibronectin mRNA Levels in Fibroblasts Cultured From Skin of Two Patients With Eosinophilic Fasciitis

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Proα1(I) Collagen mRNA</th>
<th>Fibronectin mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF-1</td>
<td>11.8 (6.2)</td>
<td>9.3 (1.9)</td>
</tr>
<tr>
<td>C-1</td>
<td>1.9 (1.0)</td>
<td>5.0 (1.0)</td>
</tr>
<tr>
<td>Patient 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF-2A</td>
<td>22.1 (3.1)</td>
<td>7.7 (3.3)</td>
</tr>
<tr>
<td>EF-2B</td>
<td>17.4 (2.4)</td>
<td>4.7 (2.0)</td>
</tr>
<tr>
<td>C-2</td>
<td>7.2 (1.0)</td>
<td>2.3 (1.0)</td>
</tr>
</tbody>
</table>

*Fibroblast cultures were established as in Table 1, and the levels of proα1(I) collagen and fibronectin messenger RNAs (mRNAs) were determined as described in the "Materials and Methods" section. The values represent the mean of two parallel cultures. The values in parentheses represent the fold activation of eosinophilic fasciitis fibroblasts over the control fibroblasts from the same patients.

<table>
<thead>
<tr>
<th>Procollagen synthesis</th>
<th>Proα1(I) collagen</th>
<th>Fibronectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF-1</td>
<td>22.1 (3.1)</td>
<td>7.7 (3.3)</td>
</tr>
<tr>
<td>EF-2B</td>
<td>17.4 (2.4)</td>
<td>4.7 (2.0)</td>
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<td>C-2</td>
<td>7.2 (1.0)</td>
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</tr>
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Histopathologic examination of skin biopsy specimens from both patients revealed alterations characteristic of eosinophilic fasciitis. In patient 1, a remarkable accumulation of collagen was detected in the lower dermis, a thick fibrotic septum was seen in the subcutaneous layer, and the fascia appeared clearly thickened. Inflammatory cell infiltrates consisting of lymphocytes, histiocytes, and eosinophils were present in the fascia and adjacent muscle. In patient 2, inflammatory cell infiltrates were detected in the lower dermis, underlying fascia, and muscle. The fascia was clearly thickened, but no excessive accumulation of collagen in the dermis could be noted.

To elucidate the mechanisms of collagen accumulation in eosinophilic fasciitis, fibroblast cultures were established from the involved skin of the two patients and compared with control fibroblasts cultured from the adjacent uninvolved skin of the same patients, with respect to collagen production and cellular proα1(I) collagen mRNA levels. Eosinophilic fasciitis fibroblasts from patient 1 exhibited a 2.0-fold increased rate of procollagen production, while the cells from patient 2 demonstrated a 2.5- to 3.7-fold increased procollagen production (Table 1). The increase in the synthesis of tritiated hydroxyproline was in each case somewhat higher than the total incorporation of tritiated proline, suggesting selectively enhanced collagen production in comparison with total protein synthesis (Table 1).

Proα1(I) collagen mRNA steady-state levels were measured by Northern blot hybridizations of total cellular RNA using a nick-translated, human-sequence–specific cDNA probe (Figure). Eosinophilic fasciitis fibroblasts exhibited 6.2-fold (patient 1) and 2.4- to 3.1-fold (patient 2) elevated proα1(I) collagen mRNA levels when compared with control fibroblasts (Table 2).

In addition, cellular mRNA levels of another major fibroblast line were determined by Northern blot hybridizations of total cellular RNA using a nick-translated, human-sequence–specific cDNA probe (Figure). Eosinophilic fasciitis fibroblasts exhibited 6.2-fold (patient 1) and 2.4- to 3.1-fold (patient 2) elevated proα1(I) collagen mRNA levels when compared with control fibroblasts (Table 2).
protein component of the extracellular matrix, fibronectin, were estimated by Northern blot hybridizations (Figure). Eosinophilic fasciitis fibroblasts also exhibited clearly elevated levels of fibronectin mRNA when compared with control cells. The degree of enhancement varied from 1.9-fold (patient 1) to 2.0- to 3.5-fold (patient 2) (Table 2).

**COMMENT**

Eosinophilic fasciitis is a connective-tissue disorder characterized by inflammation and accumulation of collagen in the lower dermis, subcutis, underlying fascia, and muscle. Eosinophilic fasciitis has been regarded as a subtype of localized scleroderma (morphea), although there are differences in the clinical course of the two disorders. Specifically, eosinophilic fasciitis is clinically characterized by rapid onset and progression and often responds favorably to systemic corticosteroid therapy. The inflammation and fibrosis involve the underlying fascia and muscle, in addition to dermis and subcutis. Localized scleroderma usually starts more insidiously and shows slow progression, and tissue fibrosis is restricted to the dermis and subcutis.

The mechanisms of collagen accumulation in localized and systemic scleroderma have been studied in considerable detail, and it has been shown that fibroblasts cultured from progressing skin lesions of patients with scleroderma produce increased amounts of collagen when compared with control cultures. On the other hand, the amount of collagenase secreted by scleroderma fibroblasts is similar to that of control cells. Thus, in scleroderma the accumulation of collagen in dermal and subcutaneous tissues appears to result from increased production of collagen on a per-cell basis, and not from decreased turnover of the deposited collagen. The activation of collagen production in scleroderma fibroblasts has been shown to accompany an increase in the steady-state levels of types I and III procollagen mRNAs, apparently reflecting the increased rate of transcription of the corresponding genes.

The results of our study indicate that fibroblasts cultured from the involved skin of two patients with eosinophilic fasciitis produce increased amounts of procollagen, when compared with control fibroblasts from uninvolved skin sites of the same patients. The activation of collagen production was found to be associated with elevated cellular levels of procollagen mRNA, indicating activation of type I procollagen gene expression at the pretranslational level. The steady-state levels of fibronectin mRNA were also found to be elevated in fibroblasts from the involved skin of patients with eosinophilic fasciitis, indicating coordinate activation of type I procollagen and fibronectin gene expression in these cells. Thus, these results suggest that fibroblasts from the involved skin of patients with eosinophilic fasciitis exhibit an activated phenotype similar to that of fibroblasts cultured from involved areas of skin of patients with scleroderma.

An interesting observation was that the fibroblast cultures established from the involved skin of patient 2 clearly exhibited activation of collagen synthesis and elevated procollagen mRNA levels, while no significant accumulation of collagen could be detected in the dermis by histopathological examination. However, numerous infiltrates of inflammatory cells were detected in the dermis, and accumulation of collagen could be seen in the fascia. Thus, it is conceivable that the fibroblasts had been activated in vivo and that they maintained the activated phenotype in culture. It is also possible that the area examined was representative of an early stage of the disease process, and that the activation of collagen gene expression would subsequently lead to histologically detectable dermal fibrosis in vivo, as was demonstrated in patient 1. Note that the fibroblast cultures were established from full-thickness skin biopsy specimens, either from lesional or uninvolved control skin. Thus, the cultures tested in our study are likely to contain cells derived from all layers of the dermis. Recent studies by us, using in situ hybridizations, have demonstrated that most of the fibroblasts actively expressing type I collagen genes in the involved skin of patients with eosinophilic fasciitis reside in the deep dermis, subcutaneous tissue, and fascia. Consequently, the activation of collagen gene expression in the lower dermis and fascia may be even more pronounced than that noted in the fibroblast cultures that we have examined.

In situ hybridizations have shown a close association between inflammatory cell infiltrates and fibroblasts actively producing type I collagen in the involved skin of patients with scleroderma, suggesting an important role for mononuclear cell factors in the activation of fibroblasts. Characterization of inflammatory cell infiltrates has revealed that they consist predominantly of lymphocytes and macrophages. It has been shown that mononuclear cells secrete soluble factors capable of stimulating collagen synthesis by fibroblasts. Recent studies have identified two of these soluble factors: T lymphocytes secrete transforming growth factor β, a potent stimulator of collagen synthesis, and monocytes produce interleukin 1, which also stimulates collagen synthesis in dermal fibroblasts. Thus, the inflammatory cells present in the skin and subcutaneous tissue of patients with eosinophilic fasciitis may release transforming growth factor β and interleukin 1, which may then activate the adjacent fibroblasts to produce elevated levels of type I collagen. The accumulation of collagen in the affected tissues would then lead to manifestation of clinical features characteristic of eosinophilic fasciitis.

Of particular interest are recent observations on an eosinophilic fasciitis-like clinical picture developing in patients with a history of L-tryptophan ingestion. These patients rapidly develop swelling and induration of the skin symmetrically on the extremities, associated with peripheral blood and tissue eosinophilia. Clinically, the latter patients are similar to the two patients described in this study. These observations
raised the possibility that our patients might also have a history of L-tryptophan ingestion. Careful and thorough inquiries failed to reveal evidence of L-tryptophan exposure in either of our patients. Consequently, eosinophilic fasciitis appears to be a multicausal syndrome, perhaps caused by different initiating factors triggering the pathomechanistic reactions leading to a clinically similar phenotype.

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References